Diploma thesis

Comparison of cardiac MRI and endomyocardial biopsy in the diagnosis of myocarditis

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Philipp Rößler

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Division of Cardiology

Under supervision of

Assoz. Prof. Priv. Doz. Dr. med univ. Dr. scient. med. Peter Rainer

Priv. Doz. Dr. med. univ. Dr. scient. med. Johannes Schmid

Graz, 7th of December 2023

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Graz, 7th of December 2023 *Philipp Rößler m.p.*

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Abstract in German

Einleitung

Die Kardio-MRT und die Endomyokardbiopsie repräsentieren derzeit den nichtinvasiven und den invasiven Goldstandard in der Myokarditis-Diagnostik. Ziel der Hauptanalyse war es, die diagnostische Übereinstimmung beider Verfahren in der Myokarditis-Diagnostik zu analysieren. In der Subanalyse untersuchten wir, ob die Durchführung der Endomyokardbiopsie vor der Kardio-MRT zu vermehrten falsch positiven Myokarditis-Diagnosen führt.

Methoden

Wir führten eine retrospektive Datenanalyse durch. In die Hauptanalyse wurden 166 Patient*innen inkludiert, die zwischen 2008 und 2020 innerhalb eines Zeitraumes von max. 30 Tagen an unserer Universitätsklinik Biopsie und MRT erhielten. Für die Subanalyse wurden 147 Patient*innen eingeschlossen, bei denen innerhalb eines Zeitraums von max. 14 Tagen beide Untersuchungen durchgeführt wurden. Unsere Patient*innen wurden in die zwei Gruppen ‚Biopsie vor MRT' und ‚MRT vor Biopsie' eingeteilt. Wir verglichen die Gruppen hinsichtlich des Auftretens der Lake-Louise-Myokarditis-Kriterien und der Myokarditis-Diagnosen in der MRT und berechneten den positiven prädiktiven Wert (PPV) und den negativen prädiktiven Wert (NPV) für die MRT bezogen auf diagnostizierte Myokarditiden in der Biopsie.

Ergebnisse

In unserer Hauptanalyse wurde bei 119 (72 %) Patient*innen eine Myokarditis in der Biopsie oder in der MRT diagnostiziert. In den MRT-Befunden wurde bei 61 (37%) Patient*innen eine Myokarditis diagnostiziert, in den Biopsie-Befunden bei 101 (61 %) Patient*innen eine inflammatorische Kardiomyopathie. Bei 90 (54%) Patienten fand hinsichtlich der Fragestellung, ob eine Myokarditis vorhanden ist eine übereinstimmende Diagnosestellung in Biopsie und MRT statt. In unserer Subanalyse war beim Vergleich der Gruppen ‚Biopsie vor MRT' (n=55) und ‚MRT vor Biopsie' (n=92) die Häufigkeit des entdeckten nicht-ischämischen LGE (60,0% versus 73,9%, p=0,078) und der Myokardödeme (16,4% vs. 24,4%, p=0,249) nicht signifikant unterschiedlich. In der Gruppe ‚Biopsie vor MRT' wurden 92% der Fälle mit MRT-Diagnose Myokarditis in der Biopsie übereinstimmend als Myokarditis diagnostiziert, mit einem NPV und PPV von

42,9% und 92,3%. Im Gegensatz dazu wurden in der Gruppe ‚MRT vor Biopsie' nur 60 % der MRT-diagnostizierten Myokarditiden in der Biopsie übereinstimmend diagnostiziert mit einem NPV und PPV von 42,3% und 60,0%.

Conclusio

Wir stellten eine moderate Übereinstimmung von MRT und Endomyokardbiopsie in der Myokarditis-Diagnostik fest. Die Durchführung der Endomyokardbiopsie vor der MRT führte weder zu einer erhöhten Inzidenz von nicht-ischämischem Late-Gadolinium-Enhancement und Myokardödemen noch zu vermehrten falsch-positiven Myokarditis-Diagnosen.

Abstract in English

Introduction

Cardiac MRI and endomyocardial biopsy represent the current noninvasive and invasive gold standard of myocarditis-diagnosis. We aimed to investigate the diagnostic concordance of endomyocardial biopsy and cardiac MRI in the diagnosis of myocarditis. In the sub analysis, we investigated whether performing endomyocardial biopsy prior to cardiac MRI leads to an increased number of false-positive myocarditis diagnoses.

Methods

We performed a retrospective data analysis. One hundred sixty-six patients were included in our main analysis who received biopsy and MRI in a time frame of 30 days between 2008 and 2020 at our university clinic. For the sub analysis we included 147 patients who received both investigations within 14 days. The patients were divided into the groups 'biopsy before MRI' and 'MRI before biopsy'. We compared the groups regarding the occurrence of Lake Louise myocarditis criteria and MRI myocarditis diagnoses and calculated the positive predictive value (PPV) and negative predictive value (NPV) for the MRI with biopsy-confirmed myocarditis as a reference.

Results

In our main analysis 119 (72%) patients were diagnosed with myocarditis in either biopsy or MRI. In the MRI reports, 61 (37%) patients were diagnosed with myocarditis, compared to 101 (61%) inflammatory cardiomyopathy diagnoses in EMB reports. In 90 (54%) patients, a concordant diagnosis was made in biopsy and MRI with regard to the question of whether myocarditis was present or absent. Comparing groups in our sub analysis, frequency of detected non-ischemic LGE (60.0% versus 73.9%, p=0.078) and edema (16.4% vs. 24.4%, p=0.249) was not significantly different. In group ‘biopsy before MRI’ (n=55), 92% of MRI-suspected myocarditis cases were concordantly diagnosed with myocarditis in their biopsy reports with a NPV and PPV of 42.9% and 92.3%. In contrast, in group ‘MRI before biopsy’ (n=92) only 60% of MRI-suspected myocarditis cases were concordantly diagnosed in their biopsy reports with a NPV and PPV of 42.3% and 60.0%.

Conclusion

We found moderate concordance of MRI and endomyocardial biopsy in the diagnosis of myocarditis. Performing endomyocardial biopsy prior to cardiac MRI did neither result in increased incidences of nonischemic late gadolinium enhancement or myocardial edema, nor did it result in an increase of false-positive diagnoses of myocarditis in MRI.

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Abbreviations

ACE *Angiotensin-converting enzyme*

AM *Acute myocarditis*

ARBs *Angiotensin 2 receptor-blockers*

ARNI *Angiotensin receptor-neprilysin-inhibitor*

B. theta *Bacteroides thetaiotaomicron*

CAD *Coronary artery disease*

CMP *Cardiomyopathy*

CMR *Cardiac magnetic resonance imaging*

CMV *Cytomegalovirus*

CRP *C-reactive protein*

CTLA- *Cytotoxic T-Lymphocyte-associated protein*

DCM *Dilated cardiomyopathy*

EBV *Epstein–Barr virus*

ECG *Electrocardiogram*

ECMO *Extra corporal membrane oxygenation*

ECV *Extracellular volume*

EF *Ejection fraction*

EGE *Early gadolinium enhancement*

ESC *European Society of Cardiology*

ESR *Erythrocyte sedimentation rate*

GCM *Giant cell myocarditis*

HF *Heart failure*

HFrEF *Heart failure with reduced ejection fraction*

HHV6 *Human Herpesvirus 6*

HIV *Human immunodeficiency virus*

HLA *Human leukocyte antigen*

hsTnT *High-sensitive troponin T*

IABP *Intra-aortic balloon pumps*

ICIs *Immune checkpoint inhibitors*

iCMP *Inflammatory cardiomyopathy*

IFNß *Interferon ß*

IKDT *Institut für kardiale Diagnostik und Therapie*

IMI *Institute of Medical Informatics and Biostatistics of the Medical University of Graz*

LAG-3 *Lymphocyte activating gene 3*

LGE *Late gadolinium enhancement*

LLC *Lake Louise Criteria*

LM *Lymphocytic myocarditis*

LVEF *Left ventricular ejection fraction*

MCS *Mechanical circulatory support*

ms *Milliseconds*

MYH 6 *Alpha-myosin heavy chain 6*

MYH 7 *Beta-myosin heavy chain*

NGS *Next generation sequencing*

NSCLC *Non-small cell lung cancer*

NT-proBNP *N-terminal pro-B-type natriuretic peptide*

PCR *Polymerase chain reaction*

PD-1 *Programmed death 1*

SARS-CoV-2 *Severe acute respiratory syndrome caused by the coronavirus 2*

SCD *Sudden cardiac death*

SGLT2 *Sodium-glucose co-transporter 2*

SLE *Systemic lupus erythematosus*

sST2 *Soluble suppression of tumorgenesis-2*

TTE *Transthoracic Echocardiography*

URL *Upper reference limit*

US *United States*

VAD *Ventricular assist device*

VT *Ventricular tachycardia*

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1 Introduction

1.1 Definition of myocarditis

Myocarditis is an inflammatory disease of the muscular portion of the heart diagnosed by established histological, immunological and immunohistochemical criteria (WHO/ISFC definition). (1) It is a widely underdiagnosed cause of acute heart failure (HF), dilated cardiomyopathy (DCM) and sudden cardiac death (SCD). (2)
Inflammatory cardiomyopathy (iCMP) is defined as myocarditis in association with cardiac dysfunction (WHO/IFSC definition). (1) In the last three years, cardiac inflammation has become a well-recognized complication of 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2) infection and of SARS-CoV-2 vaccination. (3)

1.2 Epidemiology of myocarditis

Myocarditis is a cardiac condition affecting relatively young patients with the median age of onset ranging between 30 and 45 years. (4,5) The disease is a common cause of SCD in young people causing approximately 2% of infant's, 5% of childhood's, and 10 to 20% of young adult's SCD. (6–8)

The 'Global Burden of Disease' study determined a myocarditis incidence of 22 per 100,000 persons worldwide with a prevalence estimated at 9.1 per 100,000 persons. This data is based on hospital dismissal diagnosis codes from 1990 to 2013. The incidence can be extrapolated to approximately 1.5 million cases in the 2013 world population. (9,10) However, the accuracy of this data seems to be limited because there was no differentiation between acute myocarditis (AM), chronic iCMP and other forms of cardiomyopathy (CMP) in the study. Furthermore, collected data was derived from hospital dismissal codes and mild myocarditis cases without hospital admission were not included. Nevertheless, the data originating from this study is among the most cited in recent literature.

Within the last years, several studies investigated how the epidemiology of myocarditis differs between the sexes. The prevalence of myocarditis shows a female to male ratio between 1:1.5 and 1:1.7. (11)

Although still being ranked low on the list of differential diagnosis for many doctors, AM was the second most common cardiac cause of chest pain amongst patients presenting to the emergency department in a French registry in 2018. (12) A study conducted by Pasupathy et al. found around one third of patients initially suspected as myocardial infarction with non-obstructed coronary arteries are later diagnosed as AM. (13)

Finding sound epidemiological data on myocarditis is difficult as there are confounders related to the availability and quality of surveillance, as well as limitations to establishing a diagnosis of cardiac inflammation. (14) Moreover, the disease is heterogenous in etiology, clinical presentation, severity, and temporal dynamics. An additional factor limiting access to valid in-vivo data is the fact that many studies generate their data from autopsies.

1.3 Etiology of myocarditis

A variety of factors with the ability to cause myocarditis have been identified. There are exogenous infectious pathogens such as viruses and bacteria, exogenous non-infectious pathogens such as toxic substances and drugs, and systemic immune-mediated diseases. (15,16) The incidence and etiology of myocarditis are extremely variable depending on the geographic area. In developed countries like the United States (US) and Europe, viral infections are considered the most common cause, while in Latin America Chagas disease is seen as the leading cause. Chagas disease is caused by the protozoan parasite Trypanosoma cruzi and shows a markedly higher prevalence in Latin America compared to other continents (4,17). It presents the most common cause of non-ischemic CMP in that area. (18) To this day, data for many other parts of the world are still scarce. (19) Unfortunately, the specific etiology of myocarditis remains unknown frequently throughout the diagnostic process. In these ambiguous cases, an inappropriate autoimmune reaction potentially triggered by pathogens on the basis of multifactorial pre-disposition (e.g., genetic, environmental circumstances) may be assumed.

1.3.1 Viral etiology

Myocarditis can develop until weeks after the primary viral infection. Table 1 presents an overview of the most common viral agents associated with myocarditis and iCMP. Additionally, their respective tropism and their attributed virulence are shown. (16)

Table 1: Viruses associated with myocardial inflammation.

viral tropism	virus	viral genome	virulence
Cardiotropic	Adenoviruses Enteroviruses (Coxsackieviruses, Echoviruses)	dsDNA (+) ssRNA	virulent virulent
Vasculotropic	PVB19	ssDNA	Bystander: latent; low viral DNA copy numbers in cardiac tissue; with or without cardiac inflammation Virulent: high viral DNA copy numbers in cardiac tissue (>500 DNA copies per microgram cardiac DNA) with cardiac inflammation or systemic infection
Lymphotropic	Cytomegalovirus (CMV); Epstein–Barr virus (EBV); human herpesvirus 6 (HHV6)	dsDNA	Bystander: latent, low DNA copy numbers in cardiac tissue, with or without cardiac inflammation Virulent: high cardiac DNA copy numbers, with cardiac inflammation
Cardiotoxic	Hepatitis C; human immunodeficiency virus (HIV); Influenza viruses	(+) ssRNA	Virulent: cardiac inflammation with viraemia
ACE2-tropic; cardiotoxic (?)	Coronaviruses (MERS, SARS, SARS-CoV-2)	(+) ssRNA	Virulent: cardiac inflammation with viraemia
Based on Tschöpe C et al., 2021. (16)			

When analyzing detected viruses in EMB samples, it is important to differentiate between virus-associated and virus-induced inflammation. As shown in Table 1, PVB19 and lymphotropic viruses are suspected to have a bystander role when they are prevalent in low copy numbers, whether cardiac inflammation is present or not. In case of these viruses being present in high copy numbers with coexistent inflammation they should be considered as virulent and therefore a possible cause of an ongoing inflammatory process. (16)

The proportion of detected viruses in EMB samples has shifted within the last decades. While in the late 1990s there was a shift from Cocksackie-B-viruses to Adenoviruses, mainly Parvovirus B19 and other viruses have been found since the mid-2000s. (20–22) The Marburg Myocarditis Registry, one of the biggest EMB registries to date, analyzed the etiology of 1098 patients with suspected inflammatory DCM and/or myocarditis and found Parvovirus B19 to be by far the most common detected pathogen in EMB. It was detected in 28% of patients. (23)

Obtaining a travel history is important as travel-acquired viral infections such as Dengue and Zika virus have been associated with myocarditis. In case of immunosuppressive therapy, the differential diagnosis should be broadened and certain pathogens such as EBV, CMV and fungi that are typically responsible for a relatively low number of cases in the immunocompetent patient may need to be considered. (24)

1.3.1.1 Non-viral infectious etiology

Besides viruses, there are many other pathogens capable of causing myocarditis. Among them are bacteria, protozoa, and fungi. Considering the myriad of possible pathogens, only a selection is presented here.

