Children with Dilated Cardiomyopathy

Towards predicting outcome and optimizing treatment

Marijke van der Meulen

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´The hills and the sea and the earth dance. The world of man dances in laughter and tears.´
Kabir

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Children with Dilated Cardiomyopathy

Towards predicting outcome and optimizing treatment

Kinderen met gedilateerde cardiomyopathie
Op weg naar het voorspellen van uitkomst en het optimaliseren van behandeling

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Above all else, watch over your heart, for everything you do flows from it

Proverbs 4:23

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General Introduction

1

Dilated Cardiomyopathy (DCM) is characterized by depressed myocardial function and dilation of the left ventricle. The incidence rate varies between 0.58 and 0.73 per 100,000 children, and is more likely to be diagnosed below 1 year of age than at older pediatric ages (1, 2). At diagnosis, up to 80% of children display signs and symptoms of heart failure ranging from poor feeding and growth failure, to overt failure of the circulation (1, 3, 4). A substantial number of children suffer from progressive disease and develop end-stage heart failure. DCM is the leading indication for heart transplantation and up to 50% of children die or undergo heart transplantation within 5 years after diagnosis (3, 5). On the other hand, a recent study on the long-term outcome of children with DCM, reported recovery in 33% of patients more than 10 years after diagnosis (6).

Management of dilated cardiomyopathy in children

As disease severity and outcome vary widely, the starting point for optimizing management of children with DCM is adequate risk prediction. This proves to be a great clinical challenge. Prerequisite for risk prediction is better understanding of **disease etiology** and closely linked to that, understanding pathophysiology. Also, in-depth study of the clinical course and disease parameters from diagnosis and onward is essential to obtain useful **risk factors for adverse outcome**. This has been done in adult heart failure, and also in pediatric DCM (3, 6-9). Recognition of the children at the highest risk of adverse outcome is critical: these are the children who should be monitored closely and, if medical treatment fails, should be listed for heart transplantation at a timely stage, and if needed, bridging to transplantation with mechanical support of the circulation is a viable option too. Optimizing current medical **treatment** in terms of heart failure medication and the care for patients with end stage heart failure is needed as well. Here, we will concisely introduce these three aspects of management of pediatric DCM.

The importance of understanding disease etiology

Children with DCM share a common clinical phenotype, but the underlying diagnoses varies widely. The Pediatric Cardiomyopathy Registry (PCMR) reported outcomes of a total of 1426 children with DCM in 6 diagnostic subgroups: idiopathic DCM (iDCM) (66%), myocarditis (16%), neuromuscular disorder (9%), familial DCM (5%), inborn error of metabolism (4%) and malformation syndrome (1%). Transplant free survival at 5 years after diagnosis was 47% in patients with idiopathic DCM opposed to 73% in children diagnosed with myocarditis (3). Data from the PCMR also revealed that at 3 years after presentation, 52% of children with myocarditis related DCM had achieved echocardiographic normalization, whereas only 21% of children with iDCM showed normalization (10). These studies clearly demonstrate that etiology is closely related to outcome and emphasize the importance of establishing etiology. Furthermore, when disease etiology is known, treatment and counseling can be tailored to the individual patient and family.

Genetics

Since the early 1990s, inherited gene variants have been implicated in the etiology of DCM. Recent advances in sequencing and array-based technologies have improved our understanding of the genetic basis of DCM. Genes encoding transcription factors, cytoskeletal, ion transport, nuclear membrane and mitochondrial proteins are involved in isolated DCM, while more than 200 genes are involved in syndromes or inborn errors of metabolism of which DCM can be part of the phenotype (11, 12). As in adult-onset cardiomyopathy, genetic testing in the pediatric population has now been integrated into daily clinical practice. These days, in up to 54% percent of pediatric DCM diagnoses a genetic cause can be established (13). Children, especially those under 2 years of age, display a different gene profile compared to adult-onset DCM. *MYH7*, *VCL* and *TPM1* are the most frequently affected genes in children < 2 years of age(13-15). *TTN, BM20* and *TNNT2* are the most frequently mutated genes in the age group of 2-18 years of age (16).

The importance of genetics in the management of pediatric DCM is evident. First of all, knowledge of the genetic basis of this disease gives insight into pathophysiology and can provide leads to develop targeted therapy. Second, as has recently been shown in pediatric hypertrophic cardiomyopathy, genotype-phenotype associations can be used as a tool in risk prediction for adverse outcome (17). Third, when a (likely) pathogenic variant is found, possible outcomes can be discussed with patients and families can be counseled. Conversely, in familial DCM, a negative genetic test could exempt children from life-long follow up.

Cellular patho-mechanisms of DCM in children

Medical treatment of children with DCM has unfortunately not led to substantial improvement in morbidity and mortality over the last two decades (18). This is in sharp contrast with adult DCM, where protocolized medical treatment undoubtedly has improved outcome (19, 20). Pediatric DCM might therefore be essentially different than adult DCM, and there is an urgent need for new approaches to better understand the disease process in children. Recently, multiple studies have demonstrated important differences in the molecular characteristics of pediatric and adult DCM hearts (21-25). It is hypothesized that myocardial cellular mechanisms are uniquely regulated in children with DCM. For instance, Tatman et al studied explanted hearts of children with DCM and found unique changes in gene expression that suggest maintenance of an undifferentiated state of cardiomyocytes (26). A distinctive profile in the pathophysiology of pediatric DCM is plausible and strongly motivates research in this specific area. We expect that this would provide new targets for medical treatment and also for future research on mechanisms involved in the pediatric failing heart.

How to predict outcome – risk factors for adverse outcome

When trying to identify children with DCM at the highest risk for adverse outcome, what do we look for? In general, we aim to construct a prediction tool that evaluates the severity of heart failure at diagnosis as well as the changes over time, and that couples this information with survival or heart transplantation probabilities. Such a prediction

tool would combine medical experience with a fitting statistical model and would enable physicians to make better informed decisions and thus improve outcome. In the CARS study we prospectively collected data that would enable the evaluation of severity of heart failure and to connect it to the hard endpoints of death and heart transplantation.

Risk factors for adverse outcome

Previous research has identified several risk factors for adverse outcome in children with DCM. The majority of registry based studies, like the large PCMR and the National Australian Childhood Cardiomyopathy Study (NACCS) focused on risk factors that are present at the time of diagnosis. Risk factors that were repeatedly found are older age (>6 years), congestive heart failure, severity of left ventricle dysfunction, and idiopathic and familial cardiomyopathy (3, 6, 8, 27). In addition to the risk profile present at diagnosis, the evolution over time of these same risk factors may hold prognostic information as well. As recently shown by den Boer et al, the change over time of NT-proBNP level in children with DCM was predictive for the risk of death, heart transplantation and mechanical support of the circulation (9). This concept of prognostic information in temporal evolution of risk factors proved to be useful and thus the choice of which risk factors to study builds on our previous work in CArdiomyopathy Registry Study (CARS) (9, 28-30). We included the following 7 risk factors in the analyses based on proved predictive value in both adult and pediatric heart failure.

1. **NT-proBNP**. This peptide is an inactive form of the cardiac hormone BNP, which is secreted from the myocardium into the circulation, as a response to cardiomyocyte stretch. Numerous clinical studies have demonstrated that the level of this peptide correlates well with severity of heart failure. In both adults and children, it has also been shown an independent predictor of mortality (31-34).

2. **The New York University Pediatric Heart Failure Index (NYU PHFI)**. This heart failure score is validated especially for the pediatric population (35). The well-known NYHA classification is not applicable as children display different signs and symptoms of heart failure compared to adults. Den Boer et al have shown that the NYU PHFI at diagnosis and more than 1 year after diagnosis was independently predictive for adverse outcome in children with DCM (30).

3. **Length Z-score**. Length, normalized to Z- scores, is regarded a solid proxy for overall health in children and most likely provides a long term reflection of disease severity. However, length Z-score and its evolution over time has not extensively been studied in children with DCM. In a PCMR study on risk factors for death and heart transplantation Alvarez et al showed that height-for-age at diagnosis was associated with death and stated that using length Z-score as transplantation indication might substantially improve survival (5).

4. **LVIDd.** A commonly used proxy for severity of heart failure is the end-diastolic dimension of the left ventricle (LVIDd) as measured by 2D-echocardiography. The LVIDd is easy to obtain and can be normalized to a Boston Z-score, which is based on body surface area, age and gender (36). Left ventricular diameter has been associated with

outcome in adults and children with heart failure (37, 38). In children with DCM, LVIDd had been associated with outcome as well (3, 5, 39).

5. **Global Peak Strain.** Speckle tracking echocardiography (STE) has been shown to be a reliable measure of regional and global LV systolic function. Longitudinal, circumferential and radial movements can be evaluated and subsequently quantified. Global peak strain (GPS) can be calculated as the mean of the peak strain of all left ventricular segments in a longitudinal 6-segment model, in a standardized manner (40). GPS has been associated with the risk of death and heart transplantation in both children and adults with heart failure (41-44). In pediatric DCM, STE is increasingly used to evaluate left ventricular function (45, 46). Den Boer et al recently demonstrated that in children with DCM, left ventricular GPS as measured during follow-up was predictive of death and heart transplantation (28).

6. **6MWD%.** The 6-minute walk test (6MWT) is a safe, simple and well-accepted prognostic tool in adults with heart failure(47). In children, the 6MWT is also feasible and has been shown to be predictive for outcome in patients with pulmonary hypertension (48, 49). The distance walked in a 6MWT can be expressed as a percentage of predicted, taken into account height, gender and age (6MWD%) (50). In a previous study, den Boer et al showed that in children with DCM, a single 6MWD% below 63% identified patients with the highest risk of dying or heart transplantation (29).

7. **Child Behavior Checklist.** Compelling evidence from two meta-analyses shows that adults with heart failure are at increased risk of anxiety and depression (51, 52). It is also well-established that depressive and anxiety symptoms in adults with heart failure predict mortality (53-55). Children with DCM have an impaired health related quality of life (HRQoL) and children's physical HRQoL (reported by parents) predicts mortality and cardiac transplantation, independent from heart failure severity (30). However, to the best of our knowledge, the predictive value of depressive and anxiety symptoms in children with DCM has not been studied previously.

Optimizing treatment of children with DCM

Pharmacotherapy

In general, pharmacotherapy of children with DCM mirrors adult DCM. Angiotensin-converting enzyme inhibitors (ACEi) and beta-adrenergic receptor blockers (B-blockers) clearly have improved mortality and morbidity in adults (19, 20). In children however, it is far less evident that these drugs improve prognosis. Up until now, only one randomized clinical trial on the effect of heart failure drugs in children with DCM has been performed. This trial, published in 2007, assessed the effect the B-blocker carvedilol on heart failure outcomes in children with symptomatic systolic heart failure and could not demonstrate that carvedilol improved heart failure outcome (56). Also, the shift from digoxin based medical therapy to ACEi and B-blockers in the late 1980s and 1990s, does not seem to have resulted in sustained improvement of transplant free survival (18).

Although its efficacy has not been proven in the pediatric population yet, ACEi and B-blockers are commonly prescribed in children with DCM, based on the assumption that pathophysiological mechanisms are similar to those in adults (57).

What the safety issues and adequate dosing strategy of these drugs in children with DCM would be is not fully known (58, 59). Children are not small adults, also with regard to medical therapy. Growth and development pay an important contribution to variation in the disposition and effect of most drugs in children and must therefore be taken into account (60).

To address the issue of heart failure drugs in children with DCM, we joined a larger research project to develop an age-appropriate pediatric enalapril formulation (EU FP7 LENA project). As a first start, we performed a systematic review of the literature on the safety of ACEi in children with heart failure. Knowledge on ACEi related adverse events and possible risk factors can support the design of clinical trials with ACEi, but can also be used to improve safety in clinical practice.

Mechanical support of the circulation

For children with medically incurable end-stage heart failure, heart transplantation is the only long term therapeutic option. As donor availability is limited, children face a prolonged time on the waiting list. North American studies report a waiting list mortality of 25%, which is the highest in transplantation medicine (61). In order to improve survival in patients with end-stage heart failure who fail medical therapy, much effort has been put into the development of mechanical support of the circulation. Extracorporeal membrane oxygenation (ECMO) has successfully been used to sustain the circulation for several days to weeks, but has serious limitations in providing long-term support (62). Ventricular assist devices (VADs) are nowadays a well-accepted, long-term therapeutic option in adult end-stage heart failure (63). Implementation of VADs in the pediatric population has been troublesome due to technical problems and difficulties in medical management. However, in recent years, several centers have presented their experience with Berlin Heart EXCOR VAD as long term support. They have shown that Berlin Heart EXCOR VAD is a reliable and relatively safe device in bridging children to heart transplantation or recovery (64-66). Since 1998, our institute serves as a referral hospital in the Netherlands for end-stage heart failure and heart transplantation in children. The Berlin Heart Excor Pediatric Ventricular Assist Device has become available in our hospital since 2007. We aimed to describe the outcome of children supported with a VAD in our center in terms of mortality and complications. Secondly, we aimed to determine the effect of the introduction of the VAD on waiting list mortality.

Heart transplantation

Since the first heart transplantation in 1967, the field of transplantation medicine has evolved enormously. Today, heart transplantation is a widely accepted treatment for adults with end-stage heart failure without alternative therapeutic option (67). In children, heart transplantation is a well-accepted therapy as well, and the number of transplantations yearly reported to the database of the International Society for Heart and

Lung Transplantation (ISHLT) gradually increased from around 400 in the 90s to around 600 in the last 5-10 years (67).

In 1998, the first child underwent heart transplantation in our center. When critically appraising the outcome of our program, a number of factors that determine outcome on the waiting list and after heart transplantation have to be taken into account. Firstly, who do we list: the underlying diagnosis leading to end-stage heart failure is shown to be related to outcome. Children who undergo heart transplantation for congenital heart disease have a less positive outlook than children with DCM (68, 69). Also, large volume centers have a better post heart transplantation outcome (67, 70-72). We therefore needed to evaluate the case mix and the number of yearly heart transplantation in our center. Secondly, when do we list: pre-transplantation condition and support, as well as age at transplantation affects outcome as well (73). Previously, we reported a low transplantation rate in children with DCM in the first year after diagnosis (3%), as compared to the large PCMR registry which reported an 18% transplantation rate in the 1st year after diagnosis. Similar data were shown by the Australian NACCS (3, 6). Also, median time to listing was considerably longer in our cohort, 18 months versus 1.4 months. We did not find differences in clinical characteristics of our cohort as compared to other (large) registries and importantly, we did not find an increased mortality (74). This low early transplantation rate might reflect a policy that defers listing patients for heart transplantation as long as possible, and that pursues stabilizing patients on oral heart failure therapy before listing. The question is whether this strategy leads to selection of a group of children for heart transplantation with an unfavorable risk profile that affects outcome on the waiting list as well as outcome after heart transplantation. Taken all these factors into account, we evaluated the outcome of 18 years of pediatric heart transplantation and compared our results to published international experience.

CARS cohort and statistical modelling

Study cohort CARS

The cohort of children we studied is the CARS cohort, which is the result of a unique collaboration between pediatric cardiologists of the 8 Dutch university hospitals. We aimed to include all children (0-18 year) with DCM in a time frame of 7 years, from October 2010 to July 2017. We enrolled children with a previous diagnosis of DCM until 2010, or with newly diagnosed DCM from 2010 and onward. DCM was defined as the presence of impaired systolic function (fractional shortening (FS) ≤25%) and left ventricular (LV) dilation (LV end-diastolic dimension (LVEDD)> +2 Z-score for body surface area). Patients with structural heart disease were excluded. The research program was organized in such a way that study visits coincided with routine outpatient clinic visits or hospital admissions. In the first year after diagnosis, children were evaluated by the study team 1 to 4 times per year. After the first year, children were evaluated 1 to 2 times per year, dependent on the frequency of visits. The primary study endpoint was defined as death or heart transplantation. Secondly, we defined recovery as 2 consecutive echocardiograms with normalized LVEDD and FS, the date of the first normalized echocardiogram was considered as date of recovery. Thirdly, the remaining children were categorized as having "ongoing disease".

