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Clinical Characteristics and Outcome of Biopsy-Proven Myocarditis in Children – Results of the German Prospective Multicentre Registry "MYKKE"

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Conflict of Interest

None declared.

Keywords

Paediatric, myocarditis, endomyocardial biopsy, outcome, registry

Introduction

Myocarditis is an inflammatory heart disease caused by various infectious (mostly viruses) and non-infectious triggers. It can result in both, acute and chronic heart failure and its clinical presentation is heterogeneous (1). Data on incidence of myocarditis are rare and described with 1-1.95/100000 per year in children (2, 3). In at least one third of children with a phenotype of dilated cardiomyopathy (DCM), a myocarditis could be detected as the underlying cause (4, 5). Indication for heart transplantation (HTx) ranges from 4-9% and mortality has been reported with 4-7% (6, 7).

The relationship between outcome and initial clinical presentation in paediatric myocarditis is not sufficiently described. Adults with biopsy-proven acute myocarditis and fulminant clinical presentations had a worse short- and long-term outcome compared to patients with non-fulminant myocarditis (8, 9).

Although endomyocardial biopsy (EMB) is regarded as the gold standard in the diagnosis of myocarditis, its use especially in children varies (1). In the statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology an EMB in children is recommend in unexplained cardiomyopathy (10). An important concern regarding EMB is the risk for major complications, although complication rates in adults have been reported as less than 1% when performed by experienced centres (11-13). In children, complication rates lie between 1 and 10% as retrospectively reported from single-centre studies (14-16). A German multicentre retrospective analysis reported an overall low risk of major complications of about 1% for transcatheter biopsies of the right ventricle, but a significantly higher risk in children below one year of age (17).

However, EMB is the only way to identify the underlying cause and type of myocardial inflammation. Thus, it can direct and monitor treatment strategies, which has been proven in single-centre studies in adults but not yet in children with suspected myocarditis (13, 18).

Therefore, the aim of this study was to investigate the current clinical practice of EMB, its diagnostic value, impact on therapeutic strategies, and relation to outcomes in paediatric patients with suspected myocarditis by analysing data from the prospective multicentre registry “MYKKE” (19).

Methods

MYKKE registry

MYKKE is a prospective multicentre registry for suspected myocarditis in children and adolescents, which aims to gain knowledge on incidence, pathogenesis, and outcome of paediatric myocarditis. Inclusion criteria are suspected myocarditis, hospitalization, and age <18 years (19). Its database is hosted and administered by the Competence Network for Congenital Heart Defects, Berlin, Germany. From September 2013 until January 2020, 23 centres have prospectively enrolled patients. A suspicion for myocarditis was present in patients with symptoms like angina pectoris, dyspnoea, decompensation or history of infection or fever within the last six weeks. Further, electrocardiogram (ECG) abnormalities, elevated troponin and/or N-terminal pro-B-type natriuretic peptide (NT-proBNP/pro-BNP), unexplained cardiac dysfunction or dilated left or right ventricle. Written informed consent was given from parents or legal guardians. Ethical approval was first obtained at the initiating centre (German Heart Center Berlin, Germany) from the ethics committee of the Charité - Universitätsmedizin Berlin (EA2/074/13) and subsequently confirmed by the local authorities of all other participating centres (ClinicalTrials.gov NCT02590341), following the Declaration of Helsinki.

Patient data & Follow-up

Only patients with EMB from the MYKKE registry were included in this analysis. Figure 1 shows the study inclusion criteria and dropouts. Initial clinical and follow-up data were entered into the online study database by the local physicians and monitored by the central study centre. Echocardiographic, ECG and laboratory data were recorded right after admission. Further arrhythmias, atrioventricular blockages II/III, relevant bradycardia, supraventricular and ventricular tachycardia right before or during first admission were included. Due to the multicentre approach a variety of different troponins were reported. Therefore, we converted the different values into a binary variable “troponin elevated” which describes the value of troponin above the upper reference limit. Regarding outcome, the occurrence of adverse events including mechanical circulatory support (MCS), HTx and/or death was defined as a combined endpoint.

In a sub-analysis, patients were divided into patients with a fulminant (FM) and non-fulminant (NFM) clinical course. The existence of low cardiac output syndrome requiring inotropes and/or MCS was

defined as FM. In the NFM group patients with hemodynamic stability and without need for inotropes or MCS were included (8, 20).

Analysis of endomyocardial biopsies

EMB was taken from the left, right or both ventricles. All EMB specimen were analysed histopathologically and immunohistologically as previously described (21) and by polymerase chain reaction ((RT-)PCR) for myocardial detection of viral RNA/DNA by one specialized centre for Cardiopathology (Institute for Pathology and Neuropathology, University Hospital Tübingen, Tübingen, Germany). For histopathological and immunhistological analyses usually 3 biopsies, for molecular pathological analyses 1-2 biopsies were taken.

From the biopsies, 4-µm-thick tissue sections were stained with haematoxylin and eosin, Masson's trichrome, and Giemsa and examined by light microscopy. Interstitial fibrosis was graded in Masson´s trichrome stained sections as 0: none, 1: mild, 2: moderate and 3: severe interstitial fibrosis (22). Histological analysis followed the Dallas criteria as the gold standard for the evaluation of myocarditis and was completed by immunohistochemical stainings detecting CD3+ T lymphocytes and CD68+ macrophages (23). The mononuclear infiltrates were classified as 0: no inflammation, 1: single inflammatory cells (CD3+ T-lymphocytes and CD68+ macrophages ≥14/mm2); 2: a few foci of inflammation, 3: several foci of inflammation, 4: pronounced inflammation. For a detailed definition of the grades of CD3+ T-lymphocyte and CD68+ macrophage infiltration, see the supplemental methods section and the publication on the paediatric cohort for the evaluation of inflammation in endomyocardial biopsies (21).

Only patients with EMB confirmed myocarditis were conducted to further analysis.

The diagnosis of myocarditis was confirmed according to the established criteria and grouped in accordance with the WHO definition in (24, 25):

a) Acute Myocarditis: Infiltrate of ≥14 leucocytes/mm² and presence of myocyte damage.

b) Healing/chronic Myocarditis: Infiltrate of ≥14 leucocytes/mm² and absence of myocyte damage but presence of fibrosis.

c) Healed Myocarditis: Multifocal fibrosis or scarring without inflammation (0-3 leucocytes/mm²).

Deoxyribonucleic and ribonucleic acid (DNA, RNA) was detected in the myocardium and EDTA blood by nested (RT-) PCR or quantitative PCR of the following pathogens as described (26):

Parvovirus B19 (PVB19), enteroviruses, adenoviruses, human herpesvirus 6 (HHV6) and 7 (HHV7), cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus type 1 (HSV1) and type 2 (HSV2), varicella zoster virus (VZV).

The myocardial viral load of PVB19 DNA was classified as follows (27)

a) Low: < 500 copies/µg isolated myocardial DNA

b) Moderately elevated: ≥ 500-2000 copies/µg isolated myocardial DNA

c) Severely elevated: ≥2000 copies/µg isolated myocardial DNA.

The DNA/RNA detection of the other pathogens was described as present or absent. When only detected in nested PCR the viral load was defined as low.

Statistical analysis

Categorical variables were summarized by frequencies and percentages. For continuous measures, data were presented as median values with interquartile range (IQR). Pearson's chi-square test and Fisher's exact test were used to compare dichotomous variables. For comparison of independent groups, the Mann-Whitney U and Kruskal-Wallis test were applied. Kaplan-Meier curves and log rank tests were used for survival analysis of the combined endpoints. A probability value of <0.05 was considered statistically significant. Data were analysed using IBM Corp. SPSS Version 24.0 (Armonk, NY, USA).