Bacterial infection with Borrelia burgdorferi, transmitted by tick bite, can lead to Lyme disease with possible cardiac involvement. In Europe and the US cardiac involvement is reported in 1-2% of Lyme disease patients. (25–27) Lyme disease has an endemic occurrence with distribution reported mainly in Scandinavia, Central Europe (especially Germany, Austria, Slovenia), upper mid-west and northeastern USA and some parts of

Asia. (28,29) Lyme carditis typically presents with atrioventricular conduction abnormalities in the electrocardiogram (ECG). (20,30)
Another bacterium associated with myocarditis is Corynebacterium diphtheriae, leading to almost 50 cases of myocarditis per 100 million persons per year worldwide. The diphtheria associated myocarditis incidence is much higher in countries of the former soviet union. (2,31) Moreover, fungal pathogens are also known to have the ability to cause myocardial inflammation especially in immunocompromised patients. The most common causes of fungal myocarditis are Candida albicans, followed by Aspergillus and Cryptococcus Neoformans. (32)

1.3.2 Non-infectious etiology

There are various non-infectious triggers of myocarditis including toxic substances and drugs, allergic/hypersensitivity reactions, autoimmune phenomena and systemic immune-mediated diseases. (15,24)

Substances and drugs
A detailed drug history including prescription, over the counter and recreational drug use and the recent vaccination history are critical. Numerous drugs have been linked to toxic myocarditis for example catecholamines and chemotherapy. Exemplary recreational drugs known to cause myocardial inflammation are amphetamines and cocaine. (24) Immune checkpoint inhibitors (ICIs) revolutionized the cancer treatment for many forms of cancer like malignant melanoma and non-small cell lung cancer (NSCLC). They are monoclonal antibodies directed against specific T-cell receptors. ICIs abolish the inhibitory effect of T cell receptors on the cytotoxic T cell immune response, thereby activating T cells to fight tumor cells. Antibodies currently approved as drugs include the following receptors: Programmed death 1 (PD-1), cytotoxic T-Lymphocyte-associated protein 4 (CTLA-4), PD-1 ligand and lymphocyte activating gene 3 (LAG-3). Therapeutic responses are improved by combining those agents. Unfortunately, the ICIs also interrupt immunological T-cell self-tolerance manifesting as autoimmune-like events with variable organ involvement. ICI myocarditis is an infrequent manifestation, which shows variable clinical presentation with the potential for severe cardiac inflammation with fatal outcome. (33,34) Due to the widespread use in our aging population ICI myocarditis might be one of the leading causes

of AM in the near future. Moreover, bites (e.g. snakes and spiders) and stings (for example scorpion stings (35), giant hornet stings (36) have been reported to cause myocarditis. (24)

Allergic/hypersensitivity reactions

Drugs can not only cause toxic myocarditis, but also allergic/hypersensitivity myocarditis. Antibiotics, antipsychotics and some anticonvulsants are a few of the numerous identified examples. (2,20) Furthermore, several vaccines against different diseases are linked to this subtype including vaccines against hepatitis B, meningococcal C, smallpox and COVID-19 vaccines. (37–39)

Systemic immune-mediated diseases

About 7% of patients with diagnosed myocarditis have an associated autoimmune disease and these conditions may need additional therapy. Examples for systemic immune-mediated diseases associated with myocarditis are systemic lupus erythematosus (SLE), granulomatosis with polyangiitis, sarcoidosis, psoriasis and systemic hypereosinophilic syndromes. (24) Sarcoidosis is a granulomatous multisystem disease and can be present predominantly with cardiac manifestation in about 5% of sarcoidosis-patients. (24,40,41)

(Auto-)immune phenomena

The role of autoimmunity in myocarditis is extensively investigated and established, although many questions remain unanswered. Autoantibodies specific for the heart are present in around 60% of patients with myocarditis and their relatives. (16,40,42,43) There are many cardiac epitopes which are recognized by autoantibodies, particularly ß-myosin heavy chain epitopes also known as myosin 7 (MYH 7) and alpha-myosin heavy chain epitopes known as myosin 6 (MYH 6). (16,44) Some of these antibodies seem to have direct pathogenic and prognostic role in myocarditis. (16,45,46)
In animal models the passive transfer of antibodies purified from rats previously immunized with cardiac myosin leads to myocyte apoptosis and development of CMP in recipients. (16,47) Whether cell-mediated and antibody-mediated autoimmune forms of iCMP both exist in humans is still unclear at the current state of research. In animal models both forms have already been established. (16)

Gil-Cruz et al. showed that myocarditis patients have significantly elevated IgG antibodies against the bacterium Bacteroides thetaiotaomicron (B. theta.) compared to healthy

individuals. B. theta. is a ubiquitous human gut symbiont thought to play an important role in the maintenance of the host's health. (48) A significant correlation was found between immune reactivity to B. theta. peptides and reactivity with MYH6 in a subgroup of patients with specific human leukocyte antigen (HLA)-DQA1/B1 combinations. Their findings indicate a cross-reactivity between both antigens with a potential for fatal myocardial disease progression. This cross reactivity might lead to an exacerbation of myocardial damage (virus induced or induced by myocardial infarction) as $CD4^+$ T-cells primed in the gut enter the myocardium and cross-react with heart antigens. (49)

1.4 Classification of myocarditis

Options of classifying myocarditis include classification by temporal (acute and chronic), causal, clinical, clinicopathological, histological and immunohistological criteria. (2) Due to the complexity of myocarditis, there is not one exclusively used classification. An important and established classification is the histological classification. It differentiates predominant infiltrating cell types in EMB samples. In the histological classification, the classification is usually made into the following subgroups: Cardiac sarcoidosis, lymphocytic, eosinophilic, giant cell, neutrophilic, and other granulomatous myocarditis. (50)

1.4.1 Histological classification

Lymphocytic myocarditis

Lymphocytic myocarditis (LM) is the most common histological subtype and may be associated with viral infection even when no viral agents can be identified in the analysis. Alternatively, it can occur as cardiac manifestation of a systemic immune-mediated disease or as a primary autoimmune condition. The inflammatory infiltrate consists of mononuclear cells with dominance of T Lymphocytes and a variable number of macrophages. The presence of neutrophil granulocytes depends on the degree of myocyte damage. A few eosinophil granulocytes and plasma cells are typically also present. However, if these present the dominant cell component another subtype of myocarditis should be considered. (20,51)

Eosinophilic myocarditis

Eosinophilic myocarditis represents a rare type of myocarditis. Possible causes include hypersensitivity reactions, malignancies, idiopathic hypereosinophilic syndrome, drugs, vaccines, and parasitic infections. Depending on the underlying disease, eosinophils can be the major inflammatory stimulus or be part of a complex cellular infiltrate with other cell types. Clinical suspicion of this subtype and performance of EMB are crucial to detect eosinophilic myocarditis and start early immunosuppressive treatment. (50,52)

Giant cell myocarditis

Giant cell myocarditis (GCM) is a severe type of myocarditis and is of autoimmune nature. A mixed cellular infiltrate is generally found containing multinucleated giant cells, which are macrophage derived. GCM is frequently fulminant in clinical presentation and associated with poor outcome. (50,53,54)

Neutrophilic myocarditis

This subtype of myocarditis is rare and typical for bacterial myocarditis which is observed mainly in immunocompromised patients. The neutrophils can be distributed in a patchy pattern or in micro-abscesses. Significant myocyte damage is common. Neutrophilic myocarditis can develop during fulminant bacterial pneumonia or sometimes within the scope of bacterial endocarditis. Some rare infections like tuberculosis, Lyme disease and Whipple-disease can present with special forms of neutrophilic myocarditis. (50,55)

Cardiac sarcoidosis

Sarcoidosis is a condition with largely unknown etiology and can occur as a multi-organ disease but also as isolated cardiac sarcoidosis. Clinically, sarcoidosis is difficult to detect and frequently missed. (56) The histological findings are similar to the findings of extracardiac sarcoid manifestations consisting of epithelioid, non-necrotizing granulomas. In most cases these are accompanied by giant cells. Foci of LM in absence of granulomas are not an uncommon finding in this subtype. This can lead to a misdiagnosis of LM, if due to the sampling error no granulomas are found in the obtained biopsy samples. (57,58) Therefore, integrative diagnosis is important for this entity (clinical features, pulmonary involvement, imaging like PET, MRI).

Other granulomatous myocarditis

There are other granulomatous forms of myocarditis aside from cardiac sarcoidosis. Possible causes are infections with pathogens such as tuberculosis and other mycobacteria, parasites, and fungi. Though histological features such as caseating necrosis can help in the differentiation, specific staining should always be performed additonally. Two examples for recommended staining methods are Ziehl-Neelsen staining for tuberculosis and Giemsa staining for parasitic infections. (50)

Histological variants difficult to classify

Lympho-histiocytic myocarditis, myocarditis with vasculitis and microvascular inflammation and toxic myocarditis are three variants that cannot be categorized in the categories above and therefore have a separate standing. (50)

1.5 Signs and symptoms of myocarditis

The clinical presentation of myocarditis is heterogeneous, ranging from completely asymptomatic patients to heart failure presentations to acute coronary syndrome like presentations. Myocarditis has the ability to mimic other diseases, making a clear differentiation from those diseases challenging. (50) However, the most typical symptoms at presentation include dyspnea, chest pain, fatigue, syncope, palpitations, cardiogenic shock and SCD. (16,59) Ammirati et al. found chest pain to be the most common symptom occurring in up to 95% of cases. (4) In the weeks before the acute phase, 80% of patients experience prodromal symptoms like fever, upper respiratory symptoms or gastrointestinal disorders. (60) Subclinical forms of myocarditis mostly remain undiagnosed with the potential for delayed complications.

Features suggestive of an autoimmune cause include a history of specific symptoms like fevers, musculoskeletal symptoms, fatigue, lymphadenopathy, hair loss, exanthema, mucosal ulceration, neuropathic pain, ocular manifestations (e.g., uveitis), bowel habit alterations, peripheral blood eosinophilia, impaired kidney function or a possible family history of an autoimmune disease. Sarcoidosis may present with several of the features listed above but can also be completely asymptomatic. When considering a diagnosis of sarcoidosis, extra-cardiac symptoms include respiratory manifestations like cough and

dyspnea, erythema nodosum and bilateral hilar adenopathy. However, these signs are non-specific for sarcoidosis and can occur in the course of other diseases. (24)

1.6 Diagnosis of myocarditis

In a patient with signs and symptoms suggestive of AM further investigations will be guided primarily by the patient's history, physical examination and first-line test results, such as blood test results, ECG and echocardiography findings. (24)

A differential diagnosis of coronary artery disease (CAD) should be considered and ruled out in most patients presenting with suspected AM. Depending on the CAD pretest probability there is a non-invasive option with computed tomography coronary angiography and an invasive option with invasive coronary angiogram. Furthermore, CAD can be assessed indirectly by ruling out myocardial infarction in cardiac magnetic resonance imaging (CMR) showing the absence of an ischemic subendocardial or transmural pattern of late gadolinium enhancement (LGE). (24)

1.6.1 ECG

The performance of an ECG is part of the first line testing. Abnormalities are observed frequently in myocarditis patients and therefore the ECG is widely used as a screening instrument and as a tool for risk stratification because of its prognostic relevance. The 2013 European Society of Cardiology (ESC) Guidelines recommend the performance of an ECG in all patients with clinically suspected myocarditis. (43) However, the observable changes in myocarditis are non-specific and hence the diagnosis can never be based solely upon ECG abnormalities.
Typical ECG-changes include T-wave and ST-segment changes, atrial and ventricular conduction delays, arrhythmias, and Q-waves. ST-segment changes caused by myocarditis can mimic ST-segment changes seen in myocardial infarction. (51,61)

1.6.2 Serological markers

Routinely Recommended by the ESC-Guidelines

Inflammatory markers c-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are typically elevated in myocarditis and in pericarditis, but they are non-specific. Markers for myocardial cell damage include cardiac troponins and creatin kinase-MB. Troponins are more sensitive in comparison, but they are also non-specific for myocarditis. Furthermore, normal troponin values cannot exclude the presence of myocarditis. (43,62) The correlation between troponin levels and cardiac dysfunction has been shown to be rather weak. (63)

The absence of viral genomes during EMB analysis raises suspicion of an immune mediated myocarditis/DCM. Hence detectable IgG autoantibodies can serve as biomarkers for patients who might benefit from immunosuppression. Some autoantibodies have been identified as negative predictors in DCM and myocarditis for example Anti-Beta1-AR and Anti-Alpha-MHC. The ESC recommends autoantibody-testing, if at least one of the published, recommended tests is available. Preferably only disease specific autoantibodies should be assessed. (43,64)

Not routinely recommended by the ESC Guidelines

Viral serology seems to be of limited use, mainly because circulating IgG antibodies are highly prevalent in the general population without concomitant cardiac inflammation in most of these people. In a recent study performed by Mahfoud et al., there was no correlation found between virological analysis in EMB samples and viral serology. (65) Only 4% of patients had serological evidence of the same viral infection that was detected in their EMB via polymerase chain reaction (PCR) examination. Some special indications where serological testing seems to be conceivable are rickettsial phase 1 and phase 2, Lyme disease, suspected hepatitis C and HIV in high risk patients. (43)

Other relevant markers

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a serological marker for heart failure and can be elevated in myocarditis patients. Similar to the cell damage markers discussed earlier, negative findings do not exclude the suspected diagnosis of myocarditis.

Soluble suppression of tumorgenesis-2 (sST2) is a biomarker to predict the risk of HF in men. Men under the age of 50 years with diagnosed myocarditis or clinically suspected myocarditis have a higher risk of developing more severe heart failure if sST2 serum levels are elevated. (16,66) Furthermore, S100A8-S100A9 is a heterodimer acting as a good marker for disease activity in patients with recent onset myocarditis. (16,67) In 2021 Blanco Domínguez et al found the microRNA mmu-miR-721, which is synthesized by Th17 cells, to be present in mice with acute viral or autoimmune myocarditis, while not being present in mice with myocardial infarction. The human microRNA equivalent is hsa-miR-Chr8:96 and has been evaluated in four independent patient cohorts with AM. The marker did show promising results in distinguishing the myocarditis patients from myocardial infarction patients but further evaluation will be necessary before the marker can be recommended for routine clinical practice. (68)

1.6.3 Echocardiography

Transthoracic Echocardiography (TTE) is considered the first line imaging tool for cardiac evaluation in suspected myocarditis. (69)

TTE helps to rule out certain differential diagnoses of non-inflammatory cardiac diseases, mainly CMPs like hypertrophic CMP and valvular heart disease. Intriguingly, myocarditis can mimic DCM, hypertrophic and restrictive CMP by presenting with echocardiographic features that are also found in those diseases. (43,70) On the other hand, there are often overlaps of CMP and myocarditis with CMPs presenting with additional/concomitant inflammation.

Risk stratification of patients with suspected or diagnosed myocarditis is usually performed according to left ventricular ejection fraction (LVEF). The TTE serves as the gatekeeper to further diagnostic and therapeutic steps. There are no TTE abnormalities specific for myocarditis. However, regional wall motion abnormalities, global ventricular dysfunction and diastolic dysfunction with normal LVEF are frequently observed.
In the last years, myocardial strain analysis by two-dimensional speckle tracking echocardiography emerged as a diagnostic and prognostic tool. Løgstrup et al. found longitudinal strain to be significantly correlated with the degree of edema detected by

CMR in patients with myocarditis. (69,71) However, some forms of myocarditis like ICI myocarditis can present with no or only subtle TTE abnormalities in early stages of the disease.