Statistical modelling

Since the basis of CARS study was repeated measurements of the abovementioned risk factors, we needed a statistical model that would allow interpreting in particular the repeated measurements. A statistical model that best fitted our data proved to be a so called Joint Model. This statistical model is a combination of a Mixed Effect model for repeated measurements, and a Cox Regression model for survival data. The Mixed Effect model assumes that each subject in the population has his own evolution over time. It also accounts for the correlation within the measurements obtained from the individual patients and can address dissimilar spaced patient visit times, as is common in clinical practice. A Cox model for survival data is routinely used when interest is on event outcome, in our studies defined as cardiac death: heart transplantation or death. A Cox regression model alone assumes that the level of the risk factor, say NT-proBNP, remains constant between one measurement and the next, to suddenly change at the moment of the patient visit. For markers that slowly change of time, like NT-proBNP, the Cox model alone is therefore too rough an estimate. The joint model thus elegantly couples the survival model of time to event data (heart transplantation or death) with a Mixed Effect model for the repeated measurements (75).

AIMS OF THIS THESIS

The aim of this thesis is three-fold: first, to provide better insight into the etiology of childhood DCM; second, to evaluate the contribution of temporal evolution of risk factors in predicting adverse outcome, and third, to improve treatment of children across the clinical spectrum of DCM.

OUTLINE OF THIS THESIS

Chapter 2 describes the current practice and results of genetic evaluation in our national cohort of children with DCM, and reports the relation between the presence of (likely) pathogenic variants and clinical outcome. In **Chapter 3** we explore the cellular phenotype in a unique collection of pediatric DCM myocardium samples by combining functional measurements in single isolated cardiomyocytes, protein analyses and electron microscopy. In **Chapter 4** the added value of repeated 6MWT in addition to a single 6MWT in predicting outcome is evaluated. **Chapter 5** describes the use of serial measurements of known risk factors in prediction of outcome. **Chapter 6** reviews the safety of ACE-inhibitors for the treatment of heart failure in children. In **Chapter 7** we evaluate the level of emotional and behavioral problems and whether depressive and anxiety problems are associated with outcome. **Chapter 8** describes the outcome of children supported with a VAD and the effect of the introduction of VAD on waiting list mortality is evaluated. In **Chapter 9** we evaluate the outcome of heart transplantation against the background of a restrictive early listing strategy.

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Genetic evaluation of a nation-wide Dutch pediatric DCM cohort – the use of genetic testing in risk stratification

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ABSTRACT

OBJECTIVES To describe the current practice and results of genetic evaluation in Dutch children with dilated cardiomyopathy (DCM) and evaluate genotype-phenotype correlations that may guide prognosis.

METHODS We performed a multicenter prospective observational study in children diagnosed with DCM from 2010 to 2017.

RESULTS One hundred forty-four children were included. Initial diagnostic categories were idiopathic DCM in 67 children (47%), myocarditis in 23 (16%), neuromuscular in 7 (5%), familial in 18 (13%), inborn error of metabolism in 4 (3%), malformation syndrome in 2 (1%) and 'other' in 23 (16%). Median follow-up time was 2.1 years [IQR 1.0-4.3]. Hundred-seven patients (74%) underwent genetic testing. We found a likely pathogenic (LP) or pathogenic (P) variant in 39 children (36%), most often in *MYH7* (n=9). In one patient initially diagnosed with myocarditis, a pathogenic *LMNA* variant was found. During the study, 39 patients (27%) reached study endpoint (SE: all-cause death or heart transplantation). Transplant-free survival was significantly lower in patients with a LP/P variant (P=0.005), and children with a LP/P variant were more likely to reach SE compared to children without (hazard ratio 2.8; 95% CI 1.3 to 5.8, P=0.007), while apart from left ventricle diastolic dimension, clinical characteristics at diagnosis did not differ between the two groups.

CONCLUSION Genetic testing is a valuable tool for predicting prognosis in children with DCM, with carriers of a LP/P variant having a worse prognosis overall. Genetic testing should be incorporated in clinical work-up of all children with DCM regardless of presumed disease etiology.

INTRODUCTION

Since the early 1990s, gene variants have been implicated in the etiology of dilated cardiomyopathy (DCM), which is defined as systolic dysfunction and increased ventricular chamber volume. Genetic DCM was initially thought to be a disease primarily caused by variants in genes encoding cytoskeletal and sarcomeric proteins (1-3). However, recent advances in sequencing and array-based technologies have increased our understanding of the genetic basis of DCM. In addition to genes encoding sarcomeric and cytoskeletal proteins, genes coding for transcription factors, ion channels, the nuclear membrane and mitochondrial proteins are now also known to be involved in isolated DCM. In addition, more than 200 genes are known that underlie syndromes or inborn errors of metabolism (IEMs) in which DCM can be part of the phenotype (4-6).

As in adult-onset cardiomyopathy, genetic testing has now been integrated into daily clinical practice in the pediatric population, and a genetic cause can be identified in up to 27-54% of pediatric DCM patients (7-9). *MYH7* (5.1%), *VCL* (3.2%) and *TPM1* (2.2%) are among the most frequently affected genes in children younger than two years of age, while *TTN* (10.0%), *RBM20* (6.7%) and *TNNT2* (4.7%) are the most frequently mutated genes in the 2-18 year age group(10).

The etiology of pediatric DCM is a strong predictor of long-term outcome. The 5-year transplant-free survival rate is 47% in idiopathic DCM, while it is 73% in DCM related to myocarditis. In familial DCM, the 5-year survival rate is high (94%), but the 5-year transplantation rate is also relatively high (38%). These differences emphasize the importance of establishing the genetic etiology in DCM, as it may help further guide optimal treatment (11, 12). Studies in adult DCM patients have reported a more severe phenotype and earlier onset in patients with a pathogenic genetic variant compared to variant-negative patients (13, 14). Furthermore, DCM patients with pathogenic variants in *LMNA*, *PLN*, *RBM20*, *DES* and *FLNC* are at higher risk for malignant arrhythmias and have a worse prognosis than patients with variants in other genes (13-16). Matthew *et al.* showed that the affected gene (e.g. *MYH7*), a higher variant burden and *de novo* variant status are all factors independently associated with earlier onset and higher frequency of adverse outcomes in pediatric hypertrophic cardiomyopathy (17). However, studies reporting on the utility of genetic testing for risk stratification in children with DCM are scarce.

The aims of the present study were twofold. First, we aimed to describe the current practice and results of genetic evaluation in a large cohort of pediatric DCM patients presenting to all tertiary referral hospitals in the Netherlands. Second, we evaluated these patients for potential genotype–phenotype correlations that may guide prognosis.

METHODS

Patients

Data were collected in a multicenter, prospective observational study design. Eight tertiary pediatric cardiology centers cooperated in the study. Given the structure of the Dutch health service system, these eight centers serve almost 100% of the Dutch population.

Patients (age < 18 years at diagnosis) were included from October 2010 to July 2017. In addition, we retrospectively enrolled children diagnosed with DCM before 2010. The first member of each family who presented to our services with a diagnosis of DCM was designated the proband for this analysis.

DCM was defined as the presence of impaired systolic function (fractional shortening (FS) ≤25%) and left ventricular (LV) dilation (LV end-diastolic dimension (LVEDD) *z*-score >2 for body surface area) (18, 19). Patients with additional structural heart disease that explained their LV dilation were excluded. A diagnostic work-up was performed in all patients, as previously described (11, 20). Patients were subsequently classified into an initial diagnostic category within the six months following their DCM diagnosis. Diagnostic categories consisted of: idiopathic, myocarditis, neuromuscular disease (NMD), familial, IEM, malformation syndrome or other. This classification follows the standard of the Pediatric Cardiomyopathy Registry (PCMR) (11, 21). Familial DCM is defined as two or more affected family members and/or an explanatory genetic finding.

Diagnosis of myocarditis was made based on clinical grounds and viral test results. Myocarditis was 'definite' if there was histological or immune-histological evidence of myocarditis. Myocarditis was 'probable' when blood plasma/serum or cerebrospinal fluid PCR or culture was positive for enterovirus, adenovirus, parechovirus or human parainfluenza virus, or if blood plasma/serum PCR or culture was positive for parvovirus B19, HHV6, cytomegalovirus or Epstein-Barr virus accompanied by serological proof of a primary infection (seroconversion and/or positive IgM) (20). In the patients diagnosed before 2010, data on diagnostic work-up, family history and genetic variant segregation analysis were retrospectively collected.

Study endpoint (SE) was defined as all-cause death or heart transplantation (HTx). In addition, patient status at the last follow-up visit was recorded as 'ongoing disease' or 'recovered'. Recovered was defined as two consecutive echocardiograms with normalized LVEDD and FS, with the date of the first normalized echocardiogram considered the date of recovery. Furthermore, gender, age at diagnosis, New York University Pediatric Heart Failure Score (NYUPHFI), NT-proBNP, and standardized echocardiogram (LVEDD, FS) were recorded at inclusion. All study data were collected during routine outpatient clinic visits or hospital admissions. Subjects were followed until SE was reached, the age of 18 years, or the last outpatient visit within the study window. This study was approved by the Medical Research Ethical Committee of the Erasmus University Medical Center (MEC 2014-062) and performed in accordance with the Declaration of Helsinki. All legal parents and children ≥12 years of age gave their written informed consent.

Genetic evaluation and variant classification

The genetic data we collected reflect the genetic evaluation that was common practice at that time: single gene testing (e.g. Sanger sequencing of *MYH7*), targeted next-generation sequencing (NGS) of a gene panel (typically 46-61 genes), exome sequencing (ES) with analysis of genes related to cardiomyopathy (7) or open exome analysis. Additional genetic testing (e.g. SNP-array) was performed in a subset of patients in whom a malformation syndrome was suspected. As this was an observational study, patients who had no genetic testing or a test that is now considered too limited were not actively referred for genetic (re)evaluation.

Patients were considered genetically evaluated when at least one genetic test was performed. The pathogenicity of the variants was assessed using Alamut Visual Software (Interactive Biosoftware, Rouen, France). All variants were reclassified (December 2019) by a molecular geneticist specialized in cardiogenetics (RLdD) according to ACMG criteria (22). Variants with a minor allele frequency <0.1% in the Genome Aggregation Database (gnomAD) were considered rare. Nonsense and frameshift variants were considered null variants if they occurred proximal to the last 50 bases of the penultimate exon. We defined two groups: patients with a pathogenic (P) or likely pathogenic (LP) variant (class 4 or 5) and patients without a pathogenic variant, including patients with one or more variants of unknown significance (VUS, class 3) (23).

Statistical analysis

Categorical variables were reported as numbers and percentages. Continuous variables were reported as means with standard deviation (SD) when normally distributed, or as medians with interquartile range (IQR) when non-normally distributed. To compare clinical characteristics between patients with a LP/P variant and those with negative genetic test results, the student's t-test was used in case of normally distributed variables and the Wilcoxon rank test was used when variables were non-normally distributed. A Chi-square test or two-sided Fisher exact test was performed to examine the relation between categorical data.

We used the Kaplan-Meier method to estimate transplant-free survival in the two groups. The log-rank test was used to determine whether the difference between the two curves was statistically significant. Univariate Cox regression analysis was used to test the predictive value of a LP/P variant. Proportional hazard assumptions were tested, and were not violated. The hazard ratio and 95% confidence interval (CI) were calculated. Testing was performed two-sided, and statistical significance was set at $P < 0.05$. All analyses were performed using IBM SPSS Statistics for Windows, version 24 (IMB Corp, Armonk, NY, USA).

RESULTS

Patient characteristics

Hundred forty-four children with DCM were included in the study: 97 children (67%) diagnosed during the study period and 47 patients (33%) diagnosed before the start of the study in 2010. Median age at diagnosis was 1.5 years [IQR 0.12-9.97], and 63 children (44%) were diagnosed before the age of 1 year.

Initial diagnostic categories included idiopathic DCM in 67 children (46%), myocarditis in 23 (16%), NMD in 7 (5%), familial DCM in 18 (13%), IEM in four (3%), malformation syndrome in two (1%) and 'other' in 23 (16%). The 'other' category included anthracycline-related DCM in eight (6%), LV dilation and systolic dysfunction with non-compaction cardiomyopathy (NCCM) in six (4%) (as described by van Waning *et al*.(24)), DCM based on tachyarrhythmia in three (2%), LV infarction in two (1%), vasculitis in two (1%) and congenital AV-block in one (1%).

The median follow-up time was 2.1 years [IQR 1.0-4.3]. Table 1 describes the clinical characteristics of the cohort.

Table 1. Characteristics of children with dilated cardiomyopathy stratified by LP/P variant-positive patients and variant-negative patients

Characteristic	Total	LP/P positive	Variant-negative	P-value
	n=144	**n=39**	**n=68**	
Gender, female, n (%)	68 (47)	20 (51)	32 (47)	0.49
Age at DCM diagnosis, years, median (IQR)	1.5 (0.12 to 10.0)	2.3 (0.1 to 12.4)	1.7 (0.3 to 6.0)	0.94
Heart failure score*, NYUPHFI, median (IQR)	9 (7 to 12)	9 (6 to 11)	9 (6 to 13)	0.28
Echocardiographic parameters*, mean (SD)				
LVEDD Z- score	4.8 (3.6)	4.3 (3.8)	5.6 (2.7)	0.04
SF	15.9 (7.0)	17 (7)	15 (7)	0.10
NT pro BNP*, pg/ml, median (IQR)	5244 (1893 to 21651)	4253 (895 to 28218)	6932 (1147 to 19028)	0.41
Time diagnosis till last follow-up years, median (IQR)	3.1 (1.3 to 5.7)	2.3 (0.6 to 5.4)	3.3 (1.3 to 5.8)	0.31
Status at end of study, n (%)				
Death/transplantation**	39 (27)	17 (44)	12 (18)	0.002
Ongoing disease	82 (57)	20 (50)	46 (67)	0.04
Recovered	23 (16)	2 (5)	10 (15)	0.10
Genetic evaluation, n (%)	107 (74%)			

Variant-negative: VUS or no variant
*at study inclusion
Student's T-test in normally distributed data and Wilcoxon rank test in non-normally distributed data.
** Chi Square
DCM, dilated cardiomyopathy; IQR, interquartile range; NYUPHFI, New York University Pediatric Heart Failure Index; LVEDD, Left Ventricular End Diastolic Dimension; SF, shortening fraction; NT pro BNP, N Terminal-pro brain natriuretic peptide.

Genetic findings

Hundred-seven of 144 DCM patients (74%) underwent genetic testing, with some patients undergoing more than one test. Twenty-three (20%) patients underwent Sanger sequencing of one or more genes, 82 (72%) patients had a targeted NGS gene panel, 29 patients (25%) had ES with analysis of an expanded gene panel related to cardiomyopathy and one patient had ES with comprehensive analysis of all known genes. Table 2 describes the number of genetically evaluated patients and the number of LP/P variants per diagnostic category.