Results

Clinical presentation and biopsy results of the EMB cohort

From 436 screened patients of the MYKKE registry, 260 patients (60%) with a median (IQR) age of 12.7 (1.2-15.9) years received an EMB (EMB cohort). Forty-eight percent of these patients with EMB presented with dyspnoea. Accordingly, more than one third were in NYHA class III or IV and showed signs of cardiac decompensation. The Z-score of the left ventricular internal dimension at end-diastole (LVIDd) was elevated with 2.1 (0.2-5.7) and the left ventricular ejection fraction (LVEF) impaired with 45 (25.3-60.0)%. In addition, in 39% of patients with EMB arrhythmias and in 32% ST-elevations were detected. For further characteristics, please see Table 1.

In 5 patients (1.9%) EMB-related pericardial effusion and in 2 patients (0.7%) a pericardial tamponade with need for drainage were reported. No permanent atrioventricular blockages or other complications were seen.

Mostly, EMB was taken from the right ventricle (n=227, 86%). In 28 patients, it was taken from the left ventricle (LV) and in 10 patients from both ventricles. Fifty percent of the LV biopsies were taken during ventricular assist device (VAD) implantation. The median time from admission to EMB was 3.0 (1.0-9.0) days, the median time from symptom onset to EMB was 11.0 (4.0-29.0) days.

In 209/260 patients (80%) myocarditis was diagnosed in EMB. Healing/chronic myocarditis was detected in 133 of patients (51%), followed by 47 (18%) patients with an acute myocarditis and 29 (11%) patients with healed myocarditis. DCM was found in 16 (6%) patients, 6 patients (2.3%) had other diagnoses (hypertrophic cardiomyopathy: n=3; left ventricular non-compaction cardiomyopathy: n=2, restrictive cardiomyopathy: n=1). See Figure 2 for different histopathological and immunohistochemical findings. No signs of inflammation or any other pathological findings were detected in 29 (11%) patients.

Following analyses were only performed on the patients with biopsy proven myocarditis (myocarditis group, n=209).

Clinical presentation of the myocarditis group

The median age of the myocarditis group (n=209) was 12.8 (1.4-15.9) years, 125 (60%) were male. The time between symptom onset to EMB was 11.0 (4.0-29.0) days, from admission to EMB 3.0 (1.0-10.3) days. The myocarditis subgroups are defined by different clinical characteristics: especially children with acute myocarditis were significantly younger with signs of heart failure, had lower LVEF,

LV dilatation and higher NT-proBNP levels, compared to those with healing/chronic or healed myocarditis. On the contrary, children with healed myocarditis were older with lower NYHA classes, and presented more frequently with angina pectoris, syncope and sudden cardiac death (see Table 1 and Figure 3).

According to the clinical presentation, CMR data revealed a severe left ventricular dilation and functional impairment within the acute myocarditis group. Further, CMR was able to detect myocardial inflammation according to the revised Lake Louise criteria in a large proportion of patients (see Table 1).

Comparing patients with FM and NFM clinical courses, children with FM courses were significantly younger and presented with signs of heart failure. ST-elevations in ECG were more often found in the NFM cohort, T-inversion in the FM cohort (see Table S1).

Histopathological and immunohistochemical results in paediatric myocarditis

In almost all patients (n=207, 99%) a lymphocytic myocarditis was found. Two patients had eosinophilic myocarditis, one after Clozapine intake. No giant cell myocarditis was diagnosed. Considering the mononuclear cell infiltrates, higher levels of cardiac immune cells were found in younger children (p=0.001) and were associated with the presence of heart failure symptoms like elevated NT-proBNP (p<0.001), LV dilation (p=0.004) and impaired LVEF (p<0.001, supplemental Figure S1). Regarding the detection of interstitial fibrosis in EMB we found that patients with higher grades of fibrosis were also the younger (p=0.026) and presented more often with heart failure signs as elevated NT-proBNP (p=0.042), LV dilatation (p=0.010) and impaired LVEF (p=0.003) compared to patients with less fibrosis.

Altogether, moderate and severely elevated infiltrates, CD3+ lymphocytes and CD68+ macrophages were more frequently present in acute, followed by healing/chronic myocarditis patients compared to healed myocarditis (p<0.001). In contrast, fibrosis was more often present in healing/chronic myocarditis (p<0.001, supplemental Table S2). The time between admission and EMB (p=0.114) as well as the time between onset of symptoms and EMB (p=0.088) did not differ significantly between the groups. However, patients with healed myocarditis had the longest time interval between symptom onset and EMB (see Table 1).

Viral nucleic acid detection

In 105 (50%) patients viral nucleic acid could be detected. Most frequently in EMB PVB19 DNA was found (n=60, 57%), followed by HHV6 DNA (n=20), HVV6/PVB19 DNA (n=10), enterovirus RNA (n=7), human CMV DNA (n=3), EBV DNA (n=2), HHV7 (n=1) and HHV6 and 7 DNA (n=1). In EMB of one patient PVB19, HHV6, EBV DNA and enterovirus RNA were found (supplemental Figure S2).
In patients with acute myocarditis, viral DNA/RNA could be detected more frequently (p=0.014, supplemental Table S2). In addition, a parallel detection of viral DNA/RNA within EDTA blood and myocardium was more frequently seen in acute myocarditis (p<0.001, see supplemental Table S3). Accordingly, patients with acute myocarditis more frequently had a myocardial PVB19 DNA load of ≥ 2000 copies/µg DNA compared to healing/chronic and healed myocarditis (p<0.001, supplemental Table S4). In patients with PVB19 DNA loads of ≥ 2000 copies/µg DNA, the mononuclear cell infiltrates were in 92% of the patients moderately or severely elevated, compared to 50% with viral loads of <500 copies/µg DNA and 38% in ≥500-2000 copies/µg DNA (p=0.004).
The DNA/RNA of other cardiotropic viruses was present at rather low levels as they were not found in the first PCR but only in the nested PCR with the exception of a 3-month old male patient with an acute CMV myocarditis (supplemental Figure S3 shows his histopathological findings).
A simultaneous detection of viral DNA/RNA within blood and myocardium was found in 30 patients (29%): for PVB19 (n=20), enteroviruses (n=5), CMV (n=2), HHV6/7, PVB19/HHV6 and enterovirus/PVB19/HHV6 (n=1, respectively).

Specific medical treatment

Apart from heart failure therapy (n=165, 79%) and the need for inotropic support (n=91, 44%), eighteen patients (9%) received a specific antiviral treatment with valganciclovir or ganciclovir. An immunosuppressive therapy with azathioprine and prednisolone was applied to 15 patients (7%). In nine out of these (60%), a virus was detected within the myocardium (3 x PVB19, 3x HHV6 and 3 x PVB19/HHV6; viral loads for PVB19 were 2 x < 500, 2 x ≥ 500-2000 and 2 x ≥2 000 copies/µg DNA). Patients with myocardial viral detection did not differ clinically from patients without viral detection. In all patients, the immunosuppressive therapy was started after EMB performance, in two patients after a second EMB.

Outcome of the paediatric myocarditis patients regarding clinical presentation and histopathological results

In the myocarditis group the median follow-up time was 11.1 (3.4-17.8) month. Regarding MCS, highest rate for ECMO implantation were detected in patients with acute myocarditis, reflecting a more fulminant clinical course, whereas the VAD implantation rate did not differ significantly between patients with acute and healing/chronic myocarditis. Lowest rates were seen in patients with healing myocarditis. Heart transplantation was most frequently performed in patients with healing/chronic myocarditis. Mortality did not differ significantly between the myocarditis groups (Table 1).