1.6.4 Cardiac MRI

CMR has been declared the noninvasive gold standard for diagnosis of myocarditis in the former ESC heart failure guidelines (Class 1 recommendation, level of evidence C). (16,72) In the current guidelines from 2021 use of CMR is recommend in patients with suspected myocarditis in the following scenarios:

- at baseline in all patients with clinical history + ECG, elevated troponin or echocardiographic abnormalities when CAD is unlikely or excluded.
- at follow-up in patients with persistent dysfunction at echocardiographic evaluation, persistent arrhythmias or ECG abnormalities. (73)

However, use is not recommended for patients in critical condition or patients with contraindications. A gadolinium-free approach combining T1 mapping with T2 based sequences may be useful for patients where the use of contrast agents is not possible. (74)

The CMR diagnosis of myocarditis is based on the detection of inflammatory changes in the myocardium. In 2009 the original Lake Louise Criteria (LLC) were published. (75) In the setting of clinically suspected myocarditis CMR-findings were considered as consistent with myocardial inflammation if at least two out of three criteria are present:

1. Regional or global signal intensity increase in T2-weighted sequences
2. Increased global early gadolinium enhancement (EGE) ratio between myocardium and skeletal muscle
3. At least one focal lesion with non-ischemic LGE

The LLC were revised in 2018. (74) In the updated criteria, strong evidence for acute myocardial inflammation is defined as at least one positive marker in each of the two main criteria:

1. Evidence of myocardial edema in T2-mapping or T2-weighted sequences
2. Evidence of myocardial injury in T1 mapping, calculated extracellular volume (ECV) or LGE

If only one of the two criteria shows a positive marker a diagnosis of myocarditis can still be assumed in the appropriate clinical setting. However, with less specificity compared to a positive marker in both categories. Supportive diagnostic criteria include signs of pericarditis and systolic left ventricular dysfunction.

The revised LLC have higher diagnostic sensitivity compared to the original criteria with 87.5% vs 72.5%. Specificity remains high at 96.2%. The improvement in sensitivity seems to be achieved mainly by using T1 and T2 mapping techniques. (76)

Myocardial edema in CMR sequences correlates with increased permeability of cell membranes and is hence considered a hallmark of cell injury caused by an inflammatory process. Initial membrane injury leads to intracellular edema through natrium influx, while more severe injury leads to efflux of water and larger molecules like troponin. Edematous regions present as high intensity regions in T2-weighted sequences. The edema can be focal or generalized, the latter is optimally detected via increased T2 times in T2 mapping. Conventional T2 weighted sequences can detect focal edema but are limited in the detection of diffuse/generalized edema. Therefore, skeletal muscle (e.g., serratus anterior muscle) can be used as an internal reference value and is compared to myocardial signal intensity. A ratio of myocardium to skeletal muscle of >2 indicates myocardial edema. (75) Regional edema is detectable in 36% of patients with active myocarditis according to histological Dallas criteria but not at all in patients with histological borderline myocarditis. (77) Therefore edema is considered to have low sensitivity in patients with lower grade inflammation.

LGE indicates increased extracellular accumulation of gadolinium contrast medium in the myocardium. Using an inversion recovery sequence, areas with increased accumulation of gadolinium are highlighted as bright regions in the myocardium. LGE generally indicates

irreversible necrosis and fibrosis of myocardial tissue. However, in the acute setting detectable LGE does not equate to irreversible fibrosis in all cases. Aquaro et al. postulated that LGE without concomitant edema could represent definite fibrosis, whereas the presence of concomitant edema indicates a chance of recovery. (78) The distribution pattern of LGE provides clues to the underlying pathology. Typically, non-ischemic and ischemic distribution patterns of LGE are distinguished. The non-ischemic LGE which is seen in patients with active myocarditis typically shows subepicardial distribution with variable extent through the ventricular wall. It is commonly localized in inferolateral and less frequently in anteroseptal segments. In comparison, the ischemic distribution pattern shows subendocardial distribution. (75) LGE plays an important role in the prediction of hospitalization and occurrence of adverse cardiovascular events in patients with suspected myocarditis. It is also of prognostic value for myocarditis patients (see chapter 1.8.1). (16,79–82) De Cobelli et al. found presence of LGE in 84% of patients with active myocarditis, but only in 44% of patients with borderline myocarditis. (77)

The diagnostic accuracy of CMR in myocarditis diagnosis is linked to the clinical presentation of the patient. CMR shows the highest sensitivity in infarct-like presentations, while showing worse sensitivity in cardiomyopathy-like presentations and the worst in patients presenting with arrhythmic pattern. (83) When solely using abnormalities in signal intensities, patients that overcame the first few days and progress from an acute form to a more subacute form of myocarditis, run danger of having false negative results. Another pitfall is coexisting skeletal muscle inflammation, which can lead to pseudo normalized reference values in T2 weighted images when calculating myocardium/skeletal muscle ratios. (74,84,85)

CMR can help avoid invasive procedures like EMB or coronary angiography, circumnavigating the possible complications of these procedures. Unfortunately, it is still underused in clinical practice due to limited availability, lack of appropriate training and lack of awareness of the usefulness in the diagnosis of inflammatory myocardial diseases. Another factor limiting the current use worldwide is the high cost of acquisition and maintenance. (86)

Although CMR has many advantages, many patients are dependent on the performance of EMB. A diagnosis solely based on CMR results is not capable of detecting the underlying

etiology of the inflammatory process. The specific etiology can on the other hand be crucial for optimal therapeutic management as special subtypes of myocarditis need specific treatment. These subtypes requiring specific treatment are eosinophilic, GCM, toxic myocarditis and cardiac involvement in a systemic immune-mediated disease. (15,50,51,87,88) Immunosuppressive treatment is a potent therapy for these subtypes, but the diagnosis needs to be confirmed before it can be safely administered.

Current CMR recommendations are based on the 2018 updated LLC. They are not including emerging techniques like strain analysis. Strain analysis (radial, circumferential, and longitudinal strain) allows the evaluation of contractility parameters at the level of each voxel over the whole cardiac contraction cycle. It can be acquired by using post processing software on the standard cine MRI images or by using specific MRI sequences like DENSE or fast-strain-encoded MRI. Currently, techniques are under development that apply 4D-flow-CMR to assess the kinetic energy in the left ventricle and intraventricular pressure gradients. The potential role of these techniques for noninvasive myocarditis diagnosis and monitoring of therapies is yet to be established. (16,89)

1.6.5 Endomyocardial biopsy

EMB aims to obtain cardiac tissue for pathology diagnostics and research and is performed via cardiac catheterization. It plays a vital role in the diagnosis of inflammation, infection, neoplasia, storage diseases and for the evaluation of rejection in transplant patients. (90) It represents the invasive gold standard of current myocarditis diagnosis and allows the identification of the underlying etiology. (43) Indications for EMB in myocarditis diagnosis are:

- AM presenting with severe HF or cardiogenic shock (4,59)
- AM complicated by severe myocardial dysfunction, acute HF, ventricular arrhythmia, or high-degree atrioventricular-block (4)
- AM or suspected iCMP associated with peripheral eosinophilia (4)
- AM or iCMP with persistent or relapsing release of biomarkers of myocardial necrosis, particularly if associated to an autoimmune disorder or ventricular arrhythmias or high-degree atrioventricular block (4)

- Myocarditis in the setting of immune checkpoint inhibitors, where appropriate diagnosis has implications for patients receiving additional cancer therapy (4,91)
- Chronic iCMP with severe reduction in LVEF to evaluate potential immunosuppressive therapy

In 1987 the Dallas criteria were published and laid base for the histological diagnosis of myocarditis. Active myocarditis was defined as the presence of infiltrating inflammatory mononucleated cells and myocyte necrosis with or without fibrosis at routine light microscopy evaluation of EMB samples. Borderline myocarditis was defined as an inflammatory infiltrate in the absence of myocyte necrosis. (92)
Although the Dallas criteria are the foundation for today's pathology diagnosis criteria, immunohistochemistry has a higher diagnostic sensitivity and is also of prognostic value for myocarditis patients. (16,21,93) The Dallas criteria show a high inter-observer-variance and have limitations in their ability to differentiate between different forms of myocarditis and interpret chronic and healing stages of those subtypes. (50,94) Furthermore, noncellular mediated inflammation is not considered at all. (94)

The 2013 ESC guidelines recommend the use of immunohistochemistry for the diagnosis and for the selection of specific therapeutic regimens. The ESC defines an inflammatory infiltrate as abnormal in the presence of at least 14 leukocytes/mm^2 including up to 4 monocytes with the presence of CD3-positive T lymphocytes >7 cells/mm^2. (16,43) A panel of monoclonal and polyclonal antibodies is used for the characterization of the inflammatory infiltrate including CD3, CD45, CD45RO, CD4, CD20, CD8, CD68/PGM1. Relevant markers for immune system activation are HLA-DR and HLA-ABC. (16,95)

The ESC also recommends viral genome analysis via PCR to detect cardiotropic viruses in LM. Included in current protocols are Adenoviruses, Enteroviruses, CMV, EBV, HSV 1 and 2, HHV6, Influenza A and B, PVB19, and Hepatitis C virus. (50,96) If one of the stated viruses is detected, the question, whether this virus is indeed the causative agent remains. Therefore, finding replicating forms of virus genomes should be considered the gold standard for identifying causative viral agents. (50,97) A negative PCR cannot exclude a viral origin of the disease. (98–100) In nonviral, infectious forms of myocarditis the detection of the infectious agent is performed using histology and special staining like for example Gram and Grocott staining. (50) Beyond virological and

immunohistochemical analysis, gene expression profiling is recommended to aid with the diagnosis of cardiac sarcoidosis and GCM. (101)

EMB is performed by using a bioptome and can be performed in both ventricles with left ventricular biopsy being as safe as right ventricular biopsy. (2,102–104) The most common location for sampling is the right side of the interventricular septum. Access is achieved through the femoral veins or through the right internal jugular vein. Left ventricular sampling is possible via the femoral, brachial, or radial arteries. CMR and voltage-mapping are helpful tools to guide the interventional cardiologist during the process of finding the locations most suitable for biopsy sampling. (105)

There is a fine line between the ambitions to reduce the sampling error of EMB and the efforts to keep the complication rate as low as possible. The sampling error is the most critical limitation to EMB diagnostic sensitivity, which seems to vary significantly between the subtypes of myocarditis. It seems to occur due to the patchy distribution of myocardial inflammation. Sensitivity of EMB is high for GCM (80 to 93%) and other aggressive forms such as acute necrotizing eosinophilic myocarditis, while it is much lower for LM (35%) and cardiac sarcoidosis (25%). (50,106–108) Multiple studies showed the strong influence of the number of obtained samples on diagnostic sensitivity of EMB. Single, overall biopsy sensitivity is only at around 20%, while 17 biopsies can increase the sensitivity to about 80%. Usually 4-5 biopsies are obtained with a combined overall sensitivity of 50%. (50,109,110) The complication rate of the procedure must not be neglected. At expert centers the rate is only around 1 to 2 % while in low volume centers it is up to 8.9 %. (102) Luckily, major complications only arise in less than one in 1000 patients receiving EMB. (111,112) Globally, the usage of EMB in the diagnosis of myocarditis is scarce (in the UK and in the United States only in 0.7%–3.6% of suspected myocarditis cases) and seems to be further declining. (113,114) However, in some countries like Italy, Germany and Austria the procedure is performed significantly more often.

New technologies like omics technologies (e.g., proteomics, genomics, metabolomics) and genetic testing are currently under evaluation, but have not yet reached the standard clinical practice for myocarditis patients. Van Linthout et al. showed that mass spectrometry evaluating region specific protein profiles allows successful clustering of

patients in those with and those without cardiac inflammation. (16,115) Genetic sequencing like next generation sequencing (NGS) allows the detection of further genetic high-risk variants which will benefit the clinical decision-making process. Furthermore, NGS will also allow the detection of more, to date still unknown pathogens causing myocarditis. Moreover, micro-RNA and mRNA profiling as markers for genetic and epigenetic activity seem promising with a transcriptome-based biomarker showing a 100% sensitivity and specificity for the detection of myocarditis. This biomarker can safely differentiate LM from patients with peripartum cardiomyopathy and rheumatic disease. (16,116)

1.6.6 Nuclear medicine

Nuclear imaging is not routinely recommended in the diagnostic process of myocarditis due to the limited sensitivity and lack of valid data. However, if CMR is contraindicated or if patients are under suspicion of having cardiac sarcoidosis, ^{18}F-FDG-PET is a nuclear imaging method that is very sensitive to metabolically active processes and should be considered. Furthermore, scintigraphy using Indium-111 labeled antimyosin-antibodies is an option for detecting intracellular proteins and hence localizing myocardial damage. (69)

1.7 Therapy of myocarditis

Therapy can be categorized in two categories: Supportive and etiology directed. Optimal medical treatment for heart failure is the base of the state-of-the-art treatment for all forms of myocarditis. Specific, etiology directed treatment is evidence-based only for a selected group of patients.

Figure 1: EMB results and recommended therapy.

Based on Tschöpe C et al., 2021 (16).

As illustrated in Figure 1 patients can be categorized into four groups based on their EMB results:

1. Inflammation negative and virus negative
2. Inflammation negative and virus positive
3. Inflammation positive and virus negative
4. Inflammation positive and virus positive.

For the groups 1 and 2 without detected inflammation there is no specific, etiology directed therapy. Autoantibody targeting might be considered in all groups and works via

immunoadsorption or via newly developed small molecules called aptamers which can neutralize specific autoantibodies and could therefore be beneficial for patients in whom autoimmunity plays a role. Evidence for etiology directed therapy is still weak with exception of immunosuppressive therapy for group 3. The gaps in current evidence for some of the treatment options are denoted by question marks in Figure 1. (16)

Myocarditis and exercising

During the recovery process of AM it is crucial for patients to distance from competitive athletics and intense physical activity for about 3 to 6 months. (19,117) After this period LVEF and cardiac dimensions should be reassessed. Patients with normal LVEF, cardiac dimensions, serum markers for HF and inflammation and no relevant arrhythmias can be cleared for returning to exercising. (118)

This approach is viewed controversially in the community as there is a lack of valid data comparing the outcome of patients with a 6-month physical restriction and patients who are physically restricted dependent on their clinical improvement.

1.7.1 Supportive therapy

Heart failure

Patients with myocarditis and HF should be treated according to ESC/AHA/ACC HF-Guidelines. The typical regimen consists of beta-blockade, diuretics and angiotensin-converting enzyme (ACE) inhibitors or angiotensin 2 receptor-blockers (ARBs). An exception to this combination represents acutely decompensated HF where beta-blockers should be avoided.