Table 2. Genetic evaluation and outcome per diagnostic category

Initial diagnostic DCM category	Number of patients at start n (% of all patients)	Number of patients genetically evaluated n (% of dx category)	Number of LP/P variants n (% of genetically evaluated)	Number of patients after genetic evaluation and reclassification n (% of all patients)
Idiopathic	67 (47)	56 (84)	8 (14)	64 (44)
Myocarditis	23 (16)	7 (30)	1 (14)	22 (15)
NMD	7 (5)	7 (100)	6 (88)	8 (6)
Familial	18 (13)	18 (100)	13 (74)	26 (18)
IEM	4 (3)	4 (100)	4 (100)	4 (3)
Malformation syndrome	2 (1)	2 (100)	2 (100)	2 (2)
Other	23 (16)	13 (56)	5 (38)	18 (12)

DCM, dilated cardiomyopathy; NMD, neuromuscular disease; IEM, inborn error of metabolism; LP, Likely Pathogenic; P, Pathogenic

Thirty-nine (36%) patients carried a LP/P variant, including 11 who had one or more additional VUSs. Thirty-nine patients (36%) had only one or more VUS, while 29 (27%) patients had no variant (Figure 1). No difference was observed in the percentage of genetic testing in patients who reached the SE compared to those who did not (29 of 39 (74%) versus 78 of 105 (75%), P=0.9).

LP/P variants were found in 21 different genes, with *MYH7* the largest contributor of pathogenic variants (9 LP/P variants (23%)). The second highest contributors were *TTN* and *TMP1,* each accounting for 8% of positive test results. No variants were identified for a number of cardiac genes that are part of standard gene panels (Supplementary Table 1). The clinical and genetic characteristics of the 39 patients with a LP/P variant are described in Table 3. Two patients with LP/P variants in genes related to a malformation syndrome (Alström syndrome) were found. Seven patients had LP/P variants in genes related to NMD (Duchenne disease, infantile type I muscle fiber disease and cardiomyopathy, centronuclear myopathy). Four had LP/P variants in genes related to IEM (Very Long Chain Acyl-CoA dehydrogenase Deficiency, propionic acidemia, Barth syndrome and GM1 gangliosidosis). In three patients, we found LP/P variants in two genes: *MYH7*/*RYR2*, *SCN5A*/*KCNQ1* and *TBX20*/*GLB1*. DCM-associated *SCN5A* variants have been shown to have either loss- or gain-of-function effects on cardiac sodium channel activity (25-27). In one patient, biallelic *ASNA1* variants were found (as described previously (28)). We also found six LP/P variants in four patients that we did not deem to be explanatory for the

DCM, including two compound heterozygous truncating variants in *CEP135*, a *de novo* deletion of chromosome 14q22.3q23.1 (Hg19: 57,007,506-61,613,506), two compound heterozygous pathogenic missense variants in *SLC37A4* and a *de novo* missense variant in *MAP3K7*. Five of 40 variants (13%) were proven *de novo* (in three children with LP/P variants, no data on segregation of the variants was available).

The diagnostic classifications of 20 patients changed during the study period. LP/P variants were found in 8 children (12%) who had initially been diagnosed with idiopathic DCM, and their cases were therefore reclassified to familial/genetic DCM. Four of 23 patients who were diagnosed with myocarditis underwent genetic evaluation, and a pathogenic *LMNA* variant was found in one patient. The diagnostic category of this patient was therefore reclassified as familial/genetic DCM (Table 2). In five patients with LV dilation and systolic dysfunction with NCCM classified as 'other', a LP/P variant was found (*DES, MYH7, NKX2.5*, *PLN, SCN5A,* (Table 3)). One patient initially classified as familial was reclassified as NMD after genetic evaluation (*MYL2*).

In addition, variant reclassification altered the definitive diagnosis in five patients (26% of all diagnostic reclassifications, Table 3). In these patients with a putative LP/P variant leading to allocation into the familial/genetic DCM group, the variant was reclassified as a VUS and patients were reclassified as idiopathic DCM. None of the variants initially classified as VUS were reclassified as LP/P (Table 2).

Clinical outcome

During the study period, 39 patients (27%) reached SE: 17 patients died (12%) and 22 patients (15%) underwent HTx. Median time from diagnosis to death was 0.09 years [IQR 0.03 to 1.1]. Median time to HTx was 2.9 years [IQR 1.1 to 6.1]. At the end of the study, 23 children (16%) had recovered (35% diagnosed with myocarditis), while 82 children (57%) had ongoing disease.

Association of LP/P variants with clinical outcome

17 of 39 children with a LP/P variant reached SE, while 20 had ongoing disease and two (with variants in *MYH7* and *LMNA*) recovered.

Children with a LP/P variant were more likely to die or undergo HTx compared to children without a pathogenic variant (17 of 39 (44%) versus 12 of 68 (17%), *P*=0.006). We found no differences in clinical characteristics at time of diagnosis between children with a LP/P variant and those without, with only the LV diameter higher in children who were variant-negative (*P*=0.04, Table 1). Median age at SE tended to be lower in children with a LP/P variant, however this difference was not statistically significant (*P*=0.19). Median age at SE was 10.9 years [IQR 0.6 to 16.1] in variant-positive patients, and 5 of 17 (29%) were under 1 year of age. In variant-negative patients, median age at SE was 13.3 years [IQR 6.9 to 14.6], and the age of the youngest patient at SE was 3.5 years.

Of the 17 LP/P variant-positive children reaching SE, 10 (53%) died and 7 (41%) underwent HTx. The majority of variant-negative children who reached a SE underwent HTx (8/12; 67%), while 4 of 12 children died (36%, *P*=0.26).

Table 3. Clinical and genetic characteristics of patients with identified LP/P variants

Sex	Cardiac phenotype	Initial diagnostic category	Age at diagnosis (years)	Gene	Cellular structure	Variant (class)	Method	Mode of inheritance	Outcome
F	DCM	inborn error of metabolism	15.52	*ACADVL* (NM_000018.3)	other	c.104del, p.(Pro35Leufs*26), homozygous (5)	Sanger sequencing	autosomal recessive	ongoing disease
M	DCM	malformation syndrome	0.11	*ALMS1* (NM_015120.4)	centrosome	c.6246_6247del, p.(Asp2083Cysfs*11) (5); c.10581del, p.(Met3527Ilefs*20) (5), compound heterozygous	Sanger sequencing	autosomal recessive	ongoing disease
F	DCM	malformation syndrome	0.1	*ALMS1* (NM_015120.4)	centrosome	c.8361dupT, p.(Ile2788fs*), homozygous (5)	ES	autosomal recessive	ongoing disease
F	DCM	idiopathic	0.05	*ASNA1* (NM_004317.2)	other	c.913C>T, p.(Gln305*), paternal (5); c.867C>G, p.(Cys289Trp), paternal; in cis configuration; c.488T>C, p.(Val163Ala), maternal (5)	ES (open exome)	autosomal recessive	died
F	mixed DCM / NCCM	other	8.45	*DES* (NM_001927.3)	cytoskeleton	c.1222C>G, p.(Leu408Val) (5)	targeted NGS	*de novo*	HTx
M	DCM	neuromuscular	15.79	*DMD* (NM_004006.2)	cytoskeleton	del exons 50-52 (X:31.628.821-31.754.369; Ensembl release 50) (5)	SNP-array	x-linked recessive	ongoing disease
M	DCM	neuromuscular	8.89	*DMD* (NM_004006.2)	cytoskeleton	del exons 42-43 (5)	MLPA DMD gene	x-linked recessive	ongoing disease
M	DCM	neuromuscular	15.98	*DMD* (NM_004006.2)	cytoskeleton	del exons 8-(35) (5)	multiplex PCR en southern blot analysis of *DMD* gene	x-linked recessive	ongoing disease
M	DCM	neuromuscular	12.61	*DMD* (NM_004006.2)	cytoskeleton	c.10094C>G, p.(Ser3365*) (5)	Sequencing exon 70	x-linked recessive	died
M	DCM	neuromuscular	12.44	*DMD* (NM_004006.2)	cytoskeleton	c.186+1G>C resulting in an in-frame deletion of exon 3 (5)	RT-PCR/PTT exons 2 - 79, RT-PCR exons 3-7, sequence analysis exon 3	x-linked recessive	HTx
F	DCM	familial/genetic	14.83	*DSP* (NM_004415.3)	desmosome	c.2631-2A>C, p.(?) (5)	targeted NGS	*de novo*	ongoing disease

Sex	Cardiac phenotype	Initial diagnostic category	Age at diagnosis (years)	Gene	Cellular structure	Variant (class)	Method	Mode of inheritance	Outcome
F	DCM	myocarditis	1.66	*LMNA* (NM_170707.3)	nuclear envelope	c.992G>A, p.(Arg331Gln) (5)	targeted NGS	autosomal dominant	recovered
F	mixed DCM / HCM	familial/genetic	0.11	*MYBPC3* (NM_000256.3)	sarcomere	c.2827C>T, p.(Arg943*) (5)	Sanger sequencing	autosomal recessive	died
F	DCM	familial/genetic	0.13	*MYH7* (NM_000257.2)	sarcomere	c.5754C>G, p.(Asn1918Lys) (5)	Sanger sequencing	autosomal dominant	recovered
M	DCM	familial/genetic	0.91	*MYH7* (NM_000257.2); *RYR2* (NM_001035.2)	sarcomere; calcium/sodium handling	c.5754C>G, p.(Asn1918Lys), maternal (5); c.5335A>G, p.(Ser1779Gly), *de novo* (4)	targeted NGS	autosomal dominant; *de novo*	ongoing disease
F	mixed DCM / NCCM	other	0.12	*MYH7* (NM_000257.3)	sarcomere	c.1106G>A, p.(Arg369Gln) (5)	Sanger sequencing	autosomal dominant	HTx
M	DCM	idiopathic	0.04	*MYH7* (NM_000257.3)	sarcomere	c.2711G>A, p.(Arg904His) (5)	targeted NGS	unknown	ongoing disease
F	DCM	idiopathic	2.3	*MYH7* (NM_000257.3)	sarcomere	c.5740G>A, p.(Glu1914Lys) (5)	targeted NGS	autosomal dominant	ongoing disease
M	DCM	familial/genetic	0.19	*MYH7* (NM_000257.3)	sarcomere	c.602T>C, p.(Ile201Thr) (5)	targeted NGS	autosomal dominant	ongoing disease
M	mixed DCM / NCCM	familial/genetic	0.01	*MYH7* (NM_000257.3)	sarcomere	c.495G>A, p.(Met165Ile) (4)	targeted NGS	autosomal dominant	ongoing disease
M	DCM	familial/genetic	0.07	*MYH7* (NM_000257.3)	sarcomere	c.5773C>G, p.(Arg1925Gly) (5)	targeted NGS	unknown	ongoing disease
M	DCM	familial/genetic	10.41	*MYH7* (NM_000257.3)	sarcomere	c.1106G>A, p.(Arg369Gln) (5)	Sanger sequencing	autosomal dominant	ongoing disease
M	DCM	familial/genetic	0.32	*MYL2* (NM_000432.3)	sarcomere	c.403-1G>C, homozygous (5)	Sanger sequencing	autosomal recessive	died
F	mixed DCM / NCCM	other	13.31	*NKX2.5* (NM_004387.3)	other	c.592C>T, p.(Gln198*) (5)	targeted NGS	autosomal dominant	HTx
M	DCM	inborn error of metabolism	12.3	*PCCA* (NM_000282.3)	other	c.1409T>G, p.(Leu470Arg), homozygous (5)	Sanger sequencing	autosomal recessive	ongoing disease
F	mixed DCM / NCCM	other	15.81	*PLN* (NM_002667.4)	sarcoplasmatic reticulum, calcium/sodium handling	c.25C>T, p.(Arg9Cys) (5)	targeted NGS	*de novo*	HTx

Sex	Cardiac phenotype	Initial diagnostic category	Age at diagnosis (years)	Gene	Cellular structure	Variant (class)	Method	Mode of inheritance	Outcome
F	NCCM	familial/genetic	0.08	*RYR2* (NM_001035.2)	ion channel	c.11084T>C, p.(Met3695Thr), maternal (3); del exon 19, paternal (4)	targeted NGS	autosomal recessive	died
M	mixed DCM / NCCM	other	0	*SCN5A* (NM_198056.2); *KCNQ1* (NM_000218.2)	ion channel	c.4978A>G, p.(Ile1660Val) (5); c.973G>A, p.(Gly325Arg) (5)	targeted NGS	autosomal dominant	recovered
M	DCM	neuromuscular	6.9	*SPEG* (NM_005876.4)	sarcoplasmatic reticulum	c.9185_9187del, p.(Val3062del), homozygous (4)	ES with analysis of 310 genes related to CMP	autosomal recessive	died
M	DCM	inborn error of metabolism	0.31	*TAZ* (NM_001303465.1)	mitochondrial	c.523del, p.(Val175Serfs*29) (5)	Sanger sequencing	x-linked recessive	HTx
F	DCM	inborn error of metabolism	0.27	*TBX20* (NM_001077653.2); *GLB1* (NM_000404.3)	transcription factor	c.456C>G, p.(Ile152Met) (4); c.176G>A, p.(Arg59His), homozygous (5)	WGS with analysis of 310 genes related to CMP	autosomal dominant; autosomal recessive	died
F	DCM	idiopathic	3.77	*TNNT2* (NM_000364.3)	sarcomere	c.650_652del, p.(Lys217del) (5)	targeted NGS	unknown	died
F	DCM	familial/genetic	10.86	*TNNT2* (NM_000364.3)	sarcomere	c.650_652del, p.(Lys217del) (5)	targeted NGS	autosomal dominant	died
M	DCM	idiopathic	0.15	*TPM1* (NM_000366.5)	sarcomere	c.725C>T, p.(Ala242Val) (4)	targeted NGS	*de novo*	recovered
F	DCM	familial/genetic	5.38	*TPM1* (NM_000366.5)	sarcomere	c. 688G>A, p.(Asp230Asn) (5)	targeted NGS	autosomal dominant	HTx
F	DCM	familial/genetic	0.42	*TPM1* (NM_000366.5)	sarcomere	c.250G>A, p.(Asp84Asn) (5)	targeted NGS	autosomal dominant	ongoing disease
F	DCM	idiopathic	9.94	*TTN* (NM_133378.4)	sarcomere - Z-disc	c.81610G>T, p.(Glu27204*) (4);	ES with analysis of 310 genes related to CMP;	autosomal dominant;	died
M	DCM	idiopathic	16.94	*TTN* (NM_133378.4)	sarcomere - Z-disc	c.61121-1G>A, p.(Glu20374Glyfs*7) (4)	targeted NGS	autosomal dominant	ongoing disease
F	DCM	idiopathic	13.63	*TTN* (NM_133378.4)	sarcomere - Z-disc	c.62347C>T, p.(Arg20783*) (4)	targeted NGS	autosomal dominant	ongoing disease

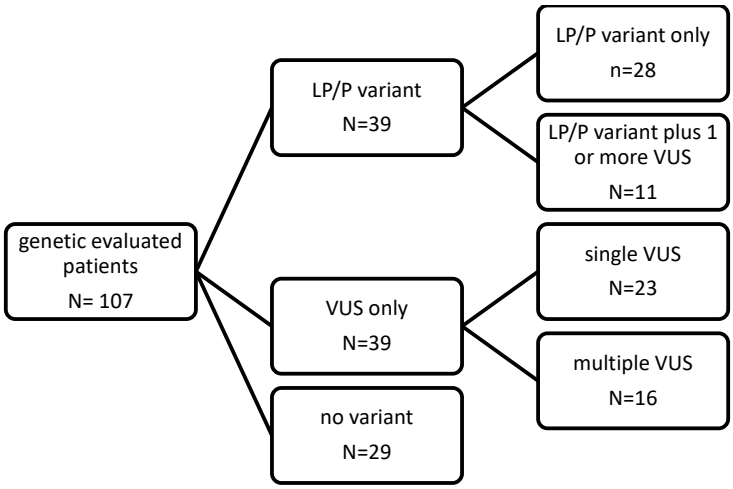
CMP, cardiomyopathy; ES, exome sequencing; F, female; M, male; HTx, heart transplantation; NGS, next-generation sequencing; PTT, protein truncation test; RT-PCR, reverse transcriptase polymerase chain reaction (52); WGS, whole genome sequencing

Transplant-free survival was significantly lower in patients with a LP/P variant compared to variant-negative patients (*P*=0.005, Figure 2). This was also true when we excluded the eight children who were diagnosed with NMD (*P*=0.04). Children with a LP/P variant had a 2.8-times increased risk of death or HTx (hazard ratio 2.8; 95% CI 1.3 to 5.8, *P*=0.007). Transplant-free survival was higher in *MYH7*-positive children compared to those with a LP/P variant in other genes (*P*=0.03, KM curve not shown).