The worst event-free survival of the combined endpoint MCS, HTx and death was seen in patients with healing/chronic myocarditis (24%) and acute myocarditis (31%) compared to healed myocarditis patients but without statistical significance (58%, p=0.294; Figure 3). The detection of myocardial virus genome alone had no significant effect on the event-free survival (p=0.726, Figure 3). The event-free survival of the combined endpoint of HTX and death was significant lower in the cohort with FM clinical courses compared to patients with NFM clinical courses (p<0.001, 68% vs. 92%, Figure 3). Patients with acute myocarditis had a MCS weaning rate of 64% (n=9) compared to 23% in healing/chronic myocarditis (n=6) and 100% in 2 patients with healed myocarditis (p=0.006, Table 1). The highest rates for heart transplantation were found in the chronic/healing myocarditis patients (p=0.035, see Table 1).

Discussion

In this study, we investigated a large number of paediatric patients with biopsy-proven myocarditis derived from a multi-centre approach and the relation to their clinical presentation, EMB results and outcome.

Clinical aspects of EMB performance

Overall, EMB was performed in 60% of the total cohort and preferably in those patients who presented with more severe clinical symptoms. This was most frequently the case in young children with impaired ejection fraction and clinical signs of heart failure or children with syncope and sudden cardiac death, and corresponds with the current guidelines for the performance of EMB and previous analysis from the MYKKE registry (1, 28). The number of patients with proven myocarditis is quite high with 80% and supports the view that EMB are a useful diagnostic tool despite the potential risk of sampling error (1). Especially young children with an acute myocarditis seem to experience fulminant clinical courses as also reported in adults (29). Their clinical deterioration is similar to children with DCM, pointing to different underlying mechanisms, which may include genetic causes (30, 31). But also different immunological processes might play an important role in mediating the myocardial inflammation in these young children compared to the adolescents (32).

EMB performance was associated with a low rate of complications, further supporting the use of EMB in children with suspected myocarditis. Despite the young mean age of the patients, major complications occurred very rarely in comparison to the published data of Mueller et al. (17).

Impact of cell infiltrates and virus genome detection

In patients with confirmed myocarditis by histology and immunohistology, 99% had a lymphocytic myocarditis. In contrast to adults, no giant cell myocarditis could be detected leading to the assumption that this type of myocarditis is very rare in children (33). Only two children revealed eosinophilic myocarditis. Our results show that with expanding time between symptom onset and EMB the natural course of myocarditis elapses – from acute to chronic and healed myocarditis with less presence of mononuclear infiltrates and viral genome in chronic and healed myocarditis. Thus, moderately and severely elevated mononuclear cell infiltrates and higher rates of myocardial virus nucleic acids are seen in patients with acute myocarditis (34). The fact that in patients with low myocardial viral loads the probability of simultaneous detection of the virus in the blood decreases, reflects persistence/latency of virus genomes in the heart in most cases. Further, a virus-triggered

immune-mediated reaction that could lead to the cardiac injury in susceptible patients with a specific genetic background needs to be considered as a potential mechanism (35). However, the presence of virus genome at low levels in the myocardium alone had no further significant impact on the combined outcome in our cohort, as already published for adult patients (26). Interestingly, the rates of myocardial virus genome detection were higher in our paediatric cohort compared to adults, and included more frequently CMV and enteroviruses (36). As virus detection is more likely in the acute phase, EMB should be performed early if a specific virostatic therapy is considered, e.g. in CMV infection. As expected, in children with healing/chronic myocarditis, the degree of interstitial fibrosis was increased in EMB. In agreement with these findings, focal myocardial fibrosis and its surrogate late gadolinium enhancement on cardiac magnetic resonance imaging have been described as independent predictors of worse outcome in both DCM and myocarditis (37, 38). Myocardial fibrosis is believed to reflect chronification and remodelling in these patients due to ongoing inflammation. Similar results were found by the Pediatric Cardiomyopathy Registry (PCMR) but were not corroborated by detailed EMB analysis (39).

Prognostic value of EMB

Looking at the clinical presentation in children with acute myocarditis, our results are in line with those from Ammirati et al. where adult patients with a fulminant clinical course had a worse outcome as defined by a combined endpoint of HTx and/or death (8). The event-free survival between the different histological myocarditis groups did not differ significantly pointing towards a higher impact of clinical characteristics. An prognostic importance of EMB can be concluded by significantly high weaning rates from MCS in children with acute myocarditis in our cohort, underlining its importance as a bridge-to-recovery. Data in children on VAD also showed that myocarditis patients are more likely to be weaned from VAD compared to non-inflammatory cardiomyopathy patients (40), while the European Registry for Patients with Mechanical Circulatory Support (EUROMACS) does not show a significant difference in the survival of VAD patients with myocarditis as compared to those with non-inflammatory cardiomyopathy (41).

Therapeutic influence of EMB

The high need of heart transplantation in children with healing/chronic myocarditis raises the question about the therapy regime. Once myocardial fibrosis has developed, anti-fibrotic pharmacotherapy seems mandatory (42). As 16% of our cohort received a specific antiviral or immunosuppressive

therapy, the therapeutic influence of EMB in this cohort exists, even though the number is rather small. Anyhow, a therapeutic effect cannot be evaluated due to the small sample size. In the ones receiving immunosuppression, therapy was started after EMB performance. As many patients had only low levels of viral DNAwithin the myocardium reflecting virus persistence/latency, it is not expedient to withdraw immunosuppression. On the other hand, we cannot support an unrestrictive immunosuppressive therapy in children with and without myocardial viral infection. The scientific basis how often a clinically relevant virus reactivation occurs is not available. Thus, it remains unclear is whether immunosuppressive therapy with prednisolone and azathioprine is able to revert myocardial remodelling or even prevent its development if administered at early stages of myocarditis (18, 43). In other words, counter-intuitively, the primary target group for immunosuppression/immunomodulation might be cases with ongoing inflammation, but also those with acute inflammation and clinical deterioration as we have seen in children with acute myocarditis. For the latter, the blockage of the interleukin (IL)-1 mechanism seems a potential target for therapy. The IL-1 receptor antagonist Anakinra showed in several severe heart failure cases positive effects (44). The application of a monoclonal anti-IL-1ß antibody, homologous to Canakinumab (Novartis, Nürnberg, Germany) led in a mouse model of enteroviral myocarditis to a reduced cell infiltration, myocardial damage and fibrosis (45).

There is a lack of reliable data on the course of myocarditis following immunosuppression, especially in children. The studies on immunosuppression in children are based on small cohorts, and only some of them performed EMB (43, 46, 47). Thus, the question whether and when immunosuppression should be started to prevent patients from getting into a chronic stage is not answered yet (1). Importantly, only EMB can differentiate the type of myocarditis and subsequently could lead to a prognostic statement. Furthermore, myocardial virus genome detection enables further therapeutic interventions with virostatic therapies like ganciclovir/valganciclovir in CMV or HHV6 myocarditis, or interferon beta in enterovirus myocarditis (48). Assuming that a child with initial myocardial inflammation continues to show a markedly restricted function in spite of an optimal heart failure therapy, a repeated EMB should be considered, for example in a time window of 3 months. In children with persistent LVEF impairment and virus negative EMB an immunosuppressive or immunomodulatory therapy should be discussed.

Limitations

EMB was performed in a large number of patients enrolled to MYKKE, but not in all of them. Thus, there is most likely a selection bias towards performing EMB in the more severe cases in our cohort. Also, while the main inclusion criteria for MYKKE requires “suspected myocarditis”, there were some cases diagnosed with DCM on EMB (6%); however, the size of that subgroup does not allow for drawing conclusions on the value of EMB in these patients.

Conclusions

Paediatric patients with fulminant clinical presentation, signs of acute or healing/chronic inflammation on EMB, and young age, have the highest risk for adverse events like MCS, HTX, or death. The probability of weaning from MCS is high in acute myocarditis patients with more mononuclear infiltrates and higher rates of myocardial virus genome detection. However, the detection of myocardial virus genome alone had no significant influence on the rate of an event-free survival. Overall, in children with suspected myocarditis and impaired ejection fraction and/or a dilated left ventricle, EMB provides important information on the type and stage of myocardial inflammation and supports further therapeutic decision-making.