Diuretics may slow the progression to DCM by decreasing volume overload. For patients with NYHA class II-IV aldosterone antagonists are additionally recommended as they increase survival and reduce hospital admissions. (19,21) In mouse models ACE-inhibitors and ARBs reduce fibrosis, necrosis and inflammation in viral and autoimmune myocarditis. Treatment with an angiotensin receptor-neprilysin-inhibitor (ARNI) is even more beneficial in the treatment of HF with reduced ejection fraction (HFrEF) compared to treatment with ACE-inhibitors, which was shown in 2014 by the PARADIGM-HF trial. (119) Additionally, sodium-glucose co-transporter 2 (SGLT2) inhibitors like dapagliflozin

or empagliflozin are recommended in combination with optimal medical therapy in patients with HFrEF regardless of their diabetes status. (73)

A subset of HF patients requires mechanical circulatory support (MCS) and/or inotropic support. The choice of the MCS mainly depends on local availability and on whether the left, right, or both ventricles are affected. (120) MCS can be temporary or long-term, depending mainly on disease progression. Temporary support includes extra corporal membrane oxygenation (ECMO), Impella devices and intra-aortic balloon pumps (IABP), while a ventricular assist device (VAD) is a permanent option. Another important factor to consider when choosing a MCS is the degree of needed circulatory support. The possible support provided by the Impella device is not as powerful as the support provided by ECMO. Furthermore, Impella devices lack oxygenation possibility compared to an ECMO. However, in addition to giving adequate support, left ventricular unloading by Impella devices exhibits disease-modifying effects important for myocardial recovery as was shown by Tschöpe et al. in 2018. (121) The duration of the recovery process with an MCS device is highly variable ranging from days over weeks to even complete absence of improvement. (19)

Arrhythmia

Myocarditis patients presenting with ventricular arrhythmia or heart block are a high risk subgroup. (118,122) It is recommended that these patients are managed according to 2006 ESC/AHA/ACC Guidelines for cardiac implantable electronic devices. (123) If heart block or bradycardia occur during the acute phase a temporary pacemaker should be installed. When non-sustained ventricular tachycardia (VT) or sustained VT occur, the use of amiodaron is recommended during the acute phase. Patients with rhythm disorders who progress to a chronic phase of myocarditis should be provided with an ICD or a permanent pacemaker. Similarly, ICD implantation should be evaluated in patients with severe chronic CMP. In patients with persistent tachyarrhythmia, electrophysiological examination and ablation should be considered. (19)

1.7.2 Etiology directed therapy

Merken et al. showed in 2018, that the virus-negative but inflammation positive group (see Figure 1, group 3) treated with prednisone and azathioprine showed improved cardiac function compared to the control group. (124) Alternative treatments are a steroid based treatment in combination with mycophenolate mofetil (125) or cyclosporin (126) or immunoadsorption with intravenous immune globulins (127,128) or small soluble molecules that target ß1-receptor-autoantibodies. (129)

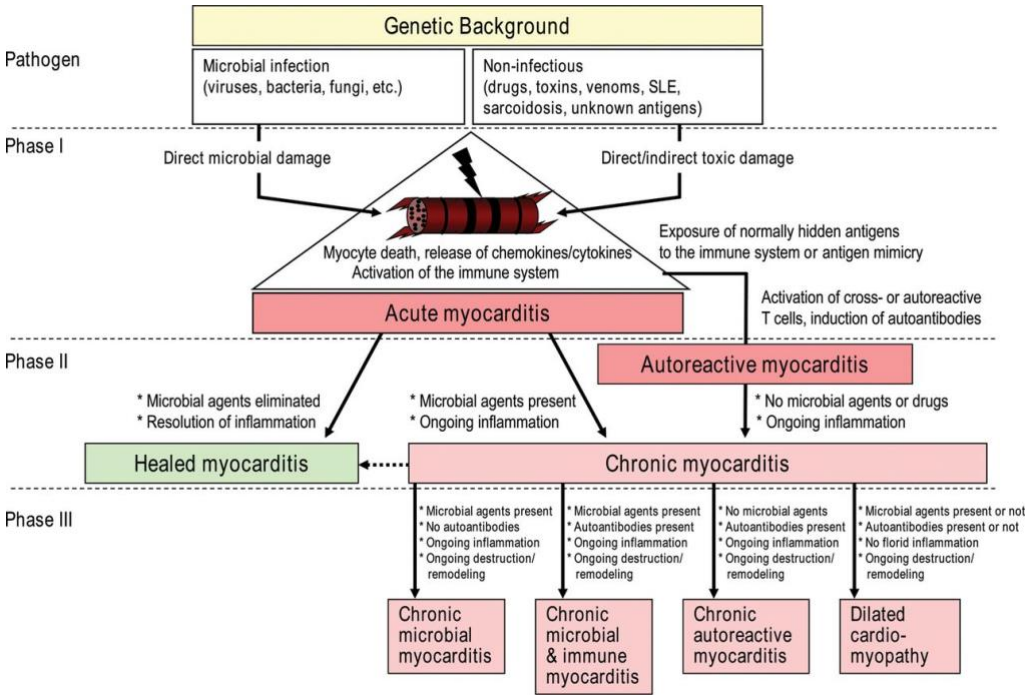
The group positive for viral genomes and positive for inflammation (see Figure 1, group 4) is the group where the differentiation between virus-associated and virus-induced active myocarditis is crucial. In virus-induced myocarditis (e.g., Enterovirus-infection), treatment with interferon-ß (IFN-ß) can lead to a reduction in viral copy numbers or even to viral clearance. In virus-associated myocarditis (e.g., latent Herpes- or PVB19-infections), this effect cannot be observed. (130) Furthermore, a study with a small series of patients performed by Tschöpe et al. has shown promising results using Rituximab for CMP with detected CD20+ lymphocytes in their biopsy samples. (131) Both treatments IFN-ß and Rituximab still lack sufficient clinical evidence for a general recommendation. Moreover, anti-Herpesvirus drugs can be used for the treatment of latent infections of CMV, EBV and HHV6 and are capable of reducing viral copy numbers. (132) As of today, there are no available treatment options for PVB19 associated CMP. However, the ESC consensus has established that no treatment is necessary in case of low PVB19 copy numbers and no present inflammation. (43) On the contrary, weak evidence exists for immunosuppressive treatment for low PVB19 DNA copy numbers with concomitant inflammation (133) and for telbivudine treatment in case of detectable RNA. (134,135)

1.8 Prognosis of myocarditis

Feared complications of myocarditis are the development of DCM and/or HF, occurrence of adverse cardiovascular events and death. The development of DCM occurs in 14%–52% of diagnosed myocarditis cases depending on the source of data. (136,137) The progression may take years, which makes the understanding of the process difficult and the development of new treatment strategies challenging. (138) Recently performed studies

showed that myocardial acute response, which could determine the prognosis and the evolution into DCM, seems to be influenced by sex hormones with the overall myocarditis prognosis being worse for women. (139,140)

Figure 2: Possible courses of AM.



Reproduced with permission (OXFORD UNIVERSITY PRESS) from Caforio et al., 2013 (43).

Figure 2 shows the variable outcomes after AM. Over the last decade, diagnosing AM has become more frequent than before, likely due to the establishment of high-sensitive troponins and CMR imaging. Therefore the proportion of diagnosed uncomplicated myocarditis cases has increased resulting in an overall improved outcome of myocarditis compared to prior decades. (60) The data regarding the frequency of complications are inconsistent. Two of the largest and well-known studies regarding the subject included their patients based on data derived from different diagnostic investigations and came to highly differing results. The first study from 2012 included 222 patients with viral AM diagnosed via EMB and registered a rate of 15% of patients requiring heart transplantation or dying within 5 years. (141) The second study from 2015 included 205 patients, this time diagnosed via CMR. A death rate of 0% over 19 months was registered among patients. (142) The more recent 2018 Multicenter Lombardy Registry by Ammirati et al. yielded cardiac death and HTX rates of 3.2% during the hospitalization period and a 4.1% rate at

5-year follow-up. (60) Comparable data was collected by Arola et al. in Finnish children with myocarditis. (143)

1.8.1 Prognostic factors

Clinical presentation

Presentation with symptoms of chest pain and a NYHA class I-II is linked to favorable outcome (21,144,145), while presentation with HF at baseline, NYHA class III-IV, acute kidney injury, sustained ventricular arrhythmias and high SOFA, SAPS II and APACHE IV admission scores are all linked to unfavorable outcome. (141,145–150)

ECG

Poor prognosis is associated with Q waves, ventricular ectopic beats, widened QRS complexes >120 milliseconds (ms) and abnormal QRS-axis. (145,151) A QRS-T-angle >100° is an independent marker for high risk of HF and death. (145,152) Furthermore, QTc prolongation >440 ms is a negative prognostic marker as a possible arrhythmogenic trigger. (51,145) If no ECG abnormalities are present or if ST-elevation with a pericarditis pattern is visible the associated prognosis is favorable. (144,145,152) ST-elevation with a pericarditis pattern is defined as upward, concave J-point elevation. QRS notching or slurring might be present or absent. (145,152)

Blood biomarkers

Troponin and NT-proBNP values are also of prognostic value. An early increase of troponin and a fast decline are linked to good outcome, while recurrently abnormal or persistently abnormal levels are linked to poor outcome. (145,153) It was recently elicited that elevated NT-proBNP levels >4245 pg/ml and low levels associated with increased troponin are of poor prognostic value. (145,154,155)

Echocardiography

The role of Echocardiography for prognostic evaluation is crucial. Negative prognostic factors are: increased left ventricular end diastolic diameter at admission (156,157), reduced LVEF at first echo (144,156), left atrial enlargement (156,158) and low strain and strain rate. (145,159) Reduced LVEF at first echo is also relevant for long-term prognosis.

If right ventricular dysfunction is present, it serves as a main predictor of transplantation and death. (136) On the other hand, positive prognostic factors are normal LVEF and wall motion (144,156) or if those are abnormal an improvement/normalization of LVEF after time is favorable for long term prognosis. (145,156,157)

CMR

Prognostically advantageous is the complete absence of LGE. (141,160) If LGE is present, it is favorable if it decreases at follow-up appointments (141) and if it is located in the inferolateral wall. (160) Similar to echocardiography, preserved baseline LVEF in CMR is linked to favorable outcome. (60) Prognostically unfavorable is presence of LGE at baseline and non-decreasing LGE at control appointments. (141) Interestingly, a combination of persistent LGE and resolving edema is a marker of poor prognosis compared to the disappearance or persistence of both. Potentially, persistent edema can indicate a still active process with a higher probability of recovery (See chapter 1.6.4 above). (78) Furthermore, mid-wall LGE in anteroseptal segments is also linked to unfavorable prognosis. (160)

EMB

To date only negative prognostic EMB markers have been established. Those are: histological subtype of GCM (4) and presence of viral genomes in patients not being treated with drugs containing specific antiviral components. (161) Moreover, Kindermann et al. have shown that histological presence of inflammation represented by invading inflammatory infiltrate and the expression of HLA-DR-alpha molecules are linked to worse prognosis compared to those markers being absent. (21)

2 Aim of this thesis

The diagnosis of myocarditis can be challenging, and an optimal diagnostic approach depends on a wide variety of factors including patient factors (e.g. the severity of the clinical presentation) and geographic factors like local availability of CMRs and catheter labs. Within the last decades, criteria for CMR and EMB myocarditis diagnosis have evolved. Most important changes for CMR-diagnosis are the formulation of the LLC in 2009 and the updated LLC in 2018. EMB-diagnosis changed from a histology-based diagnosis using the Dallas criteria to an immunohistochemistry-based diagnosis. Both investigations have their strengths and weaknesses and scenarios in which they are superior to one another. To date, MRI remains as the noninvasive gold standard and EMB as the invasive gold standard. Unfortunately, neither method has both, great sensitivity and specificity. The old LLC exhibit a sensitivity of 72.5% and a specificity of 96.2%. (74) EMB in comparison has great specificity, but a sensitivity of only around 50% with 4-5 obtained biopsy samples. (50,109,110)

The aim of this diploma thesis was to give an overview of the diagnostic results of patients that received CMR and EMB at the Cardiology Department of the Medical University of Graz. We aimed to evaluate the concordance of CMR and EMB in the diagnosis of myocarditis. In patients with divergent diagnoses, we tried to evaluate which diagnoses were actually stated. This constitutes the first, descriptive part of this diploma thesis.

An important factor to consider when comparing EMB and CMR diagnoses is the sequence in which both investigations were performed. We suspected the sequence would play an important role for the diagnostic concordance of the two investigations. In most cases CMR is performed prior to invasive EMB at our institution, but a notable portion of patients received EMB prior to CMR due to availability reasons. We suspected that if the imaging was performed shortly after the EMB procedure the septal biopsy sites could still be visible on CMR images. The biopsy sites could potentially mimic a patchy form of myocarditis, which could severely worsen the diagnostic accuracy of CMR with a possible overestimation of myocarditis by the diagnosing radiologists. Testing this hypothesis constitutes the second part of this thesis.

3 Methods

This diploma thesis is a retrospective data analysis reviewing the data of patients who received EMB and CMR at the Department of Cardiology at the Medical University of Graz between 2008 and 2020.

3.1 Collection of data

In a first step, we searched for patients who received EMB at the Department of Cardiology via a query in the electronic hospital information system openMEDOCS. The query was performed by the Institute of Medical Informatics and Biostatistics of the Medical University of Graz (IMI). Digital archiving of EMB data started in 2008 at the Department of Cardiology. From July 2008 until October 2020, 420 patients underwent EMB with the procedure code 'DA04000'. On these 420 patients, 575 EMB procedures were performed. The data was documented in a Microsoft Excel spreadsheet in a pseudonymized fashion and included general patient characteristics like patient-ID, age, sex, height, weight, available diagnoses in openMEDOCS, number of biopsies, date of biopsies, last full blood laboratory before EMB with levels of NT-proBNP, high-sensitive cardiac troponin T (hsTnT) or troponin T (before availability of hsTnT), CRP serial assessment (from 6 months prior to biopsy until 2 years after biopsy) and date of death, if deceased. Out of this list, patients who also underwent CMR were selected. This resulted in 228 patients with 263 performed CMRs.

Further data collection was performed by the author of this thesis via manual search in openMEDOCS. Data including CMR findings, EMB findings and echocardiography findings was extracted from written reports and encoded in the Microsoft Excel spreadsheet in addition to the data collected by the IMI. However, a considerable proportion of the biopsy reports was not retrievable online. Therefore, the missing reports were collected from the filed reports in the physical archive of the Department of Cardiology. About 20 biopsy reports still could not be traced and were subsequently requested at the 'Institut für kardiale Diagnostik und Therapie' (IKDT) in Berlin, Germany. The IKDT is the institute the biopsy samples obtained at our clinic are routinely sent to for further analysis. Sixteen more reports were found at the IKDT. Four patients

with still missing reports were excluded from further analysis. Literature research was performed using PubMed.

3.2 Inclusion criteria

Available data

Regarding patients with incomplete CMR data, the CMR sequences of our patients were accessible by Johannes Schmid at the Department of Radiology. This allowed collection of missing parameters in the written reports. In contrast, regarding missing EMB data, we were unable to obtain the data in retrospect. As a result, we only included patients where EMB data was sufficient to enable categorization into our diagnostic categories.

Indication for EMB

Some of the patients in our registry were cardiac transplant patients and subsequently a different analysis was performed on their biopsy samples, mainly dealing with the question of transplant rejection. These cardiac transplant patients were not suitable for our analysis and therefore excluded.

Time between CMR and EMB

As presence of inflammation is likely to change over time, we expected the concordance of CMR and EMB to suffer if the timespan between the two investigations was too extended. Thus, we tried to balance maintaining a reasonable cohort size, while keeping the timespan between examinations short. For the first, descriptive part of the analysis, we arbitrarily determined 30 days as an appropriate time frame and therefore excluded patients who had more than 30 days between their CMR and EMB examination.

For the sub analysis in which we investigated the role of the sequence of CMR and EMB, the time frame was chosen even more rigidly. Only patients who received both investigations in a maximum of 14 days were included, as our aim was to only include patients where the biopsy sites would still be visible on CMR. Patients who received both investigations on the same day were excluded because the sequence in which CMR and EMB were performed was not reliably determinable retrospectively in those patients.

3.3 EMB data

The analyses performed at the IKDT include histological, immunohistochemical and genetic analysis. All biopsy data in this thesis stems from the IKDT-reports, with exception of references to the location of the obtained biopsies, which were extracted via openMEDOCS from the catheter lab's documentation. The location of obtained biopsies was encoded as left ventricular, right ventricular or out of both ventricles. Subsequently, the histological findings, viral detection data, immunohistochemistry and the diagnostic statement were categorized for further comparison and data presentation. Histological cell numbers were not used, because data was only available in a minority of the IKDT-reports. Immunohistochemical cell content was available in almost all the reports and was therefore used instead.

Histology

Presence of fibrosis and cell necrosis was both encoded dichotomously as absent or present.