In children without LP/P variants, we did not find an association between the presence or absence of VUSs and SE: 6/39 reached a SE with one or more VUS versus 6/29 who reached a SE without VUS (*P=0.*4, Figure 1).

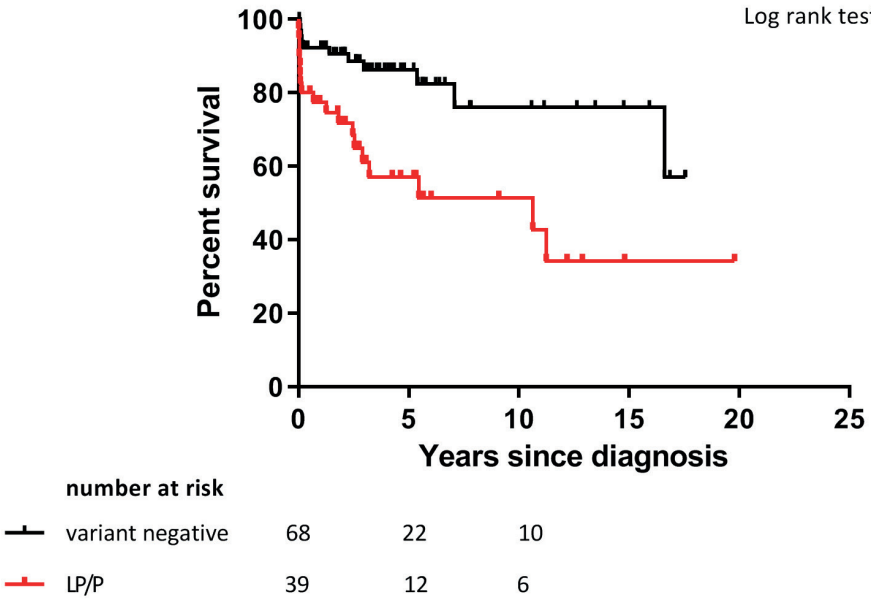
The number of patients reaching a SE and the heterogeneity of genetic findings meant that we had insufficient statistical power to explore the relationship between single affected genes or *de novo* variants and outcome.

Figure 1. Outcome genetic evaluation



LP/P variant: likely pathogenic or pathogenic (class 4 or 5 variant according to the ACMG classification)
VUS: Variant of Unknown Significance (class 3 variant according to the ACMG classification)

Figure 2. Kaplan-Meier analysis of 107 genetically evaluated children with DCM, children with a LP/P variant versus no variant or VUS



LP/P: class 4 or 5 variant according to the ACMG classification

DISCUSSION

In this cohort of 107 genetically evaluated children with DCM, 39 children (36%) carried a LP/P variant in a DCM-related gene, most often in *MYH7*. Children with DCM who carried a LP/P variant had a 2.8-times increased risk for death or HTx compared to children without such a variant, but clinical characteristics at time of diagnosis (apart from LVEDD) did not differ between the two groups. In addition, children with a LP/P variant were more likely to die or undergo HTx at an earlier age. These findings highlight the importance of early genetic testing in children with DCM, as the determination of a genetic etiology can be valuable for predicting clinical outcome.

Yield of genetic testing in children with DCM

In adults with DCM, the yield of genetic testing varies between 16-37%(29). There are only a few studies on current genetic testing in pediatric DCM. These studies differ in inclusion criteria (isolated DCM versus non-isolated DCM), the extent of genetic testing, and variant filtering and interpretation. Pugh *et al.* reported an overall yield of 37% in 766 individuals with DCM (including 286 patients younger than 18 years) using gene panels that included between 5 to 46 genes, with titin (*TTN*) being the largest contributor (up to 14%). Unfortunately, these authors did not specify the yield in the pediatric age group (10). Kühnish *et al.* found 8 LP/P variants in 34 pediatric patients with DCM (24%) using a panel-based NGS approach targeting 89 genes, which identified variants in *TTN* (1), *TNNT2* (2), *TNNI3* (1), *MYH7* (1), *MYBPC3* (1) and *ACTC1* (1)(30). In a recent study

by Herkert *et al.*, combining copy number variant analysis with stepwise trio-based ES yielded a diagnosis in more than 50% (28/44 families) of pediatric DCM patients. They also identified patients with familial and non-familial DCM and patients with extra-cardiac features or possible myocarditis (7). In a study by Long *et al.*, ES in 18 families with DCM (including three syndromic cases) yielded a genetic diagnosis in 33% when filtering for 55 known DCM genes. When they expanded their analysis by filtering the exome for compound heterozygous and *de novo* variants, they diagnosed four additional patients, including carriers of rare and syndromic genes (*ALMS1* and *PRDM16*) and two potential novel genes (*RRAGC* and *TAF1A*), resulting in a final yield of 50%. Vasilescu *et al.* reported a genetic diagnosis in 10 of 37 pediatric DCM patients (27%), of which three were in novel or less-established disease genes (*PPA2*, *TAB2* and *NRAP*) and two were in mitochondrial DNA. They also showed an increase in a genetic diagnosis with later onset of cardiomyopathy: age <1 year - 34% positive DNA diagnosis, age 1 to 5 years - 38% and age >6 years - 60%. They further showed that infants manifesting before 1 year of age had the poorest prognosis, especially when their cardiomyopathy was associated with a metabolic or syndromic origin, which is in line with previous studies (9). Finally, in a study in neonates with heart failure, ES was diagnostic in 10/15 (68%), although only 20% had a clinical diagnosis of DCM (31).

The yield of genetic testing in pediatric DCM is higher than that in adult-onset non-ischemic DCM, varying between 27 to 54% (7, 8, 10, 32, 33). In addition, the spectrum of genes involved in pediatric DCM differs from that seen in adult (non-ischemic) DCM (34, 35). Pugh *et al*. showed that the genes implicated in DCM vary with age. In adults and children 2-18 years of age, the majority of variants were located in *TTN* and *DSP*. In children under 2 years of age, *MYH7* was predominantly mutated and no *TTN* variants were found (10). This matches our findings as 7/9 children in our cohort with LP/P *MYH7* variants were under the age of 1 year at diagnosis, confirming that these variants frequently underlie DCM with infant presentation.

Our yield of 36% LP/P variants in pediatric DCM and the spectrum of genes involved are thus in line with international literature, but also leave room for further increase in yield, e.g. by systematically offering ES with analysis of an expanded gene panel or of all disease-associated genes. This is especially important for children <1 year of age at diagnosis, where the diagnostic yield of ES goes up to 55% (5/9) and three of five (60%) genes with pathogenic variants were not part of (adult-onset) cardiomyopathy panels because they are involved in metabolic and syndromic diagnoses (31).

The predictive value of the variants detected

In adults with non-ischemic DCM, there is increasing insight into the association between certain pathogenic variants and outcome. In a large meta-analysis, the highest HTx rate was found in *LMNA* mutation carriers (27%), while *RBM20* mutation carriers underwent HTx at a younger age (mean 28.5 years) than carriers of pathogenic variants in other genes (mean 41 to 43 years) (36). Another study in 5,267 individuals, ranging from healthy volunteers to end-stage DCM patients, showed that *TTN*-truncating variant-positive DCM patients reached the SE of death, HTx or ventricular assist device at earlier ages and sooner after enrolment than *TTN*-truncating variant-negative DCM patients (37).

Janswijer *et al*. found that truncating *TTN* mutations were associated with a milder form of DCM compared to that seen in patients with *LMNA* variants or idiopathic DCM (38). These findings were (partly) explained by differences in disease severity and the number of adverse events between the cohorts. In a study on the prognosis of 52 adults with DCM carrying rare variants in sarcomeric genes (*MYH6, MYH7, MYBPC3, TNNT2* and *TTN*), it was shown that death/HTx-free survival dramatically decreased after 50 years of age in variant-positive patients compared to variant-negative patients (39).

When predicting outcome in children with DCM, the genetic contribution is less clear. Specific variants (e.g. in *LMNA* or *SCN5A*) have been linked to sudden cardiac death, as this is related to malignant ventricular arrhythmias (8, 40). However, as sudden cardiac death is rare in children with DCM (5-year incidence of 3%), this is a less prevalent clinical issue than death due to heart failure or the need for HTx (41). To the best of our knowledge, only one study has systematically evaluated children with non-HCM cardiomyopathy (with 56 of 70 patients diagnosed with DCM) for genetic disease and association with outcome: Ellepola *et al*. showed that HTx were more often performed in variant-positive children than in variant-negative subgroups (48% vs. 34%), and the variant-positive children had higher mortality (17% vs. 2%). Of note, outcome was not specified for the 56 DCM patients (42).

In our study, which reports on the largest cohort of genetically tested children with DCM to date, we also observed decreased survival in children with a LP/P genetic variant. Our study is thus an important contribution to the mounting evidence that carrying a LP/P variant puts children at an increased risk of death or HTx. We also found that *MYH7* variant carriers were less likely to die or undergo HTx compared to patients with LP/P variants in other genes, although our numbers were small. Whether this truly implies that *MYH7* variants are relatively benign remains unknown. In adult DCM studies such a favorable genotype–phenotype relation could not be demonstrated for *MYH7* compared to *LMNA, PLN, RBM20, MYBPC3, TNNT*2 and *TNNI3* (29).

Clinical implications of our study

The results of our study justify incorporating genetic testing early on in the diagnostic work-up of all children with DCM (7). Since determining disease etiology is essential for prognosis and counseling, genetic testing should be offered as soon as possible after diagnosis. In both adults and children, there is also increasing evidence the presence of external causal factors (e.g. chemotherapy or myocarditis) does not preclude there being a genetic cause for the DCM (43-45). In our study we identified a pathogenic *LMNA* variant in a child diagnosed with myocarditis, which is a good example of how previously silent genetic defects might predispose to heart failure early in life when viral myocarditis acts as a second hit (46). These findings urge us to genetically evaluate *all* children diagnosed with DCM, regardless of presumed disease etiology. They also call for genetic re-evaluation of all children with DCM who have been tested previously, but to whom ES has not been offered. In our cohort, 74% percent of children were genetically evaluated, which clearly leaves room for improvement. Optimizing collaboration between pediatric cardiologists and clinical geneticists and genetic counseling of parents might increase the uptake of genetic testing.

At present, the direct translation of a genetic variant into individual clinical risk prediction is challenging. DCM in children is characterized by genetic heterogeneity, a situation contrary to that for HCM, where a relatively limited number of genes seem to be involved, predominantly those related to the sarcomere (47). Penetrance and age of presentation also vary in DCM (48-50). Based on our findings, children who carry a LP/P variant should be considered at an increased risk for adverse outcomes. The use of genetic information for better management and risk prediction will require close collaboration between research centers and analysis of pooled data. In this respect, the results of the 'PCM Genes study' of the PCMR, which aims to offer ES to 600 children with DCM, will provide more insight into genetic testing and associations with outcome (21). As long as it is not clear how to differentiate between recovery and remission, children who are variant-positive but recover should continue to receive follow-up care (51).

Limitations

Our study has limitations. Initially, diagnostic categories were assigned following the etiologic categories of the PCMR (11). However, these data pre-dated many clinically available genetic tests and, along with other epidemiologic studies, highlight the uncertain etiological basis of cardiomyopathy given that the majority of DCM patients were identified as idiopathic. Future directions of the registry include the use of ES to improve diagnostic strategies, which may lead to different etiologic classification (21). In our study, several idiopathic cases turned out to be familial/genetic. Furthermore, IEM and NMD are considered distinct categories even though a genetic diagnosis typically underlies these diseases as well. For that reason, we have included IEM and NMD as LP/P variant-positive in our analyses. It might have been more transparent if we had assigned one genetic diagnostic category that included subcategories of IEM and NMD in addition to those with a LP/P variant in an explanatory gene and those with two or more affected first degree family members. Secondly, some of our data were retrospective, and missing data might be an issue here despite the efforts we made to obtain all available data. Thirdly, genetic testing panels changed during the study period, which influences genetic yield. Furthermore, we could only be certain that a variant was *de novo* in cases with genetic evaluation of both parents, so the true number of *de novo* cases and possible relation to worse outcome remains unknown. Finally, our sample size was too small to test the associations between individual variants/genes and phenotype.

Conclusion

Genetic testing is a valuable tool to predict outcome in children with DCM, and patients with a LP/P variant have an overall worse prognosis. Genetic testing should therefore be incorporated in clinical care of all children with DCM, regardless of presumed disease etiology.

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Supplementary Table 1. Genetic findings in children with DCM. Number of likely pathogenic (LP) or pathogenic (P) variants, and variants of unknown significance (VUS).

Genes	LP/P variant	VUS
ABCC9	-	-
ACADVL	1	-
ACTC	-	-
ACTN2	-	-
ALDH3A2	-	2
ALMS 1	2	-
ALPK3	-	1 (compound heterozygous)
ANKRD	-	3
ANO5 (TMEM16E)	-	-
ASNA1	1 (compound heterozygous)	-
BAG3	-	-
CALR3	-	-
CAV3	-	1
CRYAB	-	-
CSRP3	-	-
DES	1	-
DMD	6	-
DSC2	-	1
DSG2	-	-
DSP	1	1
DTNA	-	3
EMD	-	-
FKTN	-	-
FLNC	-	1
GLA	-	-
HCN4	-	-
JPH2	-	-
JUP	-	2
KCNQ1	1	
LAMA4	-	1
LAMP2	-	-
LDB3	-	2
LDB3	-	1
LMNA	1	-
MIB1	-	-

MYBPC3	1	2
MYH6	-	2
MYH7	9	5
MYL2	1	-
MYL3	-	-
MYLK2	-	-
MYOZ1	-	-
MYOZ2	-	1
MYPN	-	2
NEXN	-	1
NKX2.5	1	-
NOTCH1	-	-
PCCA	1	-
PKP2		3 (1 compound heterozygous)
PLN	1	-
PRDM16	-	1
PRKAG2	-	-
RBM20	-	3
RYR2	1 (compound heterozygous)	1
SCN5A	2	-
SGCD	-	-
SPEG	1 (compound heterozygous)	-
TAZ (G4.5)	-	2
TBX20	1	-
TCAP	-	-
TLL1	1	-
TMEM43	-	-
TNNC1	-	4
TNNI3	-	1
TNNT2	2	-
TPM1	3	-
TTN	3	17
TTR	-	-
TXNRD2	-	-
VCL	-	1

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Cardiomyocyte hypocontractility and reduced myofibril density in pediatric cardiomyopathy

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ABSTRACT

Dilated cardiomyopathy amongst children (pediatric cardiomyopathy, pedicatric CM) is associated with a high morbidity and mortality. Moreover, treatment based on adult heart failure therapy is often ineffective. However, the reason for high morbidity and mortality is largely unknown as data on cellular pathomechanisms are limited. Here, we assessed cardiomyocyte contractility and protein expression to define cellular pathomechanisms in pediatric CM. For this study explanted heart tissue of 11 pediatric CM and 18 controls was studied. Contractility was measured in single membrane-permeabilized cardiomyocytes and protein expression was assessed with gel electrophoresis and western blot analysis. We observed increased Ca^{2+}-sensitivity of myofilaments which was due to hypohosphorylation of cardiac troponin I, a feature commonly observed in adult DCM. Unlike adult DCM we did not find an increase in compliant titin expression. We also found a significantly reduced maximal force generating capacity of cardiomyocytes, caused by reduced myofibril density. The limited ability of pediatric CM patients to induce cardiac remodeling might have contributed to their early disease onset and severity.