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Figure legends

Figure 1

Flow chart illustrating inclusion criteria and dropouts

Enrolled patients within the MYKKE-Registry with suspected myocarditis were filtered for endomyocardial biopsy (EMB). Only patients with EMB-proven myocarditis were included in further analyses.

Figure 2

Endomyocardial biopsies in children with myocarditis and dilated cardiomyopathy

Masson Trichrome staining (A-D), haematoxylin and eosin (E-H) and immunohistochemical staining of CD3+ T cells (I-L) are exemplarily shown in an 18-month-old female with acute myocarditis, a 19-month-old male with chronic myocarditis, an 8-month-old male died due to arrhythmias with healed myocarditis, and a 7-month-old female with dilative cardiomyopathy. Magnification all x400

Figure 3

Clinical presentation and outcome regarding and results of endomyocardial biopsies

Patients with acute myocarditis and high rates of mononuclear cell infiltrates were younger and presented more often with severe clinical courses and heart failure symptoms compared to healing/chronic and healed myocarditis. Overall, the myocarditis group with the worst event-free survival of the combined endpoint of MCS, HTX and death was the acute myocarditis cohort, but did not differ significantly from the other groups ($p=0.294$). Myocardial viral genome detection had no impact on the outcome either ($p=0.726$). The event-free survival of the combined endpoint of heart transplantation and death was worst in patients with fulminant clinical courses compared to Non-fulminant courses ($p<0.001$).

EMB = endomyocardial biopsies; HTX = heart transplantation; LVEDVi = indexed left ventricular enddiastolic volume; LVEF = left ventricular ejection fraction; LVIDd = left ventricular internal dimension at end-diastole; MCS = Mechanical circulatory support; NYHA = New York Heart Association.

Figure 1

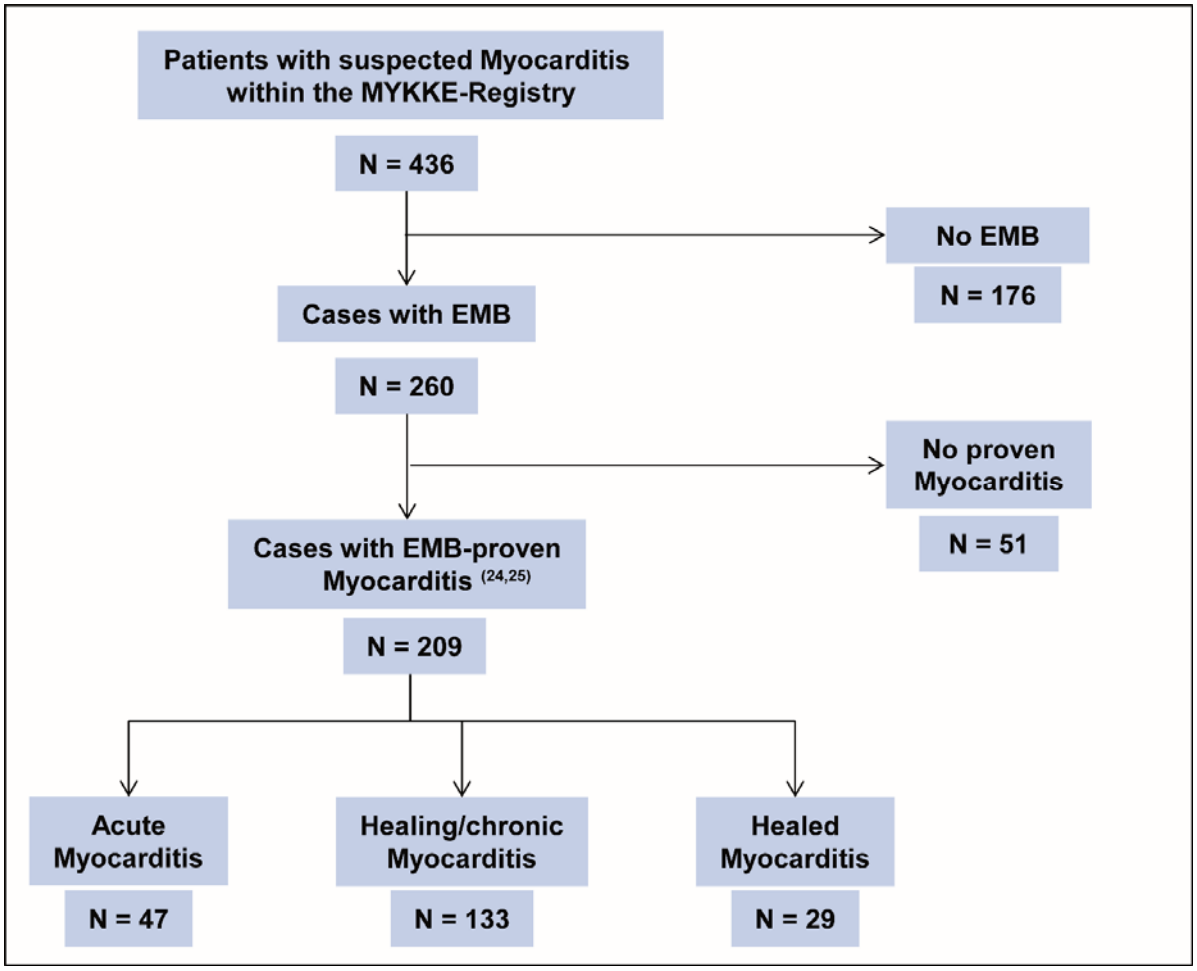

Patients with suspected Myocarditis within the MYKKE-Registry
N = 436
No EMB
N = 176
Cases with EMB
N = 260
No proven Myocarditis
N = 51
Cases with EMB-proven Myocarditis (24,25)
N = 209
Acute Myocarditis
N = 47
Healing/chronic Myocarditis
N = 133
Healed Myocarditis
N = 29