Viral detection

We dichotomously encoded whether viruses were detectable or not via PCR. If viruses were detected, we documented which ones were detected. The investigations performed by the IKDT included the detection of the following viruses: Adenovirus, Erythrovirus (including PVB19), Erythrovirus (including PVB19) in peripheral blood cells, HHV 6, HHV 6 in peripheral blood cells, EBV, EBV in peripheral blood cells and Cocksackie/Enterovirus. The presence of Erythrovirus, HHV6 and EBV was evaluated in myocardial tissue and in peripheral blood cells. The viral copy number of the respective virus was recorded as viral copies/µg of myocardial nucleic acid. For values <5 viral copies/µg myocardial nucleic acid the value 2.5 copies/µg myocardial nucleic acid was taken representatively. The presence of mRNA was encoded dichotomously as present or not present as an indication for active replication of the respective virus.

Immunohistochemistry

The presence of an abnormal inflammatory infiltrate can be detected by immunohistochemical analysis. Lymphocytes, cytotoxic T cells and monocytes/macrophages are detectable by specific immunohistochemical markers. These markers are CD3, LFA-1, CD45R0, Perforin and Mac-1. Thresholds of these markers are 14/mm^2 for CD3 and LFA-1, 40/mm^2 for CD45RO and Mac-1 and 2.9/mm^2 for Perforin (see Table 2).

Table 2: Immunohistochemical markers with thresholds for an abnormal inflammatory infiltrate

Marker	Number of cells/mm^2
CD3	14
LFA-1	14
CD45R0	40
Mac-1	40
Perforin	2,9

According to the IKDT laboratory manager Dr. Ganna Aleshcheva, an abnormal inflammatory infiltrate is assumed by the IKDT's pathologists if at least 2 markers of CD3, LFA1, CD45R0 and Mac-1 are slightly elevated, or if one is at least moderately elevated. For our categorization of EMB diagnoses (Table 3), the diagnostic algorithm of the IKDT-pathologists for the evaluation of present inflammation was adopted as they have vast experience in the diagnosis of myocarditis.

Table 3: Categorization of EMB diagnoses

Diagnosis
Borderline-myocarditis
Active myocarditis
DCM
Ischemic CMP
Amyloidosis
Specific diagnosis other than previous diagnoses
Unspecific/inconclusive findings, not sufficient for a specific diagnosis

3.4 CMR data

LLC

The presence of EGE, LGE, ECV and edema described in the radiology reports was encoded. Presence of EGE and edema were encoded dichotomously as present or not and ECV as increased or not increased. LGE was further divided into six subcategories: none, ischemic, non-ischemic, both ischemic and non-ischemic, undetermined, typical amyloidosis pattern. The latter category was added to further distinguish non-ischemic LGE seen in myocarditis from non-ischemic LGE with a pattern seen in amyloidosis. The LGE distribution was further specified dichotomously as LGE including the interventricular septum or not.

Quantitative parameters

Volumetric data including LVEF values measured in CMR were recorded.

CMR diagnosis

Final diagnoses were classified in the following groups:

Table 4: Categorization of the CMR diagnoses

Diagnoses
Normal CMR
Likely myocarditis
DCM
Specific diagnosis other than myocarditis and DCM
Unspecific/inconclusive findings, not sufficient for a specific diagnosis

The etiology of DCM cannot be reliably distinguished in CMR and can be either (post-) inflammatory or due to other non-inflammatory (e.g., genetic) causes. The category DCM was introduced to account for this ambiguous entity. To categorize CMR diagnoses, the diagnoses given by the diagnosing radiologist were adopted from the radiology reports. However, in some of the CMR reports the radiologists did not name a final diagnosis and in those cases the author of this thesis performed the classification by considering the LLC, the information given in the radiology reports and the help of Johannes Schmid who reviewed the CMR images of several cases to allow a suitable categorization.

3.5 Statistics

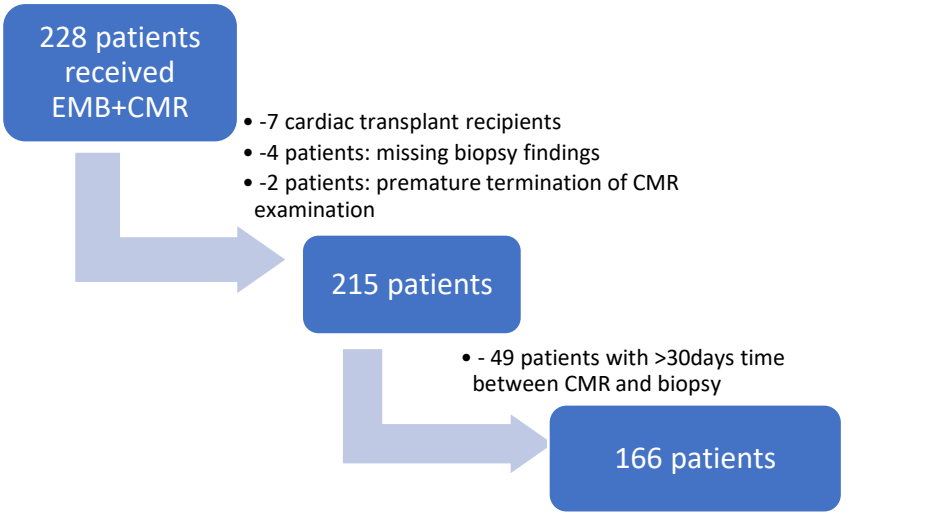
Statistical analysis was performed in Microsoft Excel and IBM SPSS Statistics Version 27. Descriptive statistics included presentation of frequency (percentage), mean ± standard deviation or median [interquartile range]. CMR and EMB results were compared by means of cross tables. Differences between groups were tested using Chi^2 test, Student's t-test or Mann-Whitney U test as appropriate. A p-value smaller 0.05 was regarded statistically significant.

3.6 Ethics Vote

The study was approved by the Ethics Committee of the Medical University of Graz (EK 32-575 ex 19/20).

4 Results

Figure 3: Inclusion process



As illustrated in Figure 3 the inclusion process started with 228 patients receiving EMB and CMR. Seven patients had to be excluded because they were cardiac transplant recipients. Four more patients had no detectable biopsy reports and were therefore excluded. Premature termination of CMR-examination led to the exclusion of 2 more patients. The criteria of a maximum of 30 days between both investigations led to the exclusion of 49 patients and to our patient final cohort of 166 remaining patients.

4.1 Descriptive statistics of the patient cohort

Table 5: Descriptive statistics of the patient cohort.

	Variables	**Value**	**Total number of patients**
Demographics	Age (years)	45.6 ± 15.6	166
	Male sex	133 (80%)	166
	BMI (kg/m^2)	28.4 ± 6.6	166
CMR	LVEF (%)	37.2 ± 16.3	161
	present LGE	135 (81.3%)	166
	non-ischemic LGE pattern	104 (62.7%)	
	Present edema	35 (21.3%)	164
	Normal MRI likely myocarditis DCM other diagnosis unspecific	3 (1.8%) 61 (36.7%) 60 (36.1%) 27 (16.3%) 15 (9.0%)	166
EMB	detected necrosis	2 (1.2%)	166
	detected fibrosis	103 (62.4%)	165
	Virus positive Positive only for PVB19 Thereof mean copy number	140 (84.3%) 86 (51.8%) 423.0±1010.8	166 166
	Active replication Thereof PVB19 pos.	35 (21.1%) 34 (97.1%)	166 35
	Active myocarditis Borderline-myocarditis DCM amyloidosis other diagnoses unspecific findings	3 (1.8%) 98 (59%) 7 (4.2%) 9 (5.4%) 14 (8.4%) 35 (21.1%)	166

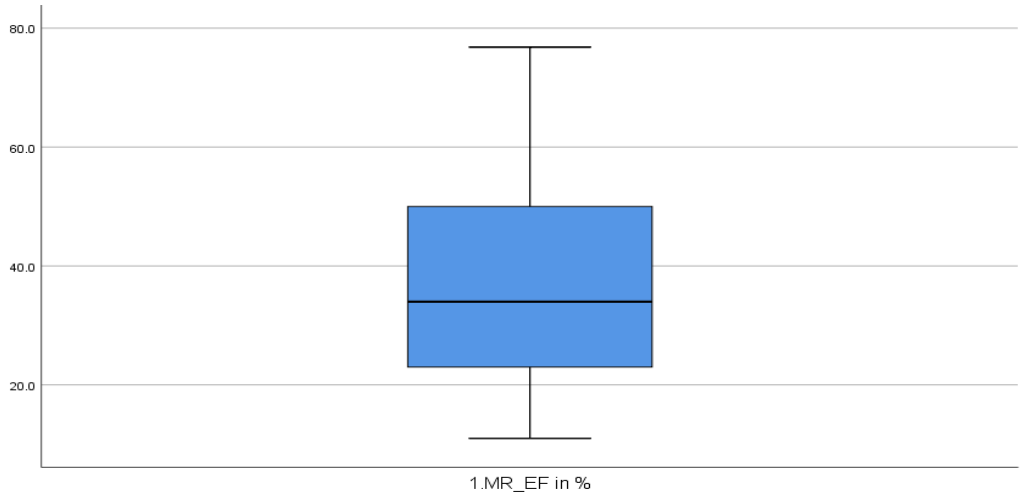
Categorial variables are presented as number (percentage), while continuous variables are presented as mean ± standard deviation.

4.1.1 General Demographics

The patient cohort consisted of 80% males and 20% females. Mean age was 45.6 ± 15.6 years with a range of 18 to 85 years. Mean BMI was 28.4 ± 6.6 kg/m^2 with a range of 16.6 to 51.6 kg/m^2.

4.1.2 CMR findings

Figure 4: EF measurements in CMR



Mean LVEF was 37.2 ± 16.3% with a range of 11.0% to 76.8%. 161 patients had valid LVEF measurements in their CMR-examination. Figure 4 shows a box plot with LVEF distribution. Figure 5 shows the distribution of LVEF values in the patient cohort.

Figure 5: Histogram of LVEF values. Values were measured via CMR.

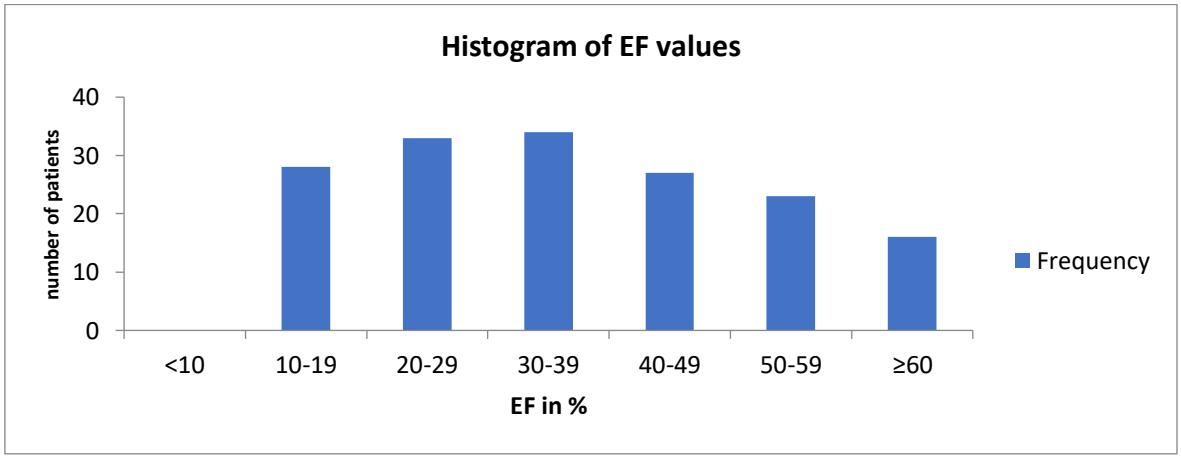
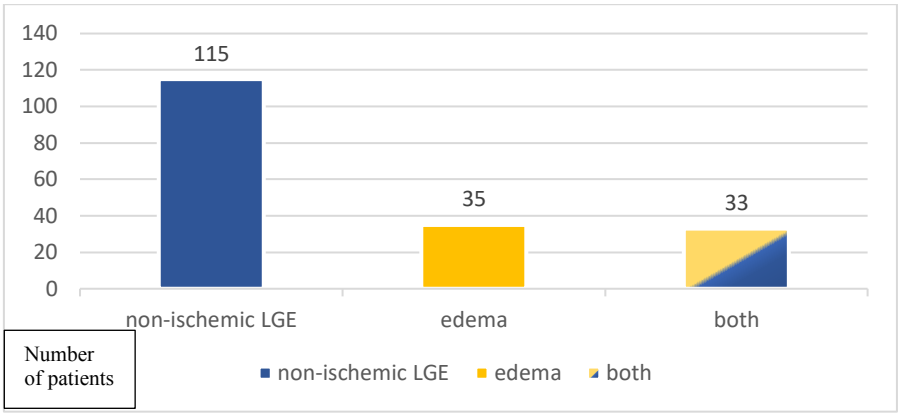
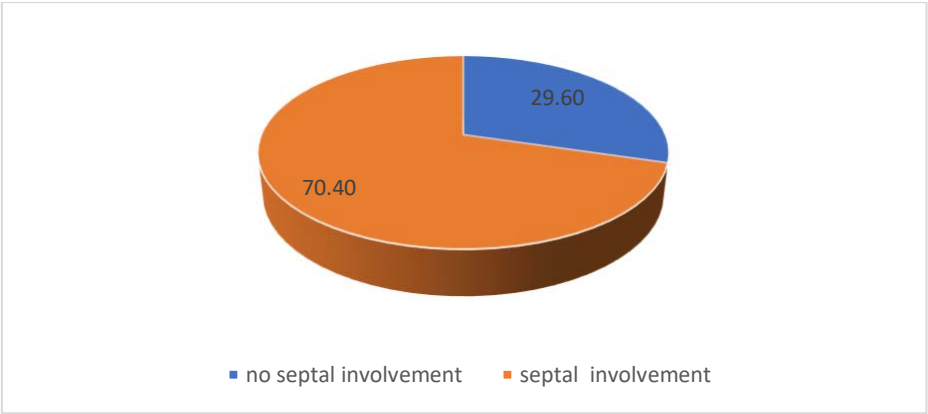


Figure 6: Prevalence of non-ischemic LGE and edema



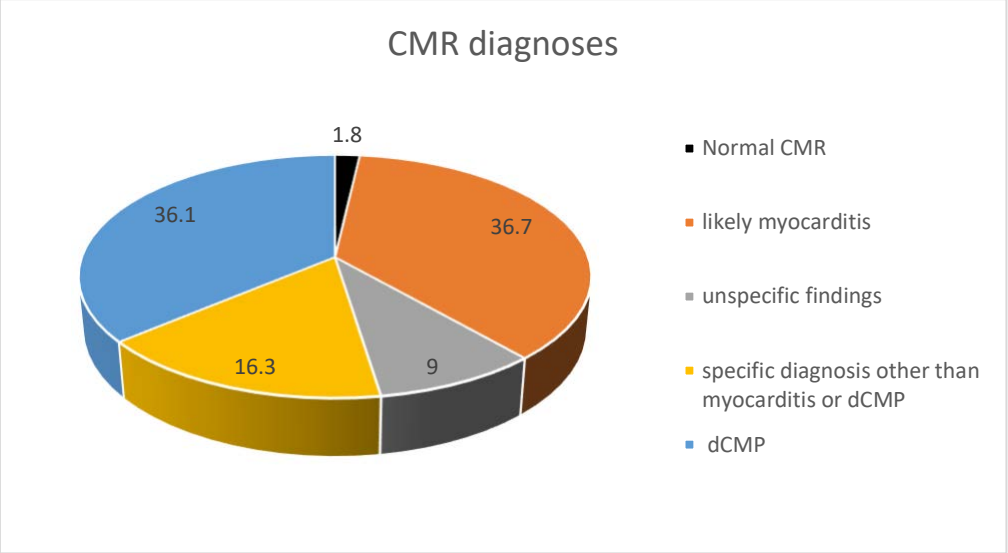
As we can see in Figure 6 a solely non-ischemic LGE pattern was detected in 104 (69.3%) of our patients (n=166), while a solely ischemic pattern was detected in 9 (5.4%) patients. Ischemic and non-ischemic LGE together appeared in 11 (6.6%) patients. Less frequently observed LGE patterns were undefined LGE and LGE typical for amyloidosis with 2 (1.2%) and 9 (5.4%) patients. Thirty-one (18.7%) patients presented without any LGE. Data on the presence of edema was available in 164 (98.8%) patients of our cohort. Out of those, 35 (21.3%) had detectable edema. Only 33 (20.1%) patients presented with edema and non-ischemic LGE.