INTRODUCTION

Dilated cardiomyopathy (DCM) is a cardiac disease characterized by the dilatation of the (left) ventricle and systolic dysfunction. A genetic cause is found in 20-50% of DCM cases (1-3). The disease often develops in adulthood and disease progression can be slow if treatment is optimized though recovery remains unlikely (4). In children, DCM is a rare disease with an annual incidence estimated around 0.6/100 000 (5) and it may be associated with an aggressive disease progression. In two large studies the 1- and 5-year transplantation-free survival rates have been reported only 69-74 % and 54-65 % respectively (5, 6). More than 5 years after presentation the rate of events is low (6). Most children die or get transplanted because of pump failure, the rate of sudden cardiac death is relatively low at 2.4% at 5 years (7). Poor prognosis in pediatric DCM patients was associated with thin left ventricular (LV) wall (LV posterior wall thickness z-score <-1.7) and age <13.1 years at time of diagnosis (5-7). In contrast, after 2 years a 22% full recovery has also been reported (8) which implies pediatric cardiomyopathy can also be reversible. The aggressive nature of DCM and the contradictory relative high recovery rate was also reported in a recent Dutch study with a 1- and 5-year survival rate of 85% and 84%, respectively, implying that most patients died within the first year after diagnosis (9). In this study a low transplantation rate in the 1st year after presentation and a 38% recovery rate, of which 50% within 1 year, was reported (9). Determinants underlying the highly diverse response to therapy, recovery rates and mortality are largely unknown. While several pathomechanisms have been elucidated in adult DCM, knowledge on pathogenesis underlying the aggressive progression of DCM at young age is scarce. A recent study showed that pediatric DCM patients harbor a different gene expression profile compared to adult onset DCM (10). pediatric CM samples were characterized by an expression profile that reflected a more undifferentiated cellular state, and reduced hypertrophy response compared to adult DCM (10).

Here we defined the cellular phenotype in a unique collection of pediatric CM samples by combining functional measurements in single isolated cardiomyocytes, protein analyses and electron microscopy. Our studies revealed highly reduced force generating capacity of single cardiomyocytes caused by significant reductions in myofibril density. We observed troponin I hypophopshorylation and an associated increased myofilament Ca^{2+}-sensitivity and impaired myofilament length-dependent activation. We did not find an increase in compliant titin in the pediatric samples, which is a common form of disease remodeling in various forms of adult heart failure (11-13). However, we did find a large spread in titin isoform composition in the pediatric CM group. Overall, our data indicate that the pediatric CM heart that progresses to end-stage failure has limited capacity to adequately respond to increased wall stress.

METHODS

Clinical characteristics

Echocardiographic examinations were performed in a uniform way. All children were at rest and in sinus rhythm during examination an a complete 2-dimensional echocardiographic study was performed. M-mode of the parasternal long-axis was used to measure LVPWs, LVPWd, LVEDD and LVESD and were expressed as Z score for body surface area. Subsequently, FS was calculated using the formula ((LVEDD-LVESD) / LVEDD) *100%.

Cardiomyocyte force measurements

Single cardiomyocytes were mechanically isolated from cardiac tissue and membrane-permeabilized as previously described(29). Maximal force (F_{max}) and passive force (F_{pass}) of sarcomeres were measured at high [Ca^{2+}] and low [Ca^{2+}] (pCa 4.5 and pCa 9.0 respectively). Force-[Ca^{2+}] curves were constructed at various submaximal [Ca^{2+}] and are shown as relative forces to F_{max}. Myofilament Ca^{2+}-sensitivity was measured as the [Ca^{2+}] needed to achieve 50% of F_{max} (EC_{50}). length-dependent activation was measured as the shift in EC_{50} (ΔEC_{50}) at a sarcomere length of 1.8 μm and 2.2 μm.

Titin expression and cTnI phosphorylation

Titin isoforms were separated on a 1% w/v agarose gel and stained with SYPRO Ruby protein gel stain (Invitrogen) as previously described (30). All samples were measured in triplicate and average of triplicate measurement per sample is shown. Phosphorylation of cTnI was assessed as previously described (31, 32).

HSP27, HSP70, LC3B-I/II and p62 expression

For HSP70 and HSP27 analysis proteins were separated on pre-cast 10% criterion gels (BioRad) and membranes were incubated with HSP70 antibody (Enzo) or HSP27 antibody (Cell Signaling), and GAPDH antibody (Cell signaling) to correct for loading differences. For LC3B-I and LC3B-II analysis proteins were separated on pre-cast 8-16% gradient TGX gels (BioRad) and membranes were incubated with LC3B-I/II antibody. Analysis of p62 expression was performed by separating proteins on a 12% acrylamide gel and membrane was cut in two pieces. The upper piece was incubated with p62 antibody (Cell signaling) and the lower piece with GAPDH antibody.

Electron microscopy

Cardiac tissue of pediatric CM and control samples were studied with transmission electron microscopy. Myocardium was fixed in 2% paraformaldehyde + 2,5% glutaraldehyde in 0,1M phosphate buffer (pH 7.4), embedded in Epon and cut in 70 nm sections. The sections were mounted onto formvar-coated copper grids and stained with a 5% solution of uranyl acetate, followed by Reynold's lead citrate. Sections were viewed with Philips CM100 Transmission Electron Microscope. The myofibril density was determined with ImageJ software and expressed as percentage of myofibrils of whole cell area in that picture of which the nucleus was excluded. For each sample 2-7 different images were analysed in order to determine average myofibril density. Maximal, and passive forces were corrected for average myofibril density of the corresponding sample.

Statistics
Graphpad Prism v7 software was used for statistical analysis. Means were compared between groups with T-test if data was confirmed to be normally distributed. Maximal force data was not normally distributed and therefore means were compared with a Mann-Whitney test. A p-value<0.05 was considered to represent a statistically significantly difference between groups. N= number of samples, n= number of cardiomyocytes measured.

Ethical approval
This study was approved by the local ethics board of the Erasmus Medical Center (protocol number MEC-2015-233) and written consent of patients and/or parents was obtained. Samples were obtained during cardiac transplantation. As we do not have access to control cardiac tissue from age-matched individuals, 18 control samples were used from explanted Left ventricular (LV) heart tissue of healthy donors (age range 21 to 65 years old); people died from a non-cardiac cause, typically motor vehicle accidents and were acquired from the University of Sydney, Australia, with the ethical approval of the Human Research Ethics Committee #2012/2814. The codes of used samples are: 6.034, 8.004, 5.086, 3.141, 3.164, 4.049, 4.104, 7.040, 6.020, 5.128, 3.160, 6.008, 7.054, 7.044, 6.056, 3.112 6.042 and 3.162. All samples were stored in liquid nitrogen or at -80^{o}C until use.

Author contributions
IB performed and analyzed the contractile force experiments and titin isoform composition analysis. IB imaged samples with TEM and analyzed myofibril density. IB performed overall data interpretation and manuscript production. MM and MD acquired patient samples and clinical data and assisted with data interpretation and manuscript production. KG performed and analyzed protein phosphorylation experiments. DK and JV were involved in overall study design, data interpretation and manuscript production.

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RESULTS

Patient characteristics

Heart tissue was obtained from 9 pediatric patients diagnosed with DCM and 2 pediatric patients diagnosed with non-compaction cardiomyopathy during cardiac transplantation. Z-scores are commonly used to define growth of the heart during development and are used to distinguish between physiological and pathological changes in pediatric patients (14). Patient characteristics are shown in Table 1. LV end systolic diameter (LVESD) and LV end diastolic diameter (LVEDD) were increased and LV posterior wall systole (LVPWs) and LV posterior wall diastole (LVPWd) were decreased, all in line with the dilated phenotype and diagnosis of DCM.

Table 1. Patient characteristics

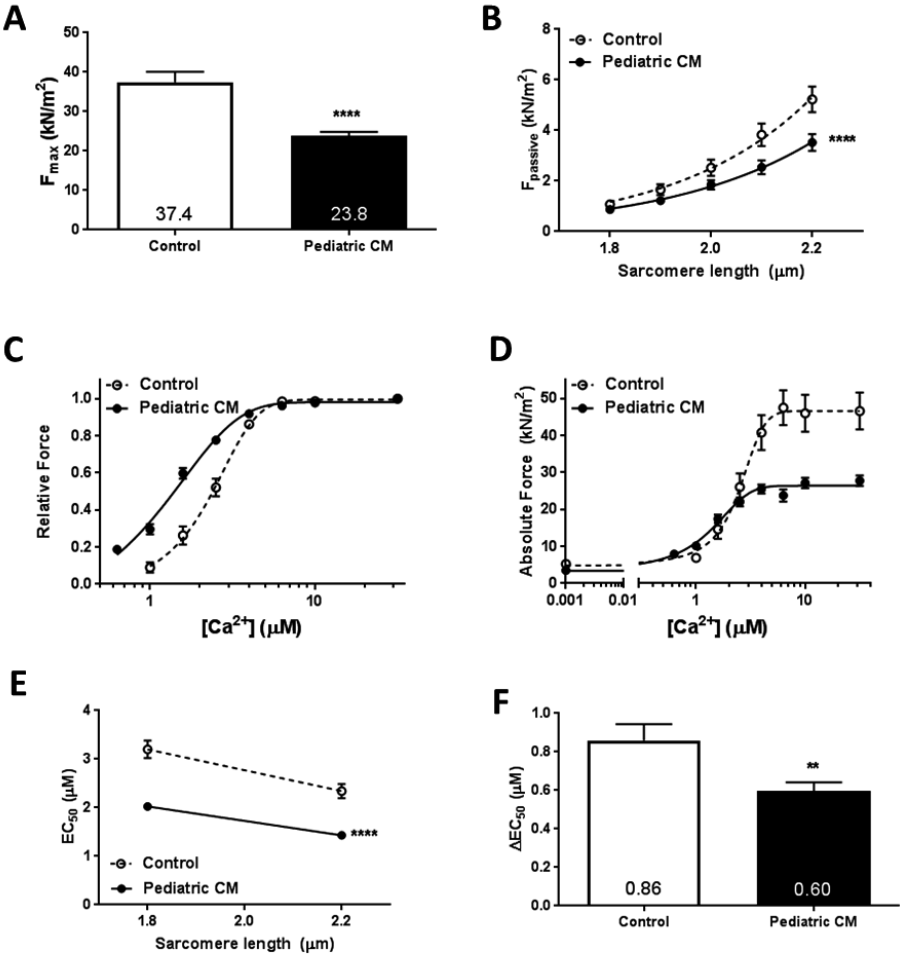
	Controls (N=18)	Pediatric CM (N=11)
Age	44,1 ± 3,1 years (N=18)	10,5 ± 1,3 years (N=11)
Sex (% male)	55,6% (N=18)	45,5% (N=11)
Time between echo and HTX		15 days (11-64)
LVEDD Z-score		7,9 ± 1.0 (N=11)
LVESD Z-score		11,7 ± 1,0 (N=11)
LVPWd Z-score		-0,8 ± 0,5 (N-11)
LVPWs Z-score		-3,4 ± 0,5 (N=8)
FS % at presentation		8 (7-10)
FS % at HTx		15 (8-19)
NT-pro-BNP at HTx		483 (206-1034)

Age, LVEDD, LVESD, LVPWd, LVPWs are shown as mean ±SEM.
Time between echo and HTX, FS% and NT-pro-BNP (pmol/L) are shown as median (interquartile range).
HTX: Cardiac transplantation.

Hypocontractility and increased myofilament Ca^{2+}-sensitivity in pediatric CM compared to controls

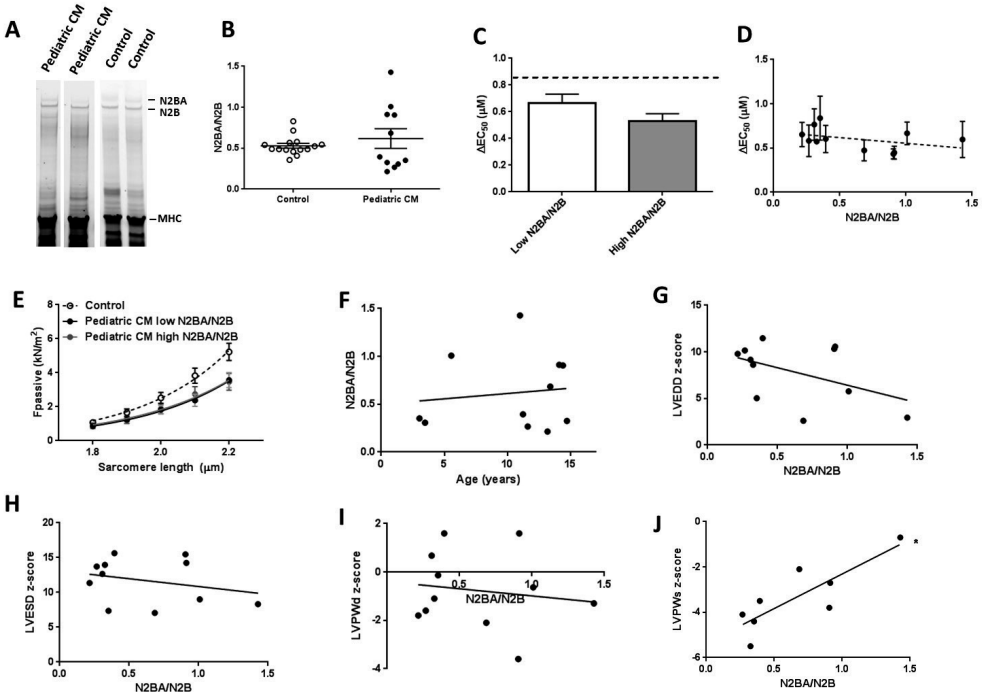
Measurements in single cardiomyocytes revealed significantly lower F_{max} (Figure 1A) and F_{pass} (Figure 1B) in pediatric CM compared to controls. A leftward shift of the force-$[Ca^{2+}]$ curve indicated an increased myofilament Ca^{2+}-sensitivity in pediatric CM compared to controls (Figure 1C). The combination of reduced F_{max}, reduced F_{pass} and increased Ca^{2+}-sensitivity of myofilaments results in lower force development at high (saturating) $[Ca^{2+}]$, lower force at very low $[Ca^{2+}]$ and higher force development at intermediate $[Ca^{2+}]$ respectively (Figure 1D). The increase in myofilament Ca^{2+}-sensitivity was evident at both sarcomere lengths (1.8 µm and 2.2 µm; Figure 1E) and associated with impaired length-dependent activation depicted as ΔEC_{50} (Figure 1F).