Figure 2

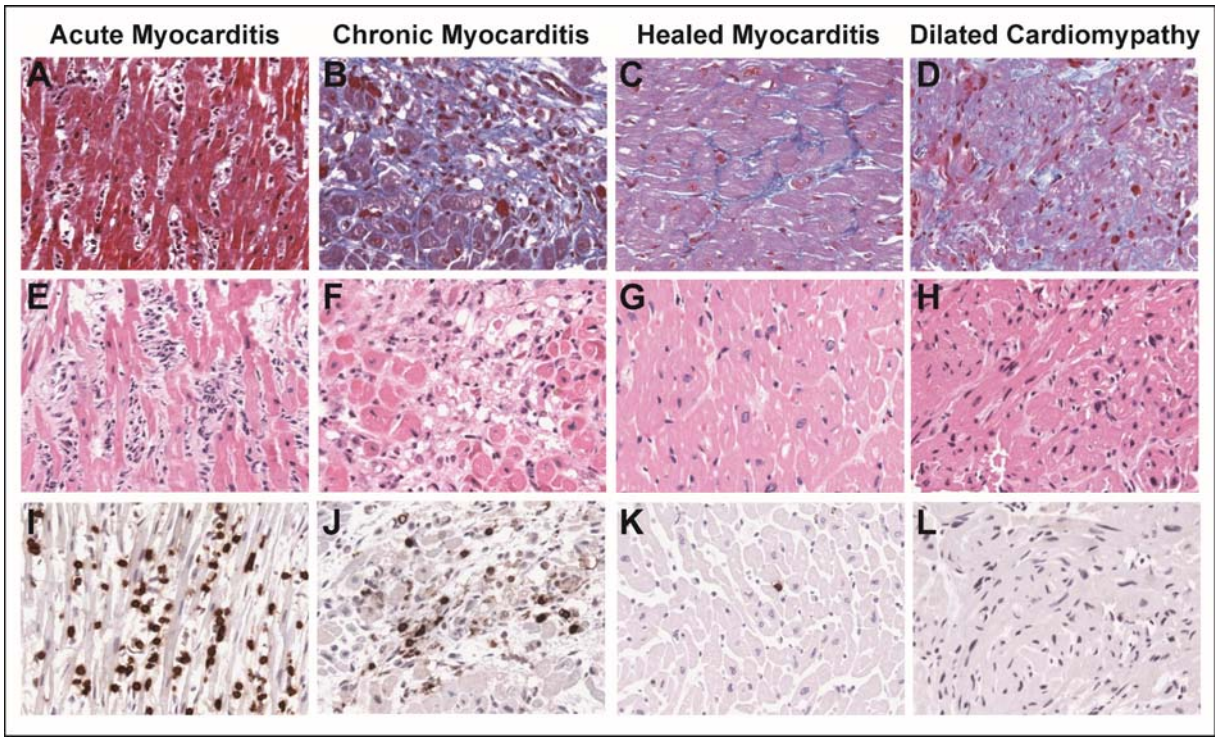
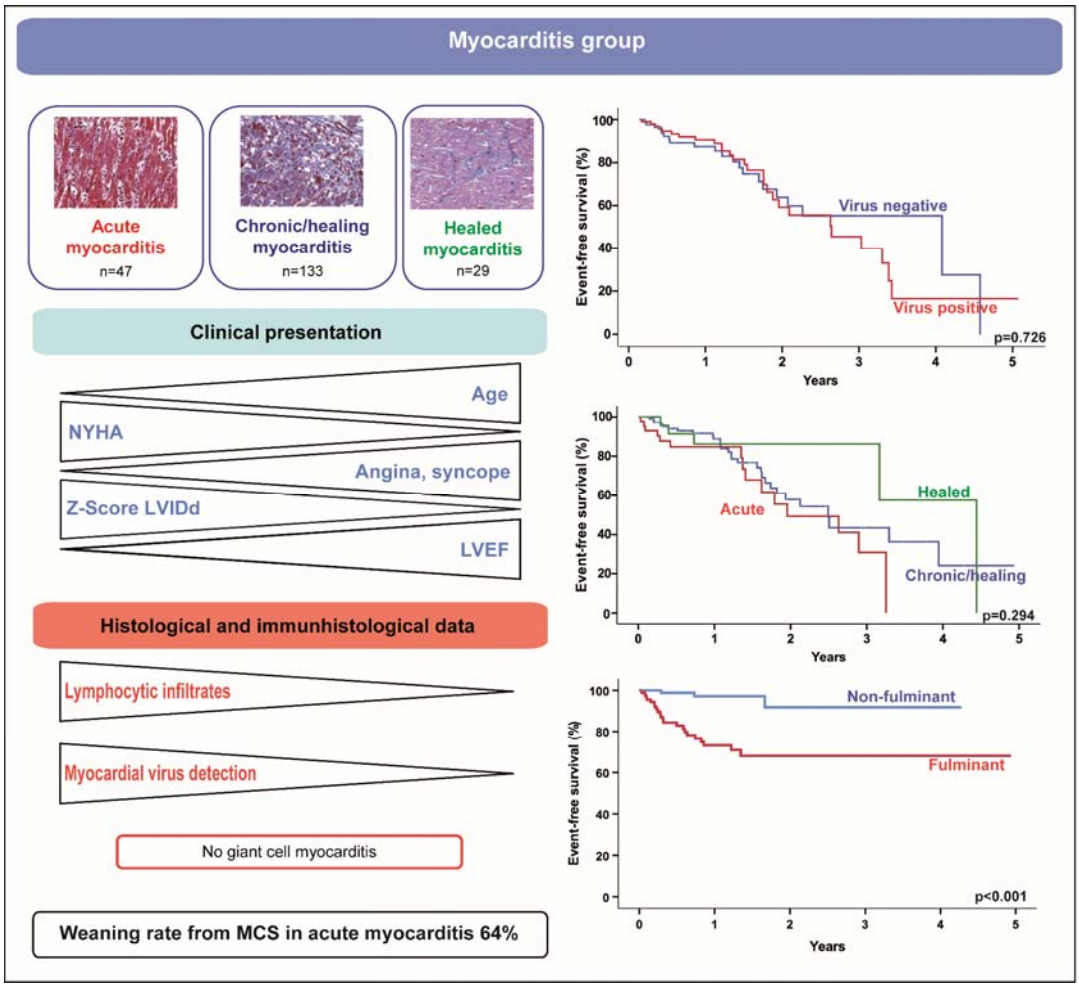

Acute Myocarditis
Chronic Myocarditis
Healed Myocarditis
Dilated Cardiomypathy
A
B
C
D
E
F
G
H
I
J
K
L

Figure 3


Myocarditis group
Acute myocarditis
n=47
Chronic/healing myocarditis
n=133
Healed myocarditis
n=29
Clinical presentation
Age
NYHA
Angina, syncope
Z-Score LVIDd
LVEF
Histological and immunhistological data
Lymphocytic infiltrates
Myocardial virus detection
No giant cell myocarditis
Weaning rate from MCS in acute myocarditis 64%
Event-free survival (%)
Virus negative
Virus positive
p=0.726
Years
Acute
Healed
Chronic/healing
p=0.294
Non-fulminant
Fulminant
p<0.001

Tables

Table 1

Table 1 Clinical characteristic according to diagnoses in endomyocardial biopsies of the myocarditis group (n=209)

	Acute myocarditis N=47	Healing/chronic myocarditis N=133	Healed myocarditis N=29	P-Value
Gender				
Male	28 (60)	79 (57)	21 (72)	0.315
Age (years)	2.1 (1.1-13.7)	13.2 (1.4-15.9)	15.2 (12.7-16.4)	**0.001**
Time to EMB (d)	3.0 (1.0-5.3)	3.0 (1.0-9.0)	6. (1.5-38.5)	0.114
Time symptom onset to EMB (d)	7.0 (4.0-18.0)	12.0 (4.0-28.0)	22.0 (5.0-79.0)	0.088
Symptoms				
NYHA				
I	11 (23)	54 (41)	20 (69)	
II	8 (17)	23 (17)	3 (10)	
III	6 (13)	17 (13)	4 (14)	**0.010**
IV	18 (38)	30 (23)	2 (7)	
n.a.	4 (9)	9 (7)	0 (0)	
Angina pectoris	10 (21)	47 (35)	16 (55)	**0.026**
Dyspnoea	29 (62)	65 (49)	8 (28)	**0.015**

Fatigue	41 (87)	102 (77)	21 (72)	0.221
Syncope	5 (11)	14 (11)	8 (28)	**0.040**
Sudden cardiac death	1 (2)	4 (3)	3 (10)	**0.010**
Feeding intolerance	18 (38)	29 (22)	2 (7)	**0.005**
Gastrointestinal symptoms	9 (19)	12 (9)	3 (10)	0.170
Decompensation	27 (57)	41 (31)	5 (17)	**<0.001**
Infection (<6 weeks)	31 (66)	70 (53)	11 (38)	0.055
Fever (<6 weeks)	20 (43)	52 (39)	7 (24)	0.241
ECG				
ST-elevation	18 (38)	41 (32)	13 (46)	0.335
T-inversion	22 (47)	50 (39)	10 (36)	0.575
Arrhythmias*	18 (38)	53 (40)	12 (41)	0.964
Laboratory				
Haemoglobin (g/dl)	10.7 (9.5-14.0)	12.9 (11.4-14.6)	13.8 (13.1-14.9)	**0.001**
Leucocytes (Tsd/µl)	11.8 (9.4-15.6)	10.1 (7.2-13.4)	8.9 (7.7-11.3)	**0.005**
Thrombocytes (Tsd/µl)	297 (234-371)	258 (216-321)	240 (201-366)	0.202
CRP (mg/l)	4.7 (2.0-18.3)	4.8 (0.8-28.9)	11.7 (0.6-58.3)	0.693
NT-proBNP (pg/ml)	14877 (3229-35001) N=31	2801 (364-26792) N=81	267 (138-1465) N=18	**<0.001**
Troponin elevated	38 (86)	95 (78)	16 (57)	**0.015**