Figure 7: Septal involvement of LGE in % of patients



Out of the patients with present LGE (n=135), 95 (70,4%) patients showed involvement of the interventricular septum.

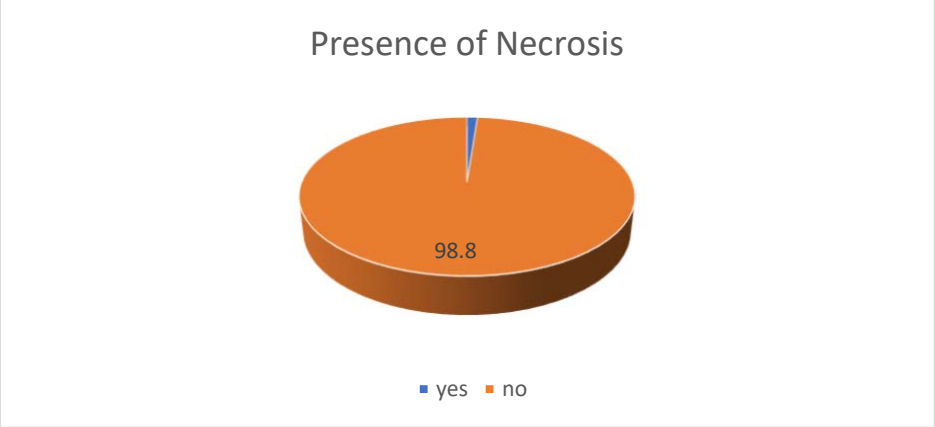
Figure 8: Overview of CMR diagnoses in % of patients



Analysis of the CMR diagnoses is presented in Figure 8. Three (1.8%) of the patients were classified as 'normal CMR findings' without visible pathologies. Patients suspicious for myocarditis and patients suspicious for DCM were almost equal in occurrence with 61 patients and 60 patients (36.7% and 36.1% of the patient cohort). Twenty-seven (16.3%) patients were found to have a specific diagnosis other than myocarditis and DCM, while 15 patients (9%) were classified with unspecific findings.

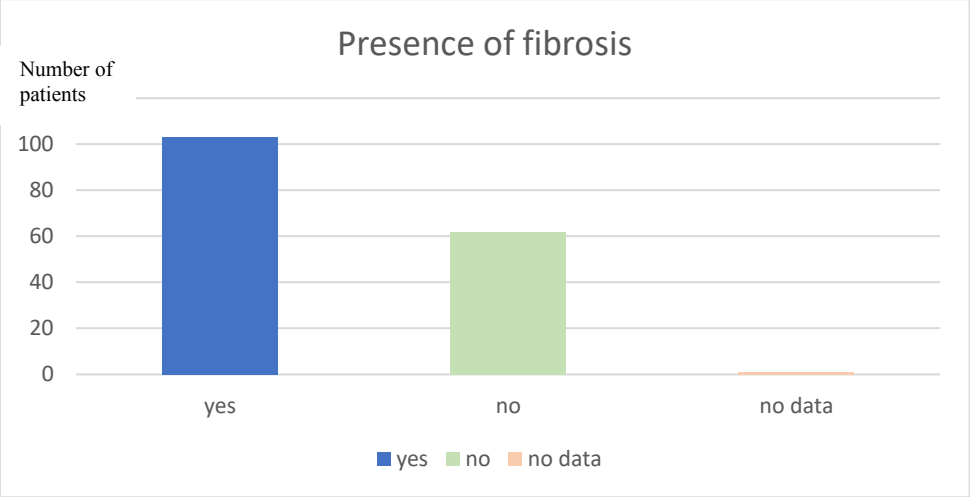
4.1.3 EMB findings

Figure 9: Presence of necrosis (% of all patients)



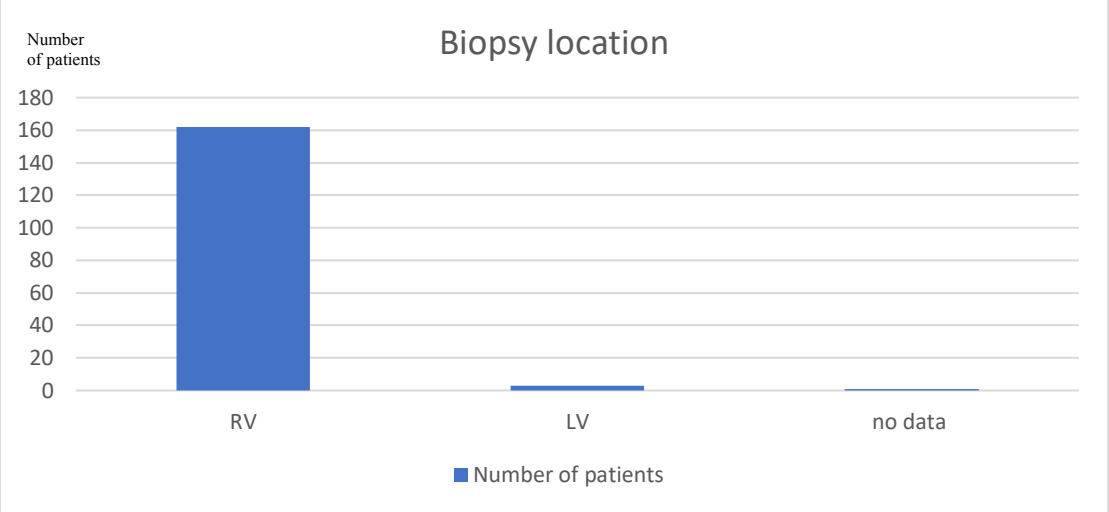
As presented in Figure 9 and Table 5 only 2 patients (1.2%) in our cohort showed visible cell necrosis.

Figure 10: Presence of fibrosis in EMB



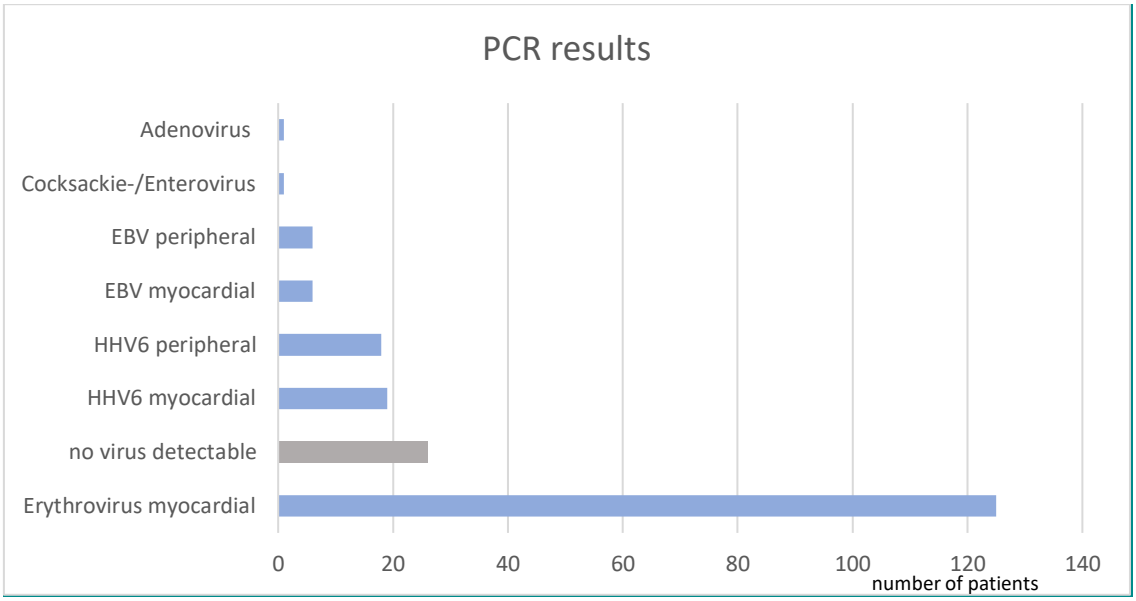
Data on the presence of fibrosis was available in 165 patients. Out of those patients 103 (62.4%) showed fibrosis in their myocardium (see Table 5 and Figure 10). The most common fibrosis localizations were perivascular, interstitial and in displacement layers.

Figure 11: Biopsy locations



As presented in Figure 11 only 3 of our patients received left ventricular biopsy while 162 patients had their biopsies obtained out of the right ventricle.

Figure 12: Detected viruses via PCR analysis



One hundred and forty (84.3%) patients were found to be positive for viral genome detection via PCR (see Figure 12 and Table 5). Viruses located in the myocardium and in peripheral blood cells were detected. In some of our patients, multiple viruses were detected. Thirty-five patients (21.1%) had at least two viruses and 7 (4.2%) patients had at least three viruses detected. There were five different viruses found to be present in our cohort. From most common to least common these were: Erythrovirus myocardial (including PVB19) in 125 (75.3%) patients, HHV6 myocardial in 19 (11.4%) patients, HHV6 peripheral in 18 (10.8%) patients, EBV myocardial and EBV in peripheral blood cells both in 6 (3.6%) patients and Adenovirus and Cocksackie/Enterovirus both in 1 (0.6%) patient. Actively replicating viruses were detected in 35 (21.1%) of patients. Active replication is synonymous with detection of mRNA via PCR.

Figure 13: IHC- markers signaling inflammatory infiltrate.

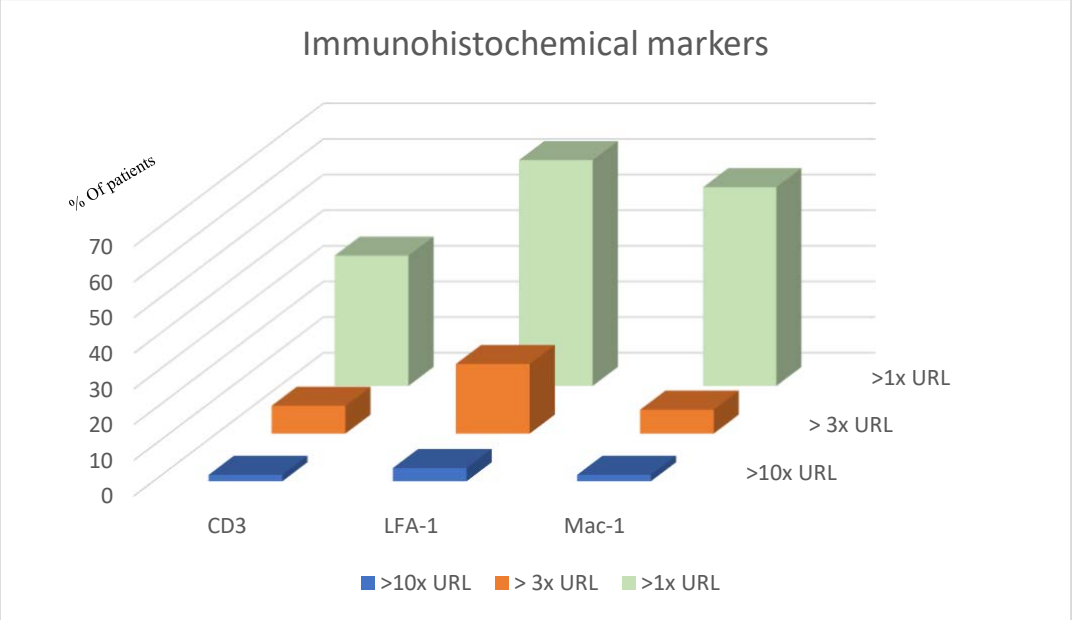
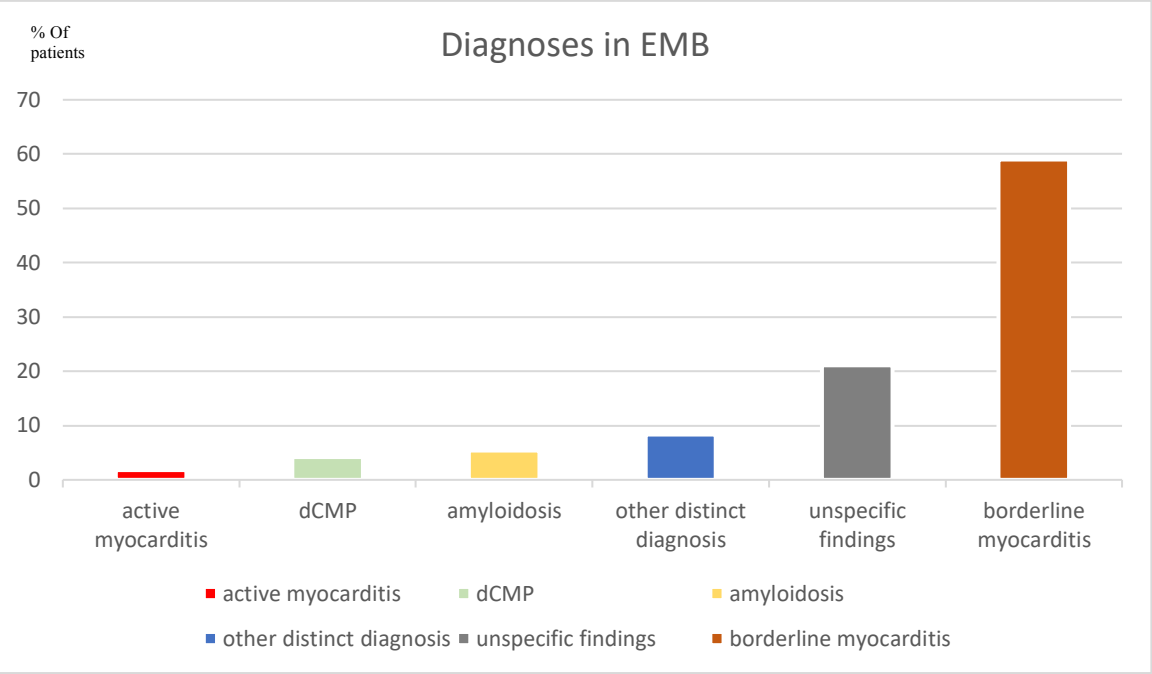


Figure 13 demonstrates the extent of inflammatory infiltrate in the patient cohort. For the immunohistochemical markers CD3, LFA-1 and Mac-1 almost all patients had data available (164, 164 and 163 patients respectively). The marker CD45R0 was only given in 102 of our patients, therefore it was not included in Figure 13.