Figure 1. Baseline characteristics



A: Maximal force was significantly lower in pediatric CM (23.8±1.1, N=11, n=78) compared to controls (37.4±2.6, N=6, n=27, p<0.0001). **B**: F_{pass} was significantly lower in pediatric CM (N=11, n=40) compared to controls (N=10, n=29, p<0.0001) over a range of sarcomere lengths. **C**: A leftward shift of the relative force vs $[Ca^{2+}]$ indicates higher myofilament Ca^{2+} -sensitivity in pediatric CM (N=11, n=38) compared to controls (N=5, n=12, p<0.0001). **D**: The absolute force development over a range of $[Ca^{2+}]$ showed pediatric CM have impaired maximal force development at saturating $[Ca^{2+}]$, increased force development at lower $[Ca^{2+}]$ and a decreased F_{pass}. **E**: Ca^{2+}-sensitivity was significantly higher at sarcomere length 1.8 and 2.2 in pediatric CM (N=11, n=38) compared to controls (N=5, n=12, p<0.0001). **F**: LDA, measured as ΔEC_{50} was significantly lower in pediatric CM (0.60±0.05, N=11, n=38) compared to controls (0.86±0.09, N=5, n=12, p=0.007). N= number of samples, n= number of cardiomyocytes measured.

Figure 2. Titin isoform composition has limited effect on contractility in pediatric CM



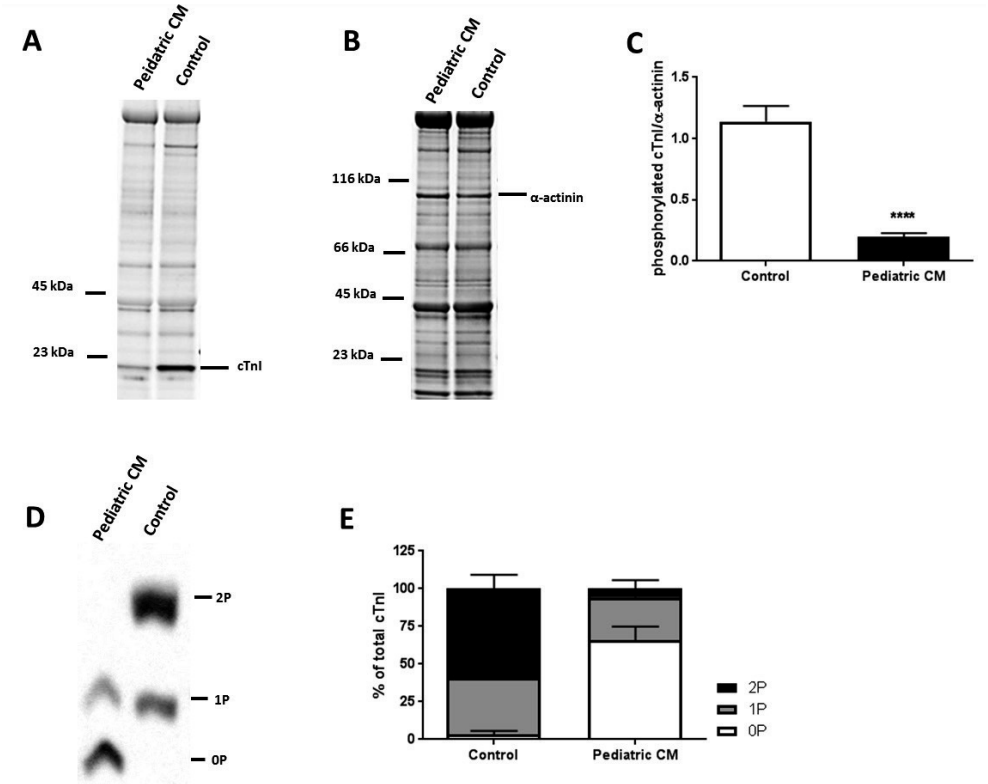
A: Separation of titin N2BA and N2B with gel electrophoresis. **B:** N2BA/N2B ratio was not significantly different between pediatric CM (0.62±0.12, N=11) and controls (0.53±0.03, N=15). **C**: LDA was mostly impaired in pediatric CM patients who had higher N2BA/N2B ratio (N2BA/N2B >0.65, N=5, n=19) compared to pediatric CM patients who had lower N2BA/N2B ratio (N2BA/N2B <0.4, N=6, n=19). Dotted line indicates control values. **D**: Mean ΔEC_{50} per sample plotted against the N2BA/N2B ratio did not show a significant correlation between ΔEC_{50} and N2BA/N2B. **E:** There was no difference in F_{pass} between pediatric CM patients who had higher N2BA/N2B ratio (N2BA/N2B >0.65, N=5, n=18) compared to pediatric CM patients who had lowerN2BA/N2B ratio (N2BA/N2B <0.4, N=6, n=22). **F**: N2BA/N2B ratio was not significantly related to age. **G:** A higher N2BA/N2B ratio was non-significantly associated with a smaller LVEDD z-score. **H:** A small non-significant trend was observed in which a higher N2BA/N2B ratio was associated with a smaller LVESD z-score. **I:** No correlation was observed between N2BA/N2B and LVPWd z-score. **J**: A significant correlation was found in which a higher N2BA/N2B ratio was associated with a less reduced LVPWs z-score ($p<0.05$). N= number of samples, n= number of cardiomyocytes measured.

Titin isoform composition variation in pediatric CM has limited effect on contractility

A shift towards more N2BA titin has been shown to lower F_{pass} (11, 12, 15) and reduce length-dependent activation (16). Analysis of titin isoform composition did not reveal a difference in the N2BA/N2B ratio between pediatric CM and controls (Figure 2A,B). However, a wide variation in titin isoform composition was observed in the pediatric CM group (Figure 2B). Since titin isoform composition has been shown to affect length-dependent activation we divided samples into a group that had a high N2BA/N2B ratio (N2BA/N2B>0.65) and a low N2BA/N2B ratio (N2BA/N2B<0.4). The group of samples with

a high N2BA/N2B ratio showed a greater reduction in ΔEC_{50} than the group of samples with a low N2BA/N2B ratio (Figure 2C). However, ΔEC_{50} was not significantly different between the two groups. In addition, ΔEC_{50} did not significantly correlate with the N2BA/N2B ratio (Figure 2D). Accordingly, F_{pass} was reduced to the same extent in the groups of pediatric CM samples with a relatively high N2BA/N2B ratio (N2BA/N2B>0.65) and a low N2BA/N2B ratio (N2BA/N2B<0.4) (Figure 2E). This indicated that other factors underlie the reduction of F_{pass}. The wide spread in age of our patient population was not responsible for the spread of titin isoform composition since no correlation was found between age and N2BA/N2B ratio (Figure 2F). We observed a significant correlation between N2BA/N2B and LVPWs (Figure 2J) in which a high N2BA/N2B ratio was associated with a less negative LVPWs z-score. This implies that patients with an increase in compliant titin had a smaller reduction in systolic LV wall thickness.

Figure 3. Hypophosphoryalation in pediatric CM compared to controls

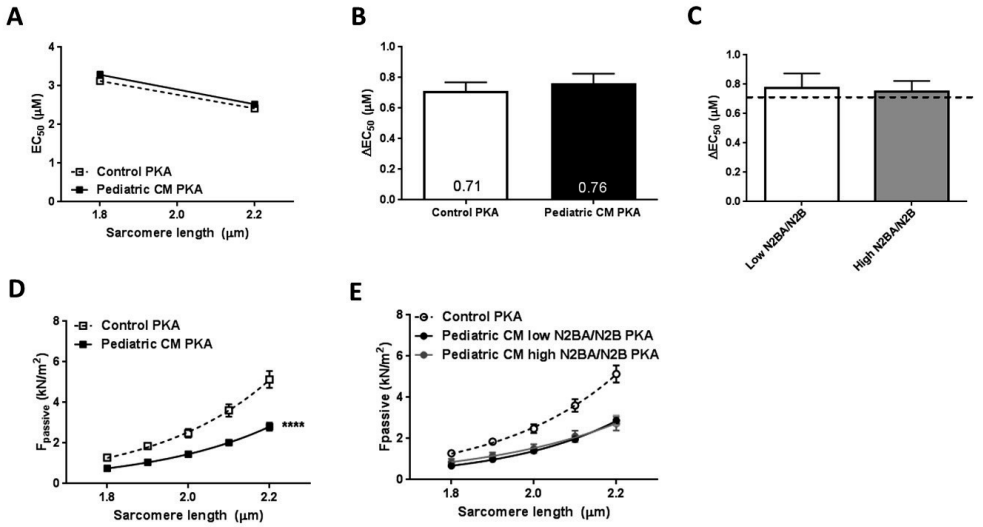


A: ProQ staining identifying phosphorylated proteins of pediatric CM and control samples. **B:** Corresponding SYPRO staining identifying proteins of pediatric CM and control samples. **C:** cTnI phosphorylation is significantly decreased in pediatric CM (N=13) compared to controls (N=13, p<0.0001). **D:** Phostag showed separation of non-, mono- and bisphosphorylated cTnI. **E:** While controls (N=11) showed predominatly mono- and bisphosphorylated cTnI, pediatric CM samples (N=11) showed mostly non-phosphorylated cTnI.

Reduced phosphorylation of cTnI in pediatric CM compared to controls

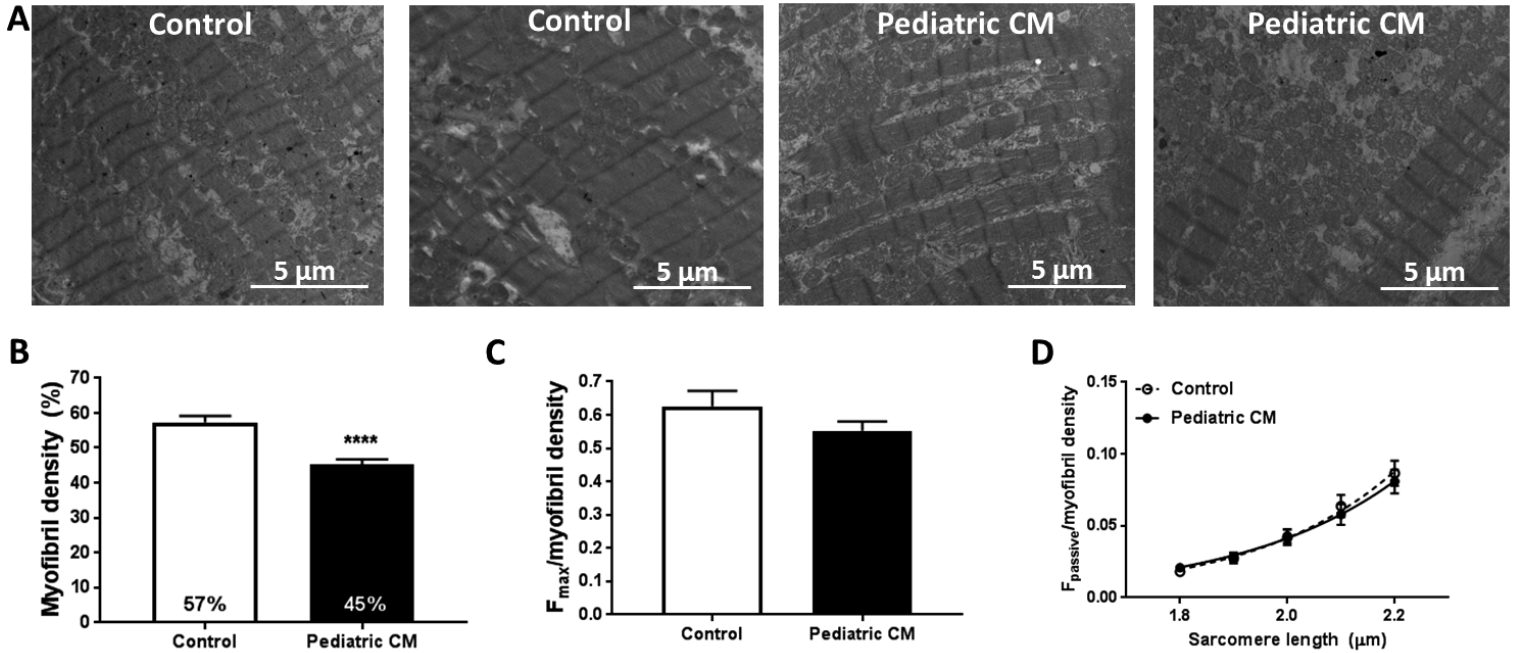
Phosphorylation of cTnI is reduced in various forms of adult heart failure and causes increased myofilament Ca^{2+}-sensitivity (13, 17-19). In line with published data in adult DCM (13, 18, 20), phosphorylation of cTnI was significantly lower in pediatric CM compared to controls (Figure 3A,B,C). PhosTag analysis showed separation of non-, mono- and bisphosphorylated cTnI (Figure 3D). While controls showed predominantly bisphosphorylated cTnI, in pediatric CM patients the non-phosphorylated cTnI was more prevalent (Figure 3E). In order to confirm that cTnI hypophosphorylation causes the high myofilament Ca^{2+}-sensitivity in pediatric CM samples, force measurements were repeated after incubation with exogenous protein kinase A which phosphorylates cTnI. Both myofilament Ca^{2+}-sensitivity and LDA normalized to control values upon incubation with exogenous PKA (Figure 4A,B). With normalized phosphorylation status of cTnI, there was no difference in ΔEC_{50} between the group of pediatric CM samples with a low N2BA/N2B ratio and a high N2BA/N2B ratio (Figure 4C). F_{pass} also remained low after incubation with exogenous PKA (Figure 4D). In addition, no difference in F_{pass} was observed between PKA-treated groups of pediatric CM samples with different N2BA/N2B ratios (Figure 4E).

Figure 4. Restoration of sarcomere function after incubation with exogenous PKA



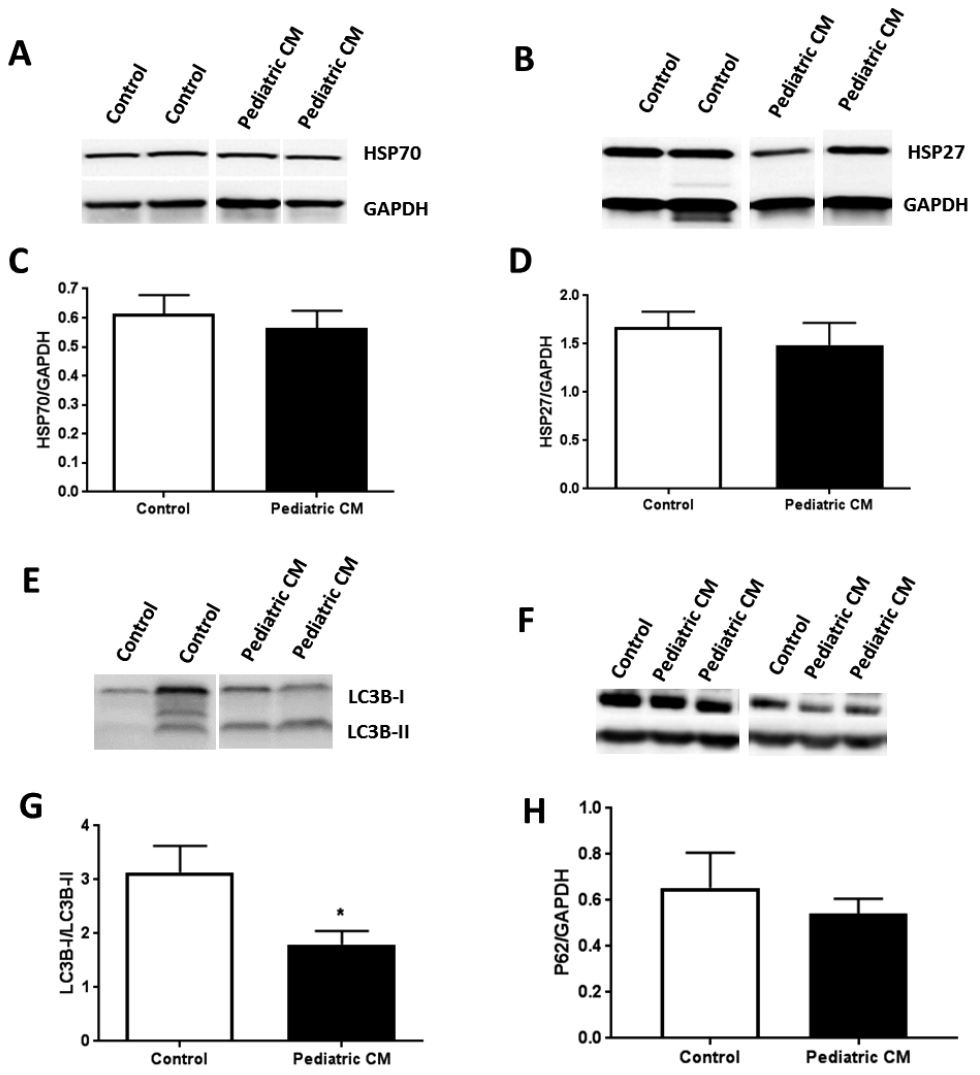
A: Exogenous PKA restored myofilament Ca^{2+}-sensitivity in pediatric CM (N=11, n=30) to control (n=5, n=12) values. **B**: Exogenous PKA eliminated the difference in length-dependent activation (ΔEC_{50}) between pediatric CM patients with higher N2BA/N2B ratio (N2BA/N2B >0.65, N=5, n=14) than pediatric CM patients who had lower N2BA/N2B ratio (N2BA/N2B <0.4, N=6, n=16) ; relative to control value. Dotted line indicates control values. **C**: There was no difference between patients with high or low N2BA/N2B ratio with respect to ΔEC_{50} after incubation with exogenous PKA. **D**: Exogenous PKA did not affect F_{pass} in controls or pediatric CM and remained significantly lower in pediatric CM compared to controls. N= number of samples, n= number of cardiomyocytes measured.