Echocardiography				
Z-score LVIDd (mm)	3.7 (1.6-6.7)	2.3 (0.3-6.1)	05. (-0.6-2.1)	**0.002**
LVEF (%)	32.0 (21.0-51.5)	48.0 (26.0-59.3)	54.9 (42.3-63.8)	**0.013**
CMR	N=22	N=86	N=19	
LVEDVi (ml/m²)	103.5 (78.8-135.6)	91.5 (76.3-113.8)	79.0 (67.5-89.7)	0.069
LVEF (%)	39.0 (26.5-60.0)	54.0 (36.8-61.0)	60.5 (53.5-66.5)	**0.010**
Oedema	10 (45)	34 (40)	6 (32)	0.627
LGE positive	15 (68)	49 (57)	11 (58)	0.658
Lake Louise criteria fulfilled[50]	16 (73)	51 (59)	12 (63)	0.336
Medical treatment				
Heart failure medication	44 (94)	106 (80)	15 (52)	**<0.001**
Inotropic medication	30 (64)	56 (42)	5 (17)	**<0.001**
Immunoglobulin	31 (66)	52 (40)	8 (28)	**0.001**
Valganciclovir/Ganciclovir	5 (11)	12 (9)	1 (3)	0.625
Azathioprine/Prednisolone	2 (4)	13 (10)	0 (0)	0.426
Devices				
ICD	1 (2)	7 (5)	4 (14)	0.097
Pacemaker	1 (2)	3 (2)	2 (7)	0.375
MCS overall	14 (30)	26 (20)	2 (7)	0.053

VAD	10 (21)	26 (20)	1 (3)	0.075
ECMO	10 (21)	9 (7)	2 (7)	**0.020**
Weaned overall	9 (64)	6 (23)	2 (100)	**0.006**
Complications				
Resuscitation	14 (31)	17 (13)	3 (10)	**0.012**
HTx	0 (0)	13 (10)	1 (3)	**0.035**
Death	4 (9)	8 (6)	2 (7)	0.782

Values are given as n (%) or median (interquartile range). *Arrhythmias were recorded with ECG and/or Holter-ECG and contained AV block II/III, relevant bradycardia, SVT, nsVT, VT.

CMR = cardiovascular magnetic resonance; CRP = C-reactive protein; ECG = Electrocardiogram; ECMO = extracorporal membrane oxygenation; HTx = heart transplantation; ICD = implantable cardioverter-defibrillator; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular enddiastolic volume; LVEF = left ventricular ejection fraction; LVIDd = left ventricular internal dimension at end-diastole; MCS = Mechanical circulatory support; nsVT = non-sustained ventricular tachycardia; n.a. = not applicable; NT-proBNP = N-terminal pro brain natriuretic peptide; NYHA = New York Heart Association; SVT = supraventricular tachycardia; VAD = ventricular assist device; VT = ventricular tachycardia.

Supplemental Material

Supplemental Methods

- CD3+ T lymphocytes presentation was graded as

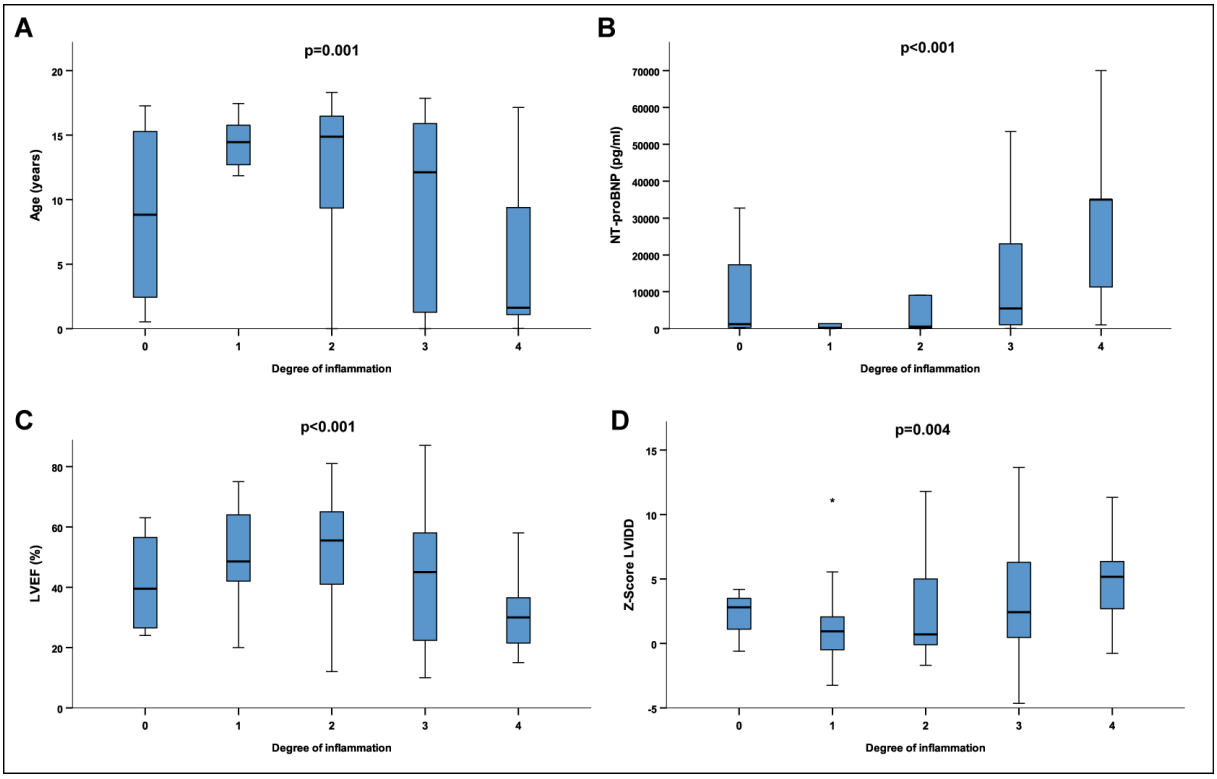
0: no inflammation, 1: single inflammatory cells (≤ 2.5 cells/mm²) (45), 2: a few foci of inflammation, 3: several foci of inflammation, 4: pronounced inflammation.

- CD68+ macrophages presentation was graded as

0: no inflammation, 1: single inflammatory cells (≤ 4.0 cells/mm²) (45), 2: a few foci of inflammation, 3: several foci of inflammation, 4: pronounced inflammation.