CD3 was elevated above the upper reference limit (URL) in 59 patients (36.6%). A threefold increase was observed in 13 patients (7.8%) and a tenfold increase in 3 patients (1.8%).
LFA-1 was elevated in an even higher proportion of patients: 104 patients (63.4%) had increased values, threefold increase was seen in 32 (19.5%) patients and 6 patients (3.7%) even showed a tenfold increase in values.
Mac-1 showed an increase in 91 patients (55.8%), while 11 patients (6.7%) presented with a threefold increase and 3 patients (1.8%) presented with a tenfold increase.
CD45R0 was only analyzed in about 2/3 of all patients and showed an elevation above the URL in 72 (70.6%) of patients, an increase above 3x the URL in 8 (4.9%) and an increase above 10x the URL in in 3 (2.9%) patients.

Figure 14: Overview of EMB-diagnoses



As shown in Figure 14, active myocarditis according to the Dallas criteria proved to be the least common diagnosis. It was only diagnosed in 3 (1.8%) patients, while borderline myocarditis without visible necrosis was the most common diagnosis, diagnosed in 98 patients (59%). In over one fifth of patients, precisely in 35 patients (21.1%), the findings were not specific enough to establish a distinct diagnosis. In 14 patients (8.4%) another specific diagnosis was stated by the pathologist. This sub group mainly includes PVB19 reactivations without evidence of inflammation, as well as CMPs without an inflammatory component (no DCM). One report with toxic/metabolic myocardial damage also belongs into this subgroup as well as one post-inflammatory finding. Furthermore, 9 (5.4%) patients were diagnosed with amyloidosis and 7 (4.2%) with DCM.

4.2 Comparison of inflammatory diagnoses in CMR and EMB

Table 6: Comparison of inflammatory diagnoses in CMR and EMB

		CMR diagnosis of myocarditis (n=61)	CMR diagnosis of no myocarditis (n=105)
EMB diagnosis of myocarditis (borderline + active, n=101)	Number of patients	43	58
	% Within EMB category	42.6%	57.4%
	% Within CMR category	70.5%	55.2%
EMB diagnosis of no myocarditis (n=65)	Number of patients	18	47
	% Within EMB category	27.7%	72.3%
	% Within CMR category	29.5%	44.8%

Table 6 shows patient numbers and percentages in the respective CMR and EMB categories.

Sixty-one (36.7%) patients were diagnosed with myocarditis in CMR and 101 (60.8%) with myocarditis in EMB. Forty-three (25.9%) patients were diagnosed with myocarditis and 47 patients (28.3%) without myocarditis congruently in both investigations, resulting in a total of 54.2% congruent diagnoses regarding the question of presence or absence of myocarditis. From Table 6, markers of diagnostic performance of CMR can be calculated when assuming EMB as the gold standard. Sensitivity of CMR was 42.6%, specificity was 72.3%, positive predictive value (PPV) was 70.5% and negative predictive value (NPV) was 44.8% with an overall diagnostic accuracy of 54.2%. The question which diagnoses were made instead in patients with incongruent diagnoses is illustrated in a larger cross table (see Table 7).

4.3 Comparison of all CMR and EMB diagnoses

Table 7: Cross table comparison of all CMR and EMB diagnoses

CMR / EMB	Normal MRI (n=3)	Likely myocarditis (n=61)	DCM (n=60)	Other distinct diagnosis (n=27)	Unspecific findings (n=15)
Borderline myocarditis (n=98)	2 (66.7%)	42 (68.9%)	37 (61.7%)	9 (33.3%)	8 (53.3%)
Active myocarditis (n=3)	0	1 (1.6%)	0	1 (3.7%)	1 (6.7%
DCM (n=7)	0	0	5 (8.3%)	1 (3.7%)	1 (6.7%)
Amyloidosis (n=9)	0	0	0	8 (29.6%)	1 (6.7%)
Other distinct diagnosis (n=14)	1 (33.3%)	4 (6.6%)	7 (11.7%)	1 (3.7%)	1 (6.7%)
Unspecific findings (n=35)	0	14 (23.0%)	11(18.3%)	7 (25.9%)	3 (20.0%)
The variables are presented as number of patients in the respective category with the proportion of the respective CMR category in parenthesis.					

Myocarditis

Table 7 compares EMB and CMR diagnosis categories. 70.5% of CMR diagnosed myocarditis was confirmed by EMB (68.9% were diagnosed as borderline myocarditis and 1.6% were evaluated as active myocarditis in EMB reports). In 6.6% of CMR-diagnosed myocarditis cases another specific diagnosis like hypertrophic cardiomyopathy was stated in EMB, while in 23% the findings were unspecific and there was no specific diagnosis made in EMB.

In contrast, only 42.6% of the EMB-diagnosed myocarditis cases were also diagnosed as myocarditis by CMR. The EMB-diagnosed myocarditis cases without congruent CMR diagnosis were mainly diagnosed as DCM (36.6%) by the radiologists. In other words, in patients with biopsy-proven inflammation radiologists diagnosed almost as many patients to have DCM as they diagnosed with myocarditis. Furthermore, with 9.2% and 8.2% respectively, considerable parts of EMB proven inflammation were classified as specific

diagnosis other than DCM and myocarditis and as unspecific findings in the CMR. The remaining 2% of biopsy proven inflammation had normal MRI-findings.

The three active myocarditis cases in EMB had congruent findings in their CMR in one case (33.3%) while the other two patients were diagnosed incongruently in CMR. One was diagnosed with unspecific findings and the other patient with questionable subacute infarct area at the anterior wall (and was therefore classified as 'specific diagnosis other than previous diagnosis' according to our CMR diagnosis groups).

No myocarditis

A frequent diagnosis in CMR reports was DCM with 60 patients, but only 8.3% of those patients were congruently diagnosed as DCM in EMB reports. The ones not congruently diagnosed by EMB were diagnosed in descending frequency as: Borderline myocarditis (61.7%), unspecific diagnosis (18.3%), specific diagnosis other than DCM and myocarditis (11.7%). These numbers suggest that in almost two out of three cases (61.7%) patients who are diagnosed with DCM in CMR show an abnormal inflammatory infiltrate in their EMB. The 7 cases that were diagnosed as DCM by EMB were mostly concordantly diagnosed by CMR (71.4%). Only two patients were not congruently diagnosed. These patients were diagnosed as 'unspecific findings' and as 'another specific diagnosis (no DCM or myocarditis)' in the EMB. In our patient cohort CMR showed a high PPV of 88.9% for EMB-proven amyloidosis as only one patient was not diagnosed concordantly in their EMB.

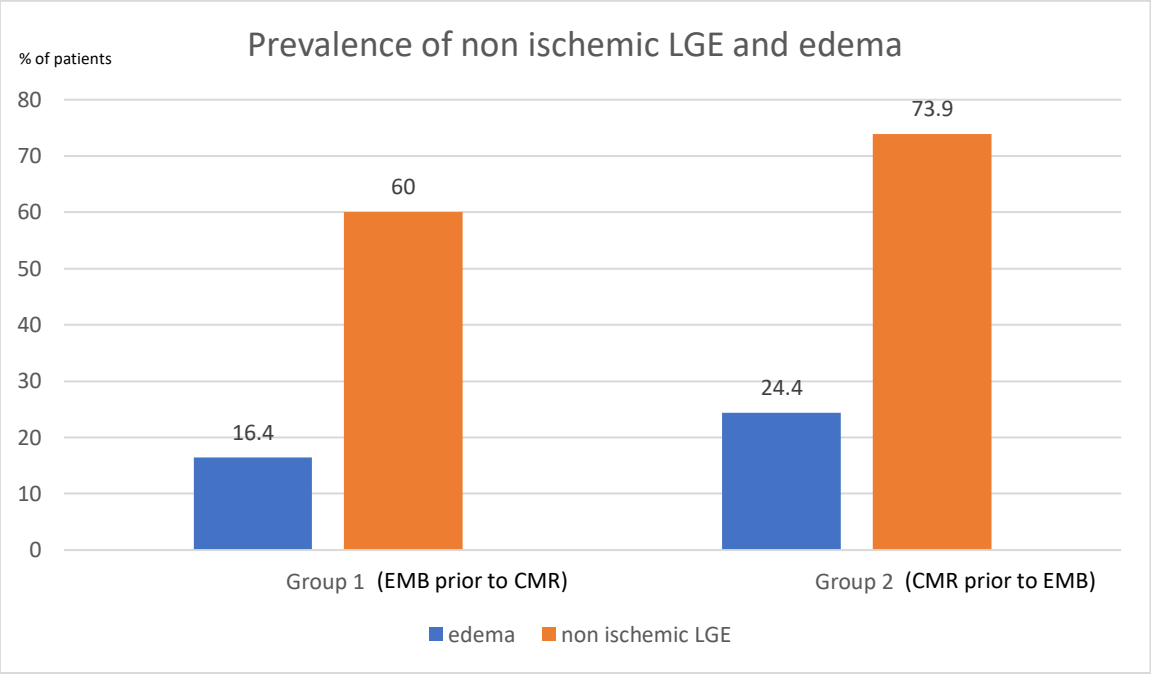
A final diagnosis of 'unspecific findings' was stated more often in EMB reports than in CMR reports (21.1% vs 9% of all patients). Patients with unspecific EMB-findings were diagnosed as myocarditis (40%), DCM (31.4%) and 'another specific diagnoses no myocarditis and DCM' (20.0%) in CMR reports. Only 8.6% of the EMB-diagnosed 'unspecific findings' were subsequently diagnosed as unspecific in CMR. Patients with unspecific CMR findings were mainly (53.3%) diagnosed as borderline myocarditis in their biopsies. The rest was diagnosed as active myocarditis, DCM', amyloidosis and as 'specific diagnosis other than myocarditis or DCM' in their EMB with 6.7% each.

4.4 Sub analysis: Does the performance of EMB prior to CMR lead to false positive myocarditis diagnoses?

We analyzed patients who received CMR and EMB within 14 days between 2008 and 2020 and divided them in two groups dependent on whether they received EMB prior to (group 1) or after CMR (group 2). Groups were compared regarding the CMR myocarditis criteria non-ischemic LGE and edema. Furthermore, groups were compared regarding the number of myocarditis diagnoses in CMR and EMB. Additionally, the concordance of CMR and EMB diagnoses was compared. Patients who received EMB and CMR on the same day were excluded because the sequence in which the investigations were performed was not determinable with certainty. Of our 215 patients, 147 passed our inclusion criteria for this sub analysis. Out of this group, 55 patients received EMB prior to and 92 patients after CMR. Patients' mean age was 45.5±16.0 years, 20% were females.

LGE and edema

Figure 15: Frequency of non-ischemic LGE and edema in study groups



The occurrence of the myocarditis CMR-criteria non-ischemic LGE and edema are presented as percentage of all patients in the respective group in Figure 15. Surprisingly, group 2 showed higher incidences of both criteria compared to group 1 with a non-

ischemic LGE frequency of 73.9% versus 60.0% and an edema frequency of 24.4% versus 16.4%.

Table 8: Cross table comparison of EMB and CMR diagnoses

		EMB findings			
		Group 1 (EMB prior to CMR, n=55)		Group 2 (EMB after CMR, n=92)	
		Myocarditis (n=36)	No myocarditis (n=19)	Myocarditis (n=54)	No myocarditis (n=38)
CMR findings	Normal CMR (n=3)	2 (66.7%)	1 (33.3%)	0	0
	Likely myocarditis (n=53)	12 (92.3%)	1 (7.7%)	24 (60.0%)	16 (40.0%)
	DCM (n=54)	11 (57.9%)	8 (42.1%)	24 (68.6%)	11 (31.4%)
	Other diagnosis (n=25)	7 (50.0%)	7 (50.0%)	2 (50.0%)	2 (50.0%)
	Unspecific findings (n=12)	4 (66.7%)	2 (33.3%)	4 (66.7%)	2 (33.3%)
EMB diagnoses have been summarized into inflammatory diagnosis (including borderline myocarditis and active myocarditis) and non-inflammatory diagnoses (all other diagnoses). For reasons of simplification the groups are just called „myocarditis“ and „no myocarditis“. The percentages given in parentheses refer to the respective CMR category for the respective study group.					

Group 1 (EMB prior to CMR)

In this group, 23.6% of patients were diagnosed with myocarditis by CMR, while almost three times as many patients (65.5%) were diagnosed with it in EMB.

The concordance between CMR and EMB regarding myocarditis diagnoses was high, with 92.3% of CMR diagnosed myocarditis cases confirmed by EMB. Out of 13 CMR diagnosed myocarditis patients only one was not confirmed via biopsy.

Similar to what we have already seen in the previous analysis in chapter 4.3, many patients diagnosed with DCM in CMR had considerable inflammation in their EMB (57.9% had inflammatory diagnoses in their EMB). Comparable observations to those in chapter 4.3 can be made for patients diagnosed with ‘specific diagnosis other than myocarditis and DCM’ in CMR, where half of the patients had detected abnormal inflammatory infiltrates in their biopsy samples.

Group 2 (CMR prior to EMB)

Regarding the second group, 43.4% of patients were diagnosed with myocarditis by CMR, while 58.7% were diagnosed with it in EMB. The difference of diagnosed myocarditis patients between both diagnostic investigations is much smaller compared to group 'CMR before EMB' (41.9% difference between modalities in group 1 vs 15.3% in group 2). The percentage of patients with myocarditis in their EMB is higher in group 'EMB before CMR' (65.5% in group 1 vs 58.7% in group 2), whereas the proportion of patients diagnosed with myocarditis in CMR is considerably higher in group 'CMR before EMB' (23.6% in group 1 and 43.4% in group 2). The concordance of CMR and EMB myocarditis diagnoses was worse in group 'CMR before EMB' with only 60% of CMR-diagnoses being confirmed by biopsy vs 92.3% in group 'EMB before CMR'. Compared to group 'EMB before CMR', an even higher portion of patients (68.6%) with MRI-diagnosed DCM had biopsy proven inflammation.

4.4.1 Significance of findings

In order to assess if the differences in our measurements were significantly different between groups 1 and 2, we used a Chi² test to test for differences in myocardial edema, non-ischemic LGE, CMR inflammatory and EMB inflammatory diagnoses.

Table 9: Significance of differences between study groups.

	variables	Group EMB before CMR	Group CMR before EMB	p-value
CMR	edema	9/55 (16.4%)	22/90 (24.4%)	0.249
	Non-ischemic LGE	33/55 (60.0%)	68/92 (73.9%)	0.078
	Inflammatory diagnosis in CMR	14/55 (25.5%)	40/92 (43.5%)	0.015*
EMB	Inflammatory diagnosis in EMB	36/55 (65.5%)	54/92 (58.7%)	0.416

Categorial variables are presented as number of patients.
** Significant values are marked specifically. p<0,05: *; p<0,01: ** ; p<0,001: ****

As illustrated in Table 9 there was no significant difference in edema prevalence (p=0.272), non-ischemic LGE prevalence (p=0.078) and number of inflammatory diagnoses in EMB (p=0.416) between study groups. However, we found a significant difference between study groups regarding the amount of CMR-diagnosed inflammatory diagnoses (p=0.012). Group 'EMB before CMR' had significantly less inflammatory diagnoses compared to group 'CMR before EMB'.

However, the established difference could probably be explained by differences in the composition of our study groups, serving as confounders. Therefore, the next step was to compare the composition of our study groups. We chose to compare the demographic parameters age, sex, BMI as well as the blood values TnT and NT-proBNP. As a functional parameter we compared CMR measured LVEF between study groups. We performed a Chi² test for differences in sex. For the variables age, BMI and EF a Student's t-test was performed since values were approximately normally distributed. We performed a Mann-Whitney-U-test for the variables TnT and NT-proBNP which is a non-parametric test as the values were not normally distributed.