Figure 5. Myofibril density is decreased in pediatric CM



A: Electron microscopy images of 2 control samples and 2 pediatric CM samples. B: Myofibril density was significantly lower (P<0.0001) in pediatric CM (N=10, 45.3%±1.4%) compared to controls (N=10, 57.3%±1.8%). C: F_{max} normalized for myofibril density of corresponding sample did not differ significantly between pediatric CM (N=10, n=71) and controls (N=6, n=27). D: F_{pass} normalized for myofibril density of corresponding sample was not significantly different between pediatric CM (N=10, n=37) and controls (N=9, n=25). N= number of samples, n= number of cardiomyocytes measured.

Figure 6. Protein quality control system in pediatric CM



A: Representative blot images for HSP27 expression. **B**: Representative blot images for HSP70 expression. **C:** Expression of HSP27 was not altered in pediatric CM (N=11) compared to controls (N=11). **D**: Expression of HSP70 was not altered in pediatric CM (N=11) compared to controls (N=11). **E:** Representative blot images for LC3BI and LC3B-II expression. **G:** Expression of LC3B-I/LC3-BII was significantly ($p<0.05$) reduced in pediatric CM (N=11) compared to controls (N=11). **F:** Representative blot images for p62 expression. **H:** Expression of p62 was not altered in pediatric CM (N=11) compared to controls (N=7).

Decreased myofibril density underlies the hypocontractility in pediatric CM
As titin isoform composition was ruled out as causes of the observed decrease in F_{pass} and F_{max}, we determined myofibril density with transmission electron microscopy. We observed a significant decrease in myofibril density in pediatric CM compared to controls (Figure 5A,B). Correction of F_{max} for myofibril density eliminated the difference between pediatric CM and controls indicating the hypocontractility was due to myolysis or the inability to create sufficient myofibrils (Figure 5C). Also F_{pass} of pediatric CM cardiomyocytes was restored to control values after correction for myofibril density (Figure 5D).

Protein quality control system is unaltered in pediatric CM
We then studied whether changes in the protein quality control system occurred, which may underlie reduced myofibril density. Heat shock proteins (HSPs) are upregulated in stressful situations in order to prevent protein denaturation and aid in refolding of misfolded proteins. We did not find an induction of HSP70 (Figure 6A,C) or the cytoskeletal HSP27 (Figure 6B,D) in our pediatric CM group compared to controls. We did find a significantly decreased LC3B1/LC3B-II ratio (Figure 6E,G), which implies an induction of autophagy. However, p62, another autophagy marker was not upregulated in pediatric CM compared to controls (Figure 6F,H).

DISCUSSION

Characterization of pediatric CM myocardium revealed reduced active and passive cardiomyocyte force development, high myofilament Ca^{2+}-sensitivity and a blunted length-dependent activation compared to controls. High myofilament Ca^{2+}-sensitivity and blunted length-dependent activation were explained by low PKA-mediated phosphorylation of cTnI, which is a general feature observed in cardiac disease. We showed that the decrease in F_{max} and F_{pass} is due to decreased myofibril density.

No indications for alterations in protein quality control system in pediatric CM
We observed a decrease in myofibril density which was causal to the hypocontractile cellular phenotype in pediatric CM. Reduced HSP expression has been shown to increase cardiac damage after brief ischemia, and pretreatment with heat to induce HSPs reduced cardiac damage after infarction. However, in conditions of continuous stress as is the case in DCM, the HSP responses are less clear. HSP27 has been shown to be upregulated in adult DCM and not in ischemic heart disease (21). There are conflicting reports about HSP70 in adult DCM ranging from no change (21), to an increased expression (22). HSPs may lose their responsiveness in conditions of continuous cardiac stress. We did not find an induction of heat shock response, and we also did not find more autophagosomes in pediatric CM compared to controls. Future studies are warranted to reveal if reduced myofibril density in pediatric CM is due to an inability of cardioymocytes to increase myofibril synthesis and/or is caused by increased myofibril degradation.

Low PKA-mediated phosphorylation, high myofilament Ca^{2+}-sensitivity and blunted length-dependent activation

In line with what has been found in adult DCM patients (13, 17), we observed decreased PKA-mediated phosphorylation and coincident increased Ca^{2+}-sensitivity due to low cTnI phosphorylation in pediatric CM patients. Hypophosphorylation of cTnI has been shown to occur in various forms of heart failure. It is likely due to desensitization of the β-adrenergic receptor signaling pathway and subsequent decrease in PKA-mediated phosphorylation (23). Hypophosphorylation of cTnI has been shown to underlie a blunted length-dependent activation (20, 24, 25). Treatment of cardiomyocytes with exogenous PKA corrected both Ca^{2+}-sensitivity of myofilaments and length-dependent activation, independent of the titin isoform composition present in the heart.

Titin isoform composition is highly diverse in pediatric CM

Titin is composed of two isoforms, a compliant N2BA and a stiff N2B isoform. On average we observed no significant change in titin isoform composition in pediatric CM compared to controls. An increase in N2BA/N2B ratio has been reported in various forms of heart failure including adult DCM (11-13) and is considered a general hallmark of DCM. However, in our pediatric CM study population we observed a wide variation of N2BA/N2B ratios: 5 patients showed a higher N2BA/N2B ratio, while 6 patients showed a normal or even lower N2BA/N2B ratio compared to controls. An increase in N2BA titin has been shown to cause a blunted length-dependent activation in animal models, (16, 24, 26, 27) while we only observed a modest effect of N2BA on length-dependent activation of myofilaments in human pediatric CM samples. The length-dependent increase in myofilament Ca^{2+}-sensitivity was slightly, though not significantly lower in pediatric CM samples with a high N2BA/N2B compared to samples with a low N2BA/N2B ratio. Length-dependent activation of myofilaments was normalized in all pediatric CM samples to control values after incubation with PKA which indicates that the increase in compliant titin only affects length-dependent activation when cTnI is hypophosphorylated. This is in line with what has been reported in adult DCM (13, 18). While increased N2BA/N2B ratio has been associated with impaired systolic function, a low N2BA/N2B ratio is associated with improved diastolic function and a positive correlation between N2BA/N2B and peak oxygen consumption, a measure for exercise tolerance, in DCM patients has been found (11). We observed that a high N2BA/N2B ratio coincided with a smaller reduction in LV wall thickness during systole (LVPWs). An increase in N2BA/N2B ratio may represent a compensatory mechanism in order to cope with altered cardiac stress. Overall, the increase in N2BA/N2B did not have a large impact on sarcomere function. However, it should be stated that all studied heart tissue was derived from end-stage pediatric CM patients and therefore might suffer from severe cardiac remodeling. It might be possible that N2BA/N2B aids in this remodeling in a positive way by creating more flexibility in the sarcomeric structure to function under overstretched conditions at the initial disease stage.

Cardiac remodeling: friend or foe?

The increase in compliant titin might not have a direct causal role in disease pathogenesis, but may rather represent an adaptive response in order to cope with altered cardiac demand. The inability of pediatric CM patients to upregulate N2BA expression might be a reflection of their limited capability to adapt to altered cardiac demands. This is in line with

Patel et al. who recently published limited adverse remodeling in pediatric CM patients compared to adult onset DCM (28). They showed that hypertrophy and perivascular and interstitial fibrosis increased in adult DCM but not in pediatric CM patients compared age-matched controls. In addition, they showed sarcomere thickness is increased in adult DCM, but not in pediatric CM (28). Together with our results this implies that limited cardiac remodeling in pediatric CM patients might hamper the hearts to cope with altered cardiac demands and might have contributed to their early disease onset and severity. However, whether the limited adaptive capabilities of the heart are indeed causal to the early and progressive disease onset warrants further research.

Limitations

We have compared pediatric DCM patients with healthy adult controls since acquisition of healthy control tissue of children is near impossible. However, we did not find any correlations between age and protein expression or sarcomere function. Therefore we believe the observed effects are not related to difference in age alone.

Three patients were supported with a ventricular assist device (VAD) prior to transplantation. The duration of VAD support was short, 1-2 months. We did not see a difference in any parameter between patients that were supported with a VAD or not prior to transplantation. However, we can not exclude that despite the short VAD support duration, VAD induced cardiac remodeling.

Conclusion

In summary, we show that pediatric CM patients harbor similar changes in protein modifications and sarcomeric function compared to adult DCM. Hypophoshorylation of cTnI, most likely due to secondary disease remodeling and desensitized β-adrenergic receptor signaling, led to increased Ca^{2+}-sensitivity and blunted length-dependent activation of myofilaments. However, we did not find a consistent upregulation of compliant N2BA titin as has been observed in adult DCM. Increased N2BA/N2B ratio was significantly related to a lower LVPWs z-score. The limited cardiac remodeling in pediatric CM patients, illustrated in this study by the limited shift in titin isoform composition, might have hampered the ability to cope with altered cardiac demands and might have contributed to their early disease onset and progression. Cardiomyocyte hypocontractility was observed which was due to a decrease in myofibril density. The severe reduction in force generating capacity of cardiomyocytes may underlie the fast progression of cardiac disease in pediatric patients.

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Does repeated measurement of a six-minute walk test contribute to risk prediction in children with dilated cardiomyopathy?

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ABSTRACT

BACKGROUND A single six-minute walk test (6MWT) can be used to identify children with dilated cardiomyopathy (DCM) with a high risk of death or heart transplantation.

OBJECTIVES To determine if repeated 6MWT has added value in addition to a single 6MWT in predicting death or heart transplantation in children with DCM.

METHODS Prospective multi-center cohort study including ambulatory DCM patients ≥ 6 years. A 6MWT was performed 1 to 4 times per year. The distance walked was expressed as percentage of predicted (6MWD%) . We compared the temporal evolution of 6MWD% in patients with and without the study endpoint (SE: all-cause death or heart transplantation), using a linear mixed effects model.

RESULTS In 57 patients, we obtained a median of 4 (IQR 2-6) 6MWTs per patient during a median of 3.0 years of observation (IQR 1.5-5.1). Fourteen patients reached a SE (3 deaths, 11 heart transplantations). At any time during follow-up, the average estimate of 6MWD% was significantly lower in patients with a SE compared to patients without a SE. In both patients groups, 6MWD% remained constant over time. An absolute 1% lower 6MWD% was associated with an 11% higher risk (hazard) of the SE (HR 0.90, 95% CI 0.86-0.95 $p<0.001$).

CONCLUSION Children with DCM who died or underwent heart transplantation, had systematically reduced 6MWD%. The performance of all patients was stable over time, so repeated measurement of 6MWT within this time frame had little added value over a single test.

INTRODUCTION

The 6-minute walk test (6MWT) is a safe, simple and well-accepted prognostic tool in adults with heart failure (1). The test is used as an outcome measure in clinical trials, and short-term change in 6MWT is considered an indicator of prognosis as well (2-4).

In children, the 6MWT is also feasible and has been shown to be reproducible (5). The distance walked in a 6MWT can be expressed as a percentage of predicted, taken into account height, gender and age (6MWD%) (6). The 6MWT has been successfully used to predict outcome in pediatric patients with pulmonary hypertension (7) and to evaluate therapy effects in patients with Duchenne muscular dystrophy (8) and pulmonary hypertension (9). Previously, we showed that in children with dilated cardiomyopathy (DCM), a single 6MWD% below 63% identified patients with the highest risk of dying or heart transplantation (HTx)(10).

In this study we report the results of repeated 6MWT in children with DCM. We studied the evolution of 6MWD% over time in relation to death or HTx. We aim to determine whether 6MWD% changes over time, and if so, if this change is associated with the risk of dying or HTx. We hypothesize that repeated measurement of 6MWT has added value over a single measurement in predicting these clinical outcomes.

METHODS

Data were collected in a multi-center, prospective study design. In total, patients from eight tertiary cardiac centers were included, covering potentially the whole Dutch population of children with DCM. Our research program started in October 2010 and ended July 2017. In this period, we enrolled children with a previous diagnosis of DCM until 2010, relatively late after diagnosis, or with a new diagnosis from 2010 and onward, early after diagnosis. DCM was defined as the presence of impaired systolic function (fractional shortening (FS) ≤25%) and left ventricular (LV) dilation (LV end-diastolic dimension (LVEDD)> +2 Z-score for body surface area). Patients with neuromuscular disease, cognitive impairment or structural heart disease were excluded. The research program was organized in such a way that study visits coincided with routine outpatient clinic visits. In the first year after diagnosis 6MWTs were obtained 1 to 4 times per year, and 1 to 2 times per year thereafter, dependent on the frequency of visits. This study was approved by the medical ethical committee of the Erasmus MC (MEC 2014-062) and performed in accordance with the declaration of Helsinki. All parents and children ≥12 years gave their written informed consent.

All included patients were asked to perform 6MWTs as part of our follow-up study program, at the outpatient clinic in the participating centers. The 6MWT was conducted according to the guidelines of the American Thoracic Society (11). The local conditions at the outpatient clinics required adaptation of the walking track, as described previously (10). In summary, patients were instructed to walk as far as possible on an 8-meter track within a 6-minute time frame. Patients were instructed and encouraged in a standardized

manner. Running was not allowed, if necessary, patients could slow down or stop walking. After 6 minutes, the total amount of 16 meter 'laps' was counted and the distance walked in a partially finished lap was added, which resulted in a total walking distance (6 MWD). If the patient stopped walking before the end of 6 minutes, e.g. due to fatigue, we accepted the walked distance as 6MWD. All 6MWT study data were collected by study personnel and stored in the study database, the treating cardiologists were blinded to the study results. The distance covered was expressed as percentage of predicted (6MWD%) according to Geiger et al, accounting for height, gender and age (6). Calculation of 6MWD% was done after closure of the database and used for study purposes only.

As part of the follow up study, in addition to the 6MWT, multiple data were recorded on the same patient visit: weight and height, current heart failure medication and dosage, New York Pediatric Heart Failure Index (NYPHFI), NT pro-BNP and a standardized echocardiogram including left ventricular end-diastolic dimension (LVEDD) and fractional shortening (FS).