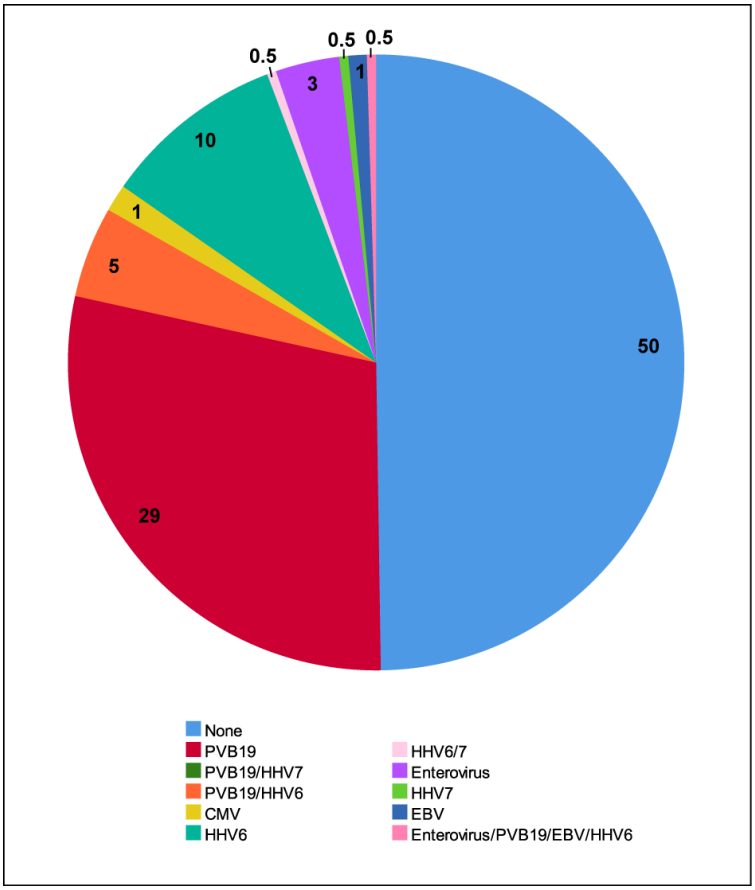
Supplemental Figures

Suppl. Figure S1 Distribution of clinical parameter concerning inflammatory infiltrates in endomyocardial biopsies in the myocarditis group



Patients with young age **(A)**, higher N-terminal pro brain natriuretic peptide (NT-proBNP) levels **(B)**, lower left ventricular ejection fraction (LVEF) **(C**) and dilated left ventricles with higher Z-Score of the left ventricular internal dimension at end-diastole (LVIDd) **(D)** showed higher grades of mononuclear cell infiltrates within endomyocardial biopsies. Inflammatory infiltrates were graded as **0:** no inflammation, **1:** single inflammatory cells (CD3+ T-lymphocytes and CD68+ macrophages ≥14/mm2); **2:** a few foci of inflammation, 3**:** several foci of inflammation, **4:** pronounced inflammation.

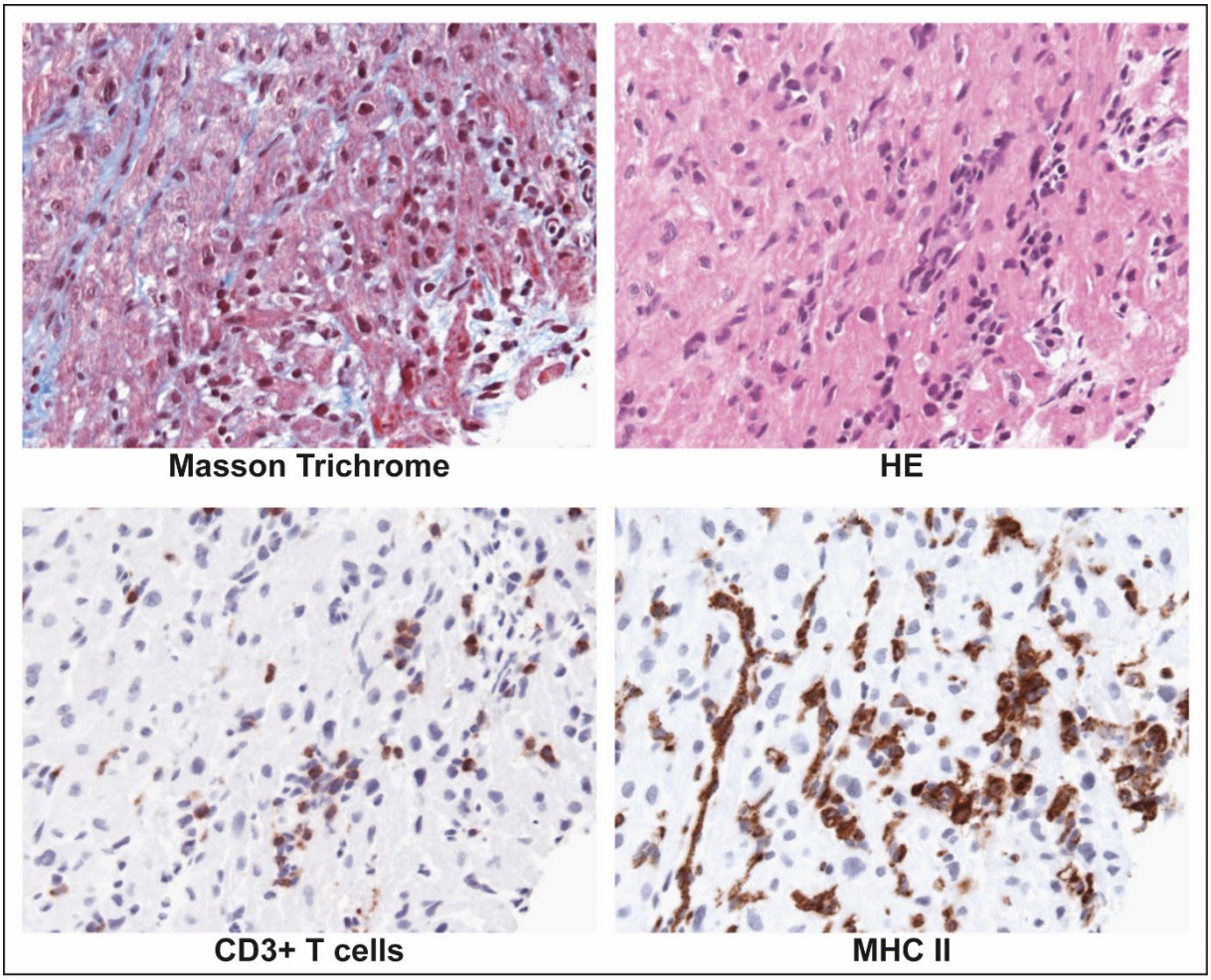
Suppl. Figure S2. Myocardial virus DNA/RNA detection within endomyocardial biopsies of the myocarditis group



In 50% (n=105) of the myocarditis group virus DNA/RNA could be detected by PCR within the endomyocardial biopsies (EMB), mostly PVB19 DNA was found with 57%.

CMV = human cytomegalovirus; DNA = deoxyribonucleic acid; EBV = Epstein-Barr virus, HHV6/7 = human herpesvirus 6/7; PCR = polymerase chain reaction; PVB19 = parvovirus B19, RNA = ribonucleic acid.

Suppl. Figure S3. Endomyocardial biopsy of a 3-month-old boy with acute lymphocytic cytomegalovirus myocarditis



Different routine stainings (Masson Trichrome, HE) and immunhistochemical stainings (CD3+ T cells, MHC II) in endomyocardial biopsies of a 3-month-old male patient with acute lymphocytic cytomegalovirus myocarditis. Magnification x400. HE: haematoxylin and eosin; MHC = major histocompatibility complex.