Table 10: Significance of differences between study groups.

	variables	Group 1 "EMB before CMR"	Group 2 "CMR before EMB"	p-value
Demographics	Age (years)	46.6 ± 16.0	44.9 ± 16.0	0.544
	Female Sex (%)	14.5	22.8	0.222
	BMI (kg/m^2)	28.8 ± 6.0	28.7 ± 7.3	0.988
Blood tests	TnT (pg/ml)	243.7	156.4	0.142
	NT-proBNP (pg/ml)	2857.5	1705.7	0.012*
CMR	LVEF (CMR measured in %)	35.4	38.2	0.317
** Significant values are marked specifically. p<0,05: *; p<0,01: ** ; p<0,001: ****				

As we can see in Table 10 there was no significant difference in age (p=0.544), sex (p=0.222), BMI (p=0.988), TnT (p=0.142) and EF (p=0.317). However, group 1, which

received their EMB prior to CMR had significantly higher NT-proBNP levels compared to group 2.

5 Discussion

Myocarditis is a disease, which likely occurs far more frequently than it is currently diagnosed. The official incidence of 22 per 100.000 persons per year reported in the Global Burden of Disease Study (10) is solely derived from hospital dismissal codes. Consequently, these numbers miss fatal cases happening outside of hospitals on the one hand and subclinical cases which are not treated in hospitals on the other hand. The consequences of the disease can be life-threatening involving CMP, HF and SCD (as illustrated in Figure 2). The diagnostic process remains challenging as the two diagnostic modalities representing the current invasive and non-invasive gold standard have limitations regarding their sensitivity and specificity e.g., the sampling error of EMB and the low sensitivity of CMR when performed in patients with low grade inflammation (see chapter 1.6.4 and chapter 1.6.5 above).

The aim of this retrospective data analysis was first and foremost to create a registry of all patients who received both, EMB and CMR investigation at the Department of Cardiology of the Medical University of Graz. Furthermore, we aimed to present the findings of these diagnostic investigations with a special focus on myocarditis-diagnoses. In the next step, EMB and CMR diagnoses were checked for congruence regarding diagnosed myocarditis. In cases of incongruent diagnoses in the two investigations, the alternatively made diagnoses were presented. As it became obvious during the creation of the registry, that about one third of the patients received their EMB prior to receiving their CMR, we assumed that this scenario might lead to an overdiagnosis of myocarditis as the fresh biopsy sites could potentially mimic spots of myocardial inflammation. Therefore, the second part of this thesis had the aim of testing this hypothesis.

5.1 Study population

Our study population included adults of all age groups. The mean age of 45.6 ± 15.6 years in our patient cohort is close to the mean age of 30-45 years of myocarditis patients reported in the literature. (4,5) The mean BMI (28.4 kg/m^2 in the main analysis) seems to be comparable to the mean BMI in the general Austrian population. However, with only 20% women and 80% males, the proportion of the female sex was significantly smaller in our patient cohort than in the literature, where 1:1.5 to 1:1.7 women to men ratios are stated. (139) The gender proportion stayed at 1:4 for the subgroup of patients who received a final diagnosis of myocarditis. However, there are a few studies who described similar sex distributions to ours, e.g. Berg et al. in Switzerland with 87% males (162) and Younis et al. in Israel with 84% males. (5). Ozierański et al. recently described in a Polish myocarditis registry, that although women were more symptomatic and had more comorbidities than men, they were less likely to be admitted to a cardiology unit or undergo diagnostic testing for myocarditis. (140) This phenomenon might contribute to the gender asymmetry in myocarditis patients.

Whether our observed gender proportion represents an adequate ratio of myocarditis patients in Styria or whether some kind of selection bias at our institution leads to women receiving less CMR and EMB is unknown and should be further evaluated. However, the gap between 1:1.5 to 1:1.7 and 1:4 in gender seems so significant that an explanation solely based on different local epidemiology seems less likely than the presence of some kind of selection bias.

5.2 *Myocarditis diagnoses in EMB and CMR*

In the CMR-reports, 61 (36.7%) patients were diagnosed with myocarditis. However, in the EMB-reports 98 (59%) patients were diagnosed with borderline myocarditis, which was by far the most common EMB-diagnosis in our cohort. Surprisingly, only 3 (1.8%) patients were diagnosed with active myocarditis, making a total of 101 (60.8%) myocarditis diagnoses in our EMB-reports.

Dallas criteria and active myocarditis

As described by Kenneth L. Baughman in 2006 in ‘Death of Dallas Criteria‘(94), the Dallas criteria no longer seem sensitive and adequate enough to diagnose inflammation in histological samples. Purely relying on H&E staining is not adequate anymore and has been replaced by IHC. Still the vocabulary used in pathology reports stayed more or less the same. In our data set, where only 3% of histological inflammation was labeled as active myocarditis, the pathologists’ terminology seems to be misleading. This becomes obvious as 97% of histological inflammation was labeled as ‘borderline myocarditis’. Although the term insinuates only marginal inflammation, abnormal inflammatory infiltrates are present and can be substantial. The low numbers of active myocarditis cases are explainable by the requirement of fresh cell necroses for this diagnosis, which seem to be rarely observed by diagnosing pathologists.

5.3 Concordance of CMR and EMB diagnoses

Forty-three (25.9%) patients were diagnosed with myocarditis and 47 patients (28.3%) without myocarditis congruently in CMR and EMB. Consequently, 54.2% of our patients received a congruent diagnosis regarding the question of myocarditis or no myocarditis.

Fifty-eight patients who received an EMB myocarditis-diagnosis were not diagnosed as myocarditis by CMR. On the other hand, 18 patients in whom CMR was positive for myocarditis were not detected by EMB. Bottom line: only 43 (25.9 %) patients were diagnosed with a myocarditis diagnosis in both, CMR and EMB, while 119 (71.7%) were diagnosed with myocarditis in either CMR or EMB. This means that only a small fraction of patients who were diagnosed with myocarditis by either CMR or EMB were diagnosed congruently.

We did not differentiate between AM and chronic iCMP in this analysis, which explains the large number of 101 EMB-diagnosed myocarditis cases and the considerably lower number of 61 CMR-diagnosed myocarditis cases. The low grade inflammation, which EMB shows higher sensitivity for (see chapter 1.6.4 and chapter 1.6.5) may be detectable in IHC but is not necessarily related to neither clinical symptoms nor visible changes in

CMR. This fact may have negatively influenced diagnostic concordance of EMB and CMR.

Almost all the available studies comparing concordance of CMR and EMB in performing myocarditis-diagnoses refer to clinical criteria as the gold standard of diagnosis. However, the MyoRacer Trial from 2016 is a trial using EMB-diagnosed myocarditis cases as the gold standard of myocarditis diagnoses which is comparable to our own approach. In patients with biopsy proven myocarditis they found a sensitivity of 66% and a specificity of 47% when applying the old LLC. (163) Our numbers were different. Although we had a lower sensitivity of 43%, our specificity of 72% was considerably higher. Overall diagnostic accuracy was comparable with 59% compared to 54% in our study. An important difference to the MyoRacer trial is that they used biventricular biopsy in 93% of included patients, while we used exclusively right ventricular biopsies in 97.6% of patients. Their approach might improve diagnostic EMB sensitivity by limiting the sampling error compared to our EMB approach. Moreover, they used the full LLC including EGE in their diagnostic process while in our cohort EGE data was mentioned in only 17.5% of CMR-reports and therefore not routinely used. When comparing our patient cohort with the MyoRacer study cohort, it is notable that their patient population included more acute cases with myocardial necrosis. Four percent of the patients had detectable cell necrosis, while only 1.8 percent of our cohort had myocardial necrosis. We seem to have had a less acute patient collective compared to the MyoRacer trial with less AM which could explain the poorer sensitivity of CMR (see chapter 1.6.4).

Alternative diagnoses

In order to find factors that may have negatively influenced diagnostic concordance of CMR and EMB regarding myocarditis diagnoses, it seems logical to have a look at the diagnoses made instead in cases with incongruent diagnoses. More precisely: Which alternative diagnoses were made by CMR in EMB-diagnosed myocarditis cases and vice versa?

Of the patients diagnosed with myocarditis in EMB, 36.6% received a diagnosis of DCM in CMR. It is well established that there is an overlap between myocarditis and DCM.

Myocarditis can lead to DCM and DCM patients may suffer from chronic inflammatory CMP.

On the other hand, if we have a look at patients who were diagnosed with myocarditis by CMR but were not confirmed by EMB, 73.7% of them had nonspecific biopsy-findings in their EMB, insufficient for a specific diagnosis. A reasonable explanation for this phenomenon is the sampling error of EMB (described in chapter 1.6.5), which is composed of the overall limited sensitivity of EMB in the diagnosis of LM, the low number of biopsies taken in EMB and the patchy distribution of some forms of myocarditis. With 8-15 obtained samples per EMB procedure, we take significantly more biopsies at our university clinic than the average 4-5 samples, but even with 17 samples per EMB, the sensitivity gets only to around 80% (cf. chapter 1.6.5). The combination of these factors might have led to the negative EMB results.

5.4 Impact of performing EMB prior to or after CMR on diagnostic concordance

In the sub analysis investigating the influence of the sequence of EMB and CMR on false positive myocarditis diagnoses, group 1 (n=55) had EMB performed prior to, group 2 (n=92) after CMR. Comparing groups, the frequency of detected non-ischemic LGE (60.0% versus 73.9%, p=0.078) and edema (16.4% vs. 24.4%, p=0.249) showed no significant difference. Neither did the amount of biopsy proven inflammation (65.5% versus 58.7%, p=0.416). The latter is an important finding as it creates an equal base of comparable histological inflammation between the groups for further comparing the amount of CMR-diagnosed inflammation. Surprisingly, there was significantly more CMR-diagnosed myocarditis in group “CMR before EMB” compared to group “EMB before CMR” (23.6% vs. 43.5%, p=0.015).
Contrary to our expectations, the amount of diagnosed myocarditis in CMR was higher in group “CMR before EMB” although we suspected it to be the other way around before performing the analysis. Hence performing EMB prior to the CMR did not create more myocarditis diagnoses compared to performing CMR prior to EMB. Our results suggest that the biopsy sites were not misinterpreted as myocarditis by the diagnosing radiologists.

Concordance of EMB and CMR between study groups

Concordance of CMR and EMB diagnosed myocarditis was much higher in group “EMB before CMR” where 92% of CMR-diagnosed myocarditis cases were confirmed by EMB while only 60% of CMR-suspected myocarditis cases were confirmed by EMB in group “CMR before EMB”. In group “EMB before CMR”, PPV and NPV of the CMR were at 92.3% and 42.9% in relation to biopsy-confirmed myocarditis, while in contrast, PPV and NPV were only 60.0% and 42.3%, in group “CMR before EMB”.
Performing EMB shortly prior to CMR investigation did not negatively affect the concordance between both diagnostic methods.

An explanation for the higher concordance of EMB- and CMR-diagnoses in group “EMB before CMR” could be found in the selection process of the patients who received EMB prior to CMR. The indications for EMB are to a great extent in the highly acute, fulminant setting of myocarditis (see chapter 1.6.5). In this patient population, we could assume more inflammation in the myocardium compared to patients in whom CMR is performed first. The limitations of CMR in the diagnosis of subacute, low-grade inflammation are established. (83) Therefore, it is quite reasonable to believe that concordance will be worse in patients who are not as acutely ill and are not selected for the "EMB first" strategy. The significant difference in NT-proBNP values (see chapter 4.4.1) supports this hypothesis as the group “EMB before CMR” showed significantly higher NT-proBNP values compared to group “CMR before EMB”.

5.5 Strengths and limitations

5.5.1 Study design

As our analysis was performed monocentric the retrospective design allowed us to create a large, representative patient cohort. Yet, the retrospective nature and mono centric study introduce inevitable selection and information bias. Prospective multicenter studies would be an appropriate option to confirm our results. Regarding our sub analysis that examined the influence of the sequence of EMB and CMR, an ideal study would be to perform CMR on the same patient before EMB and after EMB.

5.5.2 Inclusion process / study group

For our main analysis the chosen maximum time span between CMR and EMB was 30 days. For our sub analysis in chapter 4.4 the chosen maximum time span was 14 days. Those intervals may have been already too long to create comparable conditions at the time where the investigations were performed. Resolution or exacerbation of inflammation within this time frame is possible and could lead to physiological findings in one investigation and abnormal findings in the other, consequently leading to incongruent diagnoses between both investigations.

5.5.3 Myocarditis diagnoses in CMR

EGE was a criterion used in the old LLC. According to the 'two out of three' rule, two criteria from the list of three (EGE, LGE, edema) were required to form a diagnosis of myocarditis. (75) Unfortunately, EGE was not explicitly mentioned in 137 out of 166 MRI reports. Although the diagnosing radiologists might have included EGE in their assessment without mentioning it in their reports, it cannot be excluded that EGE was not considered at all in the diagnostic process. Younger CMR-criteria from the revised LLC such as ECV and mapping sequences were only mentioned in the most recent MRI reports as they were not yet established in older reports. However, these criteria are proven for myocarditis diagnosis as was shown by the updated LLC in 2018. (74) The new criteria have better sensitivity than the old LLC with comparable specificity. However, most of our patients received their CMR before 2018 and therefore the old LLC with worse sensitivity were used.

5.5.4 Suggestions for additional analyses

To generate more accurate CMR diagnoses, CMR sequences could be reassessed in a standardized fashion, which was beyond the scope of the present thesis:

- Older MRI sequences dating from before 2018 need to be reassessed and EGE data needs to be collected. Then recategorization in suitable diagnosis categories should be performed in a standardized fashion according to old LLC.
- newer MRI images dating from the years after 2018, in which categorization into our diagnoses categories was not unequivocal, should be reassessed for the presence of the revised LLC if the sequences are available. ECV, LGE, T1 and T2 mapping should be systematically evaluated, and categorization should be repeated with the new data according to the updated LLC.

5.6 Implications for theory and clinical practice

There are some implications of this thesis that are of interest to Clinical practice:

- Our patient cohort was composed of 80% males and only 20% females. The gender distribution was similar in the subgroup of patients receiving a diagnosis of myocarditis. This gender distribution is significantly different from the one given in recent epidemiological studies. Therefore, a selection bias in choosing patients for CMR and EMB procedures at our university clinic should be considered and should lead to further analysis in clinical practice.

- The concordance of CMR and EMB diagnoses was only moderate our main analysis. Reasonable explanations are on the one hand the sampling error of EMB, leading to false negative EMB results, and on the other hand histological low-grade myocarditis that remained undetected or resembled DCM on CMR. Furthermore, the chosen maximum time interval of 30 days between the two examinations may already have been too long to achieve comparable diagnostic conditions in both examinations. The concordance was worse when CMR was performed prior to

EMB compared to EMB prior to CMR. These factors should be considered in the diagnostic process of myocarditis patients.

- Contrary to our expectations, performing EMB shortly prior to CMR did not lead to an increase of non-ischemic LGE and edema. Furthermore, it did not lead to an increase in myocarditis diagnoses in CMR and did not worsen the concordance between CMR and EMB diagnoses. We can assume that the biopsy sites were not misinterpreted as myocarditis by the diagnosing radiologists in the "EMB first" approach. This conclusion is an important finding for the interpretation of the diagnostic results when EMB is performed early and prior to the CMR.

5.7 Conclusion

Concordance of CMR and EMB in the diagnosis of myocarditis was moderate in our cohort. There was no increase in false positive myocarditis diagnoses when EMB was performed prior to CMR compared to EMB being performed after CMR.

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