Supplementary Tables

Table S1

Table S1 Clinical characteristics regarding fulminant (FM) and non-fulminant (NFM) clinical courses of the EMB cohort (n=209)

	FM N=92	NFM N=117	P-Value
Gender			
Male	41 (45)	84 (72)	**<0.001**
Age (years)	1.5 (0.6-12.3)	15.2 (12.0-16.4)	**<0.001**
Time to EMB (d)	3.0 (1.0-14.3)	3.0 (1.0-7.0)	0.142
Time symptom onset to EMB (d)	15.0 (5.0-28.3)	8.0 (3.0-31.8)	0.126
Symptoms			
NYHA			
I	7 (8)	78 (67)	
II	8 (9)	26 (22)	
III	20 (22)	7 (6)	**<0.001**
IV	45 (49)	5 (1	
n.a.	12 (13)	1 (1)	
Angina pectoris	5 (5)	68 (58)	**<0.001**
Dyspnoea	65 (71)	37 (32)	**<0.001**
Fatigue	85 (92)	79 (68)	**<0.001**
Syncope	8 (9)	19 (16)	0.107
Sudden cardiac death	6 (7)	2 (2)	0.142
Feeding intolerance	42 (46)	7 (6)	**<0.001**
Gastrointestinal symptoms	15 (16)	9 (8)	0.053
Decompensation	67 (73)	6 (5)	**<0.001**
Infection (<6 weeks)	50 (54)	62 (53)	0.845
Fever (<6 weeks)	32 (35)	47 (40)	0.425
ECG			
ST-elevation	13 (15)	59 (52)	**<0.001**
T-inversion	44 (50)	38 (33)	**0.017**

Arrhythmias*	42 (46)	41 (35)	0.120
Laboratory			
Haemoglobin (g/dl)	11.4 (9.6-13.9)	13.8 (12.1-14.6)	**<0.001**
Leucocytes (Tsd/µl)	11.8 (8.7-15.0)	9.5 (7.0-11.6)	**<0.001**
Thrombocytes (Tsd/µl)	297 (221-370)	246 (208-296)	**0.016**
CRP (mg/l)	3.0 (0.9-8.8)	10.2 (0.9-62.0)	**0.005**
NT-proBNP (pg/ml)	26334 (6672-35001) N=59	487 (151-3229) N=71	**<0.001**
Troponin elevated	66 (79)	83 (76)	0.610
Echocardiography			
Z-score LVIDd (mm)	5.7 (3.3-8.0)	0.8 (-0.1-2.3)	**<0.001**
LVEF (%)	25.0 (20.0-35.0)	57.0 (48.0-63.0)	**<0.001**
CMR	N=35	N=86	
LVEDVi (ml/m²)	124.0 (80.0-170.8)	87.0 (75.5-97.0)	**<0.001**
LVEF (%)	25.0 (16.0-46.0)	58.0 (51.0-64.0)	**<0.001**
Oedema	15 (48)	35 (44)	0.699
LGE positive	16 (47)	59 (66)	0.051
Medical treatment			
Heart failure medication	89 (97)	76 (65)	**<0.001**
Inotropic medication	92 (100)	0 (0)	**<0.001**
Immunoglobulin	58 (63)	31 (27)	**<0.001**
Valganciclovir/Ganciclovir	16 (17)	2 (2)	**<0.001**
Azathioprine/Prednisolone	9 (10)	2 (2)	**0.012**
Devices			
ICD	7 (8)	5 (4)	0.304
Pacemaker	3 (3)	3 (3)	1.000
MCS overall	42 (6)	0 (0)	**<0.001**
VAD	37 (40)	0 (0)	**<0.001**
ECMO	21 (23)	0 (0)	**<0.001**
Weaned overall	17 (19)	0 (0)	**<0.001**
Complications			
Resuscitation	32 (36)	2 (2)	**<0.001**
HTx	14 (15)	0 (0)	**<0.001**
Death	11 (12)	3 (3)	**0.007**

Values are given as n (%) or median (interquartile range). *Arrhythmias were recorded with ECG and/or Holter-ECG and contained AV block II/III, relevant bradycardia, SVT, nsVT, VT.

CMR = cardiovascular magnetic resonance; CRP = C-reactive protein; ECG = Electrocardiogram; ECMO = extracorporal membrane oxygenation; EMB = Endomyocardial biopsy; HTx = heart transplantation; ICD = implantable cardioverter-defibrillator; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular enddiastolic volume; LVEF = left ventricular ejection fraction; LVIDd = left ventricular internal dimension at end-diastole; MCS = Mechanical circulatory support; nsVT = non-sustained ventricular tachycardia; n.a. = not applicable; NT-proBNP = N-terminal pro brain natriuretic peptide; NYHA = New York Heart Association; SVT = supraventricular tachycardia; VAD = ventricular assist device; VT = ventricular tachycardia.

Table S2

Table S2 Histological and immunohistological results of endomyocardial biopsies of the myocarditis group (n=209)

	Acute myocarditis N=47	**Healing/chronic myocarditis N=133**	**Healed myocarditis N=29**	**P-Value**
Mononuclear infiltrates				
None	0 (0.0)	2 (1.5)	2 (6.9)	
Normal	0 (0.0)	3 (2.3)	13 (44.8)	
Mild	3 (6.4)	38 (28.6)	11 (37.9)	**<0.001**
Moderate	14 (29.8)	69 (51.9)	1 (3.5)	
Severe	23 (48.9)	10 (7.5)	0 (0.0)	
CD3+ T lymphocytes				
None	0 (0.0)	2 (1.5)	4 (13.8)	
Normal	2 (4.3)	32 (24.1)	16 (55.2)	**<0.001**
Mild	4 (8.5)	38 (28.6)	3 (10.3)	
Moderate	12 (25.5)	26 (19.6)	0 (0.0)	

Severe	17 (36.2)	7 (5.3)	0 (0.0)	
CD68+ macrophages				
None	0 (0.0)	2 (1.5)	1 (3.5)	
Normal	1 (2.1)	3 (2.3)	10 (34.5)	
Mild	3 (6.4)	29 (21.8)	11 (37.9)	**<0.001**
Moderate	11 (23.4)	63 (47.4)	1 (3.5)	
Severe	18 (38.3)	10 (7.5)	0 (0.0)	
Interstitial fibrosis				
None	13 (27.7)	10 (7.5)	5 (17.2)	
Mild	11 (23.4)	44 (33.1)	14 (48.3)	
Moderate	15 (31.9)	60 (45.1)	10 (34.5)	**0.011**
Severe	2 (4.3)	4 (3.0)	0 (0.0)	
Virus DNA/RNA	32 (68.1)	62 (46.6)	11 (37.9)	**0.014**

Values are given as n (%) or median and interquartile range. DCM = dilated cardiomyopathy; DNA = deoxyribonucleic acid; EMB = endomyocardial biopsy; RNA = ribonucleic acid

Table S3

Table S3 Virus detection within myocardium and blood

	Detected myocardial Viruses N=105	**Virus simultaneously detected in EDTA blood N=30**
PVB19	60 (57)	18 (30)
<500 copies/µg DNA	*20 (33)*	*1 (5)*
≥500-2000 copies/µg DNA	*18 (30)*	*5 (28)*
≥2000 copies/µg DNA	*21 (35)*	*12 (57)*
HHV6	20 (19)	0 (0)
PVB19/HHV6	10 (10)	3 (30)
Enterovirus	7 (7)	5 (71)
CMV	3 (3)	2 (67)
EBV	2 (2)	0 (0)
HHV6/7	1 (1)	1 (100)
HHV7	1 (1)	0 (0)
Enterovirus/PVB19/EBV/HHV6	1 (1)	1 (100)

Values are given as n (%). CMV = human cytomegalovirus; DNA = deoxyribonucleic acid; EBV = Epstein-Barr virus, HHV6/7 = human herpesvirus 6/7; PVB19 = parvovirus B19.

Table S4

Table S4 Myocardial PVB19 DNA load according to diagnoses in endomyocardial biopsies

Copies/µg DNA	Acute myocarditis N=22	Healing/chronic myocarditis N=40	Healed myocarditis N=8	P-Value
< 500	2 (9.1)	17 (42.5)	5 (62.5)	
≥ 500-2000	3 (13.6)	14 (35.0)	3 (37.5)	**<0.001**
≥ 2000	17 (77.3)	9 (22.5)	0 (0.0)	

Values are given as n (%). DCM = dilated cardiomyopathy; DNA = deoxyribonucleic acid; PVB19 = Parvovirus B19.