The study endpoint was death or heart transplantation (HTx). The decision to list a patient for HTx was made by a team of treating physicians based on commonly accepted criteria (12). The team was blinded to the results of the 6MWT. In addition, we recorded the status of the patients at the end of the study, after their last study visit: ongoing disease or recovered. Recovery was defined as 2 consecutive echocardiograms with normalized LVEDD and FS, the date of the first normalized echocardiogram was considered as date of recovery. Echocardiograms were analyzed and reviewed by study personnel who were blinded to the patient's name, previous echocardiograms, and other study results.

Continuous variables with normal distribution are described as mean (standard deviation, sd), or as median (interquartile range, IQR) otherwise. Categorical variables are described as numbers and percentages. Differences in 6MWT characteristics between patients with and without the study endpoint were compared by Student's t-tests (normal distribution) or Mann-Whitney tests.

We applied a linear mixed effects model (LMEM) for longitudinal data to study the temporal evolution of 6MWD%, while accounting for the correlation between measurements in the individual patient. Subsequently, the LMEM was combined with a Cox proportional hazard regression model in a so-called joint model (JM) to explore the association between 6MWD% (repeatedly measured) and the study endpoint. We included time since diagnosis as covariate in the JM.

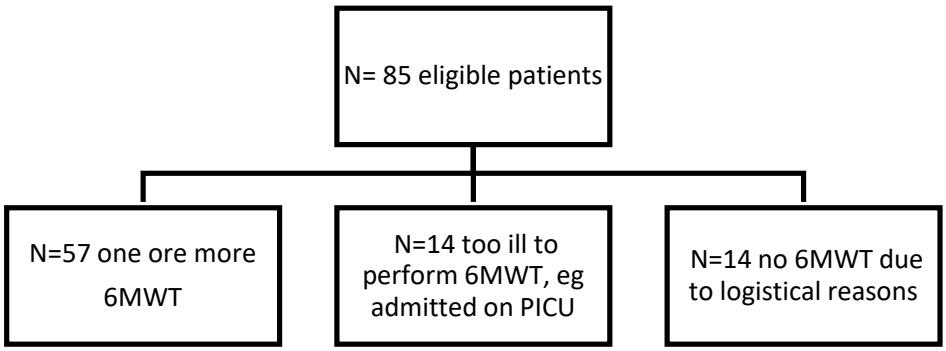
We studied cumulative event-free survival of the study endpoint by the method of Kaplan-Meier. In a previous study we showed that pediatric DCM patients with baseline 6MWD% <63% have high risk of death or heart transplant during prolonged follow-up (10). Against this background, we separated our study cohort according to this threshold, and compared cumulative event-free survival by a log-rank test.

The level of statistical significance for all analyses was set at p=0.05. Analyses were performed using IBM SPSS statistics 24 (IBM, New York, USA) and R statistical software version 3.5.1 (package JMbayes).

RESULTS

Eighty-five patients met the inclusion criteria, of which 57 performed at least one 6MWT. Twenty-eight patients did not perform a 6MWT: 14 patients were too ill (eg admitted at the PICU) and reached an endpoint before a 6MWT could be obtained. Another 14 patients did not perform a 6MWT due to logistical reasons (Figure 1.). Nineteen of the 57 patients were included early after diagnosis, whereas 38 patients had been diagnosed before 2010 and were included relatively late after diagnosis. Patient characteristics are described in Table 1. At the first 6MWT, the median age was 11.1 years (IQR 7.3-14.5), median time since diagnosis was 3.6 years (IQR 0.5-7.1) and median time since study inclusion was 0.1 years (IQR 0.0-0.9). Idiopathic DCM was diagnosed in 47%, and the majority of patients was treated with ACE- inhibitors (90%) and β-blockers (78%). The median NYPHFI at the baseline 6MWT was 8 (IQR 4-11;Table 1). For 49 patients we previously reported the results of a single 6MWT and outcome(10).

Figure 1. Flow diagram of eligible patients and included patients.



6MWT 6-minute walk test, PICU pediatric intensive care unit

Median observation time per patient was 3.0 years, (IQR 1.5-5.1), in patients with the study endpoint median observation was 1.3 years (IQR 0.2-2.1) and in patients without the study endpoint median observation was 3.8 years (IQR 2.1-5.3; p= 0.001). In this time frame, 277 6MWTs were performed, and 47 of the 57 patients performed more than one 6MWT. The median number of 6MWTs per patient was 4 (IQR 2-6). The median number of 6MWTs per year follow up was 2.7 (IQR 1.0-2.9). The mean distance walked, including all available tests, was 462 meters (±122), the mean 6MWD% was 69% (±17) (Table 1.).

Table 1. Characteristics of the study cohort (n=57) at the moment of baseline 6-minute walk test

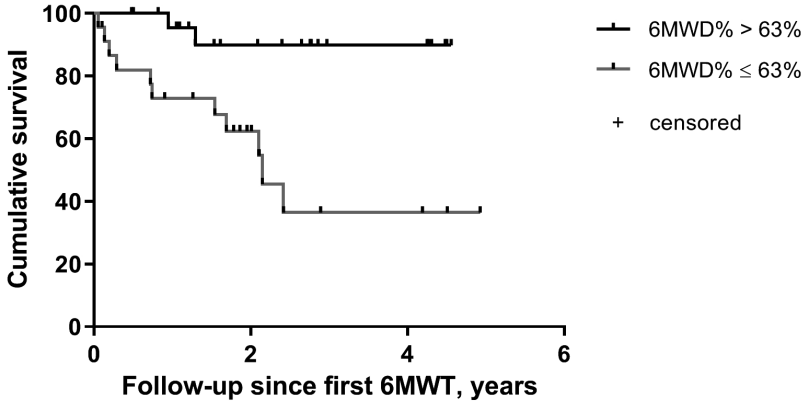
Time since diagnosis, years, median (IQR)	**3.5 (0.5-7.1)**
Time since study inclusion, years, median (IQR)	0.1 (0.1-0.9)
Gender, female , n(%)	68 (47%)
Age, years, median IQR	11.1 (IQR 7.3-14.5)
Cause of DCM (n,%)	
Idiopathic	26 (47)
Genetic or familial	8 (14)
Other	23 (40)
Heart failure score, NYPHFI median (IQR)	8 (4-11)
Medication, n(%)	
β-blockers	45 (78%)
Ace inhibitors	51 (90%)
Anti-coagulants	29 (51%)
Diuretics	32 (56%)
Echocardiografic parameters, mean(sd)	
LVEDD z score	4.7 (2.9)
SF	18.3 (6.7)
NT-pro BNP, pg/ml, median (IQR)	1873 (390-5021)
Endpoint	
Death/transplantation	14
Ongoing disease	39
Recovered	4
Number of 6MWT per patient, median (IQR)	4 (2-6)
6MWT, meters, mean (sd)	462 (122)
6MWD% of predicted, %, mean (sd)	69 (17)
Total follow up time per patient, years, median (IQR)	3.0 (1.5-5.1)

DCM dilated cardiomyopathy, 6MWT six-minute walk test, NYPHF New York University Pediatric Heart Failure Index, range 0-30, LVEDD Left Ventricular End Diastolic Dimension, SF Shortening Fraction

In the course of the study, 14 of the 57 patients in whom a 6MWT was available, reached a study endpoint: 11 patients were transplanted at a median of 5.7 years after diagnosis (IQR 2.5-11.0) and 3 patients died, 1.3 and 2.3 and 8.3 years after diagnosis. Death was caused by DCM related ventricular arrhythmia in one patient, end-stage heart failure in a setting of a contra-indication for HTx in a second patient, and multi-organ failure in a patient with an additional glycogen storage disease.

In patients who reached a study endpoint, median time from the last 6MWT to the study endpoint was 0.25 years (IQR 0.16-0.77). Median time since diagnosis to the first 6MWT was 3.2 years (IQR 0.4-6.8), which was the same as in patients who did not reach a study endpoint. The median number of 6MWTs was the same in patients with and without a study endpoint (Table 2). At the last follow up visit, 4 of the remaining 43 patients had recovered, whereas 39 patients had ongoing disease. Three recovered children showed an increase in 6MWD%, the fourth performed only one test. The low number of recovered patients did not allow for statistical analysis.

Figure 2. Transplant-free survival curves of DCM patients with 6MWD ≤ 63% of predicted compared to patients with 6MWD > 63% of predicted.



number at risk

6 MWD% > 63%	32	18	11
6 MWD% ≤ 63%	23	9	3

At the first 6MWT, median 6MWD% was 68% (IQR 53-82%). In patients with a study endpoint, 6MWD% was 53% (IQR 33-61), compared to 74% (IQR 60-84) in patients without a study endpoint (p=0.003) (Table 2.). Transplant free survival was significantly higher in patients with a first 6MWD% ≥ 63%. In children with a first 6MWD% ≥ 63%, one-year transplant-free survival was 96% (95% CI 89 to 100), two-year transplant-free survival was 92% (95% CI 81 to 100) in contrast to children with a first 6MWD% < 63% in whom one-year transplant-free survival was 74% (95% CI 56 to 92) and two-year transplant-free survival was 64% (95% CI 44 to 84) (log rank test p=0.002; Figure 2). Thus, a 6MWD% lower than 63% was associated with an increased risk of heart transplantation or death (hazard ratio 10.8; 95% CI 2.4 to 49).

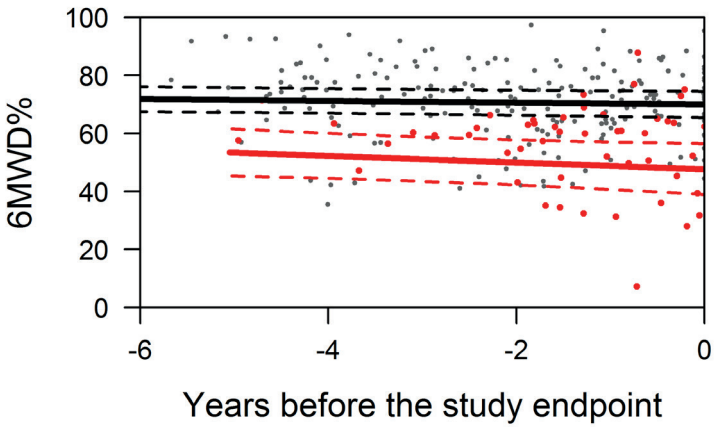
Table 2. Repeated measurement of 6MWT, comparing patients with a study endpoint to patients without a study endpoint

	All patients (n=57)	SE (n=14)	no SE (n=43)	p-value
Time Dx-fist 6MWT[b]	3.6 (0.5-7.1)	3.2 (0.4-6.8)	3.6 (0.5-7.4)	0.81
Time last 6MWD% to PEP or end of study[b]	0.08 (0.0-0.46)	0.25(0.16-0.77)	0.00(0.00-0.38)	0.004
Total FU time[b]	3.0 (1.3-5.1)	1.3 (0.2-2.1)	3.8 (2.1-5.3)	0.007
Number of 6MWT per patient	4 (2-6)	3 (1-6)	4 (2-7)	0.70
Number of 6MWT per year follow-up	2.7 (1.0-2.9)	3.7 (2.4-6.6)	1.6 (0.9-2.5)	0.004

SE Study Endpoint, Dx diagnosis
All numbers are median and Inter Quartile Range (IQR)
[a] percentage of predicted, calculated according to Geiger(6)
[b] years
Comparison of patients with SE vs patients without SE using Mann-Whitney test

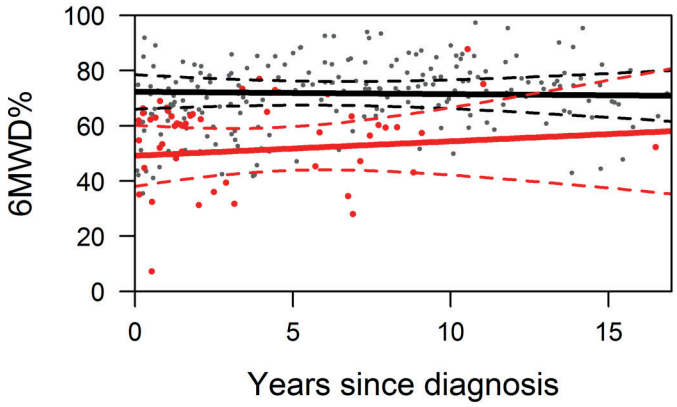
At any time during follow-up, the average estimate of 6MWD% was significantly lower in patients with a study endpoint compared to patients without a study endpoint (Figure 3.). Furthermore, we found no meaningful change in 6MWD% over time both in patients with and without a study endpoint (Figure 3.). Notably, in patients who reached an endpoint, 6MWD% did not change over time and did not suddenly decrease prior to the endpoint. When we plotted the results of the 6MWD% against years since diagnosis, we also found that they were constant over time (Figure 4.), indicating that there was no systematic difference in the results obtained early and later after diagnosis. The variability of the 6MWD% within the individual patient was considerable (Fig 5.), suggesting that obtaining more than one 6MWT would be useful to obtain a reliable mean estimate of the patient. An absolute 1% lower 6MWD% was associated with a 11% higher risk (hazard) of the study endpoint (HR 0.90, 95% CI 0.86-0.95 p<0.001). In clinical practice this means that when comparing 2 patients at any given moment after diagnosis, the one with a 1% lower 6MDW% has an 11% increased risk of death or HTx.

Figure 3. Serial measurement of 6MWD expressed as percentage of predicted, time before study endpoint.



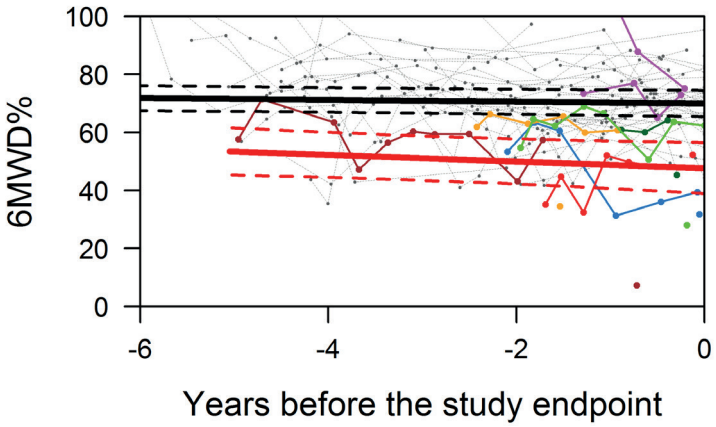
The average estimates of the longitudinal trajectory of 6MWD%: the black line indicates the patients without a study endpoint, the red line the patients with a study endpoint. The dashed lines depict the 95% confidence interval.

Figure 4. Serial measurement of 6MWD expressed as percentage of predicted, time since diagnosis



The average estimates of the longitudinal trajectory of 6MWD%: the black line indicates the patients without a study endpoint, the red line the patients with a study endpoint. The dashed lines depict the 95% confidence interval.

Figure 5 Serial measurement of 6MWD expressed as percentage of predicted, time before study endpoint.



The average estimates of the longitudinal trajectory of 6MWD%: the black line indicates the patients without a study endpoint, the red line the patients with a study endpoint. The dashed lines depict the 95% confidence interval. The individual patients are plotted, the colored lines indicate the patients with the study endpoint.

DISCUSSION

In this study we confirm the usefulness of the 6MWT as a tool to identify children with DCM at a high risk of adverse outcome. In the studied timeframe, 6MWD% was significantly lower in patients who reached the study endpoint of death or HTx, compared to those who did not. The 6MWD% remained constant throughout three years of observation, both in patients who reached the study endpoint, and in those who did not. As 6MWD% tends to vary within the individual patient, at least two 6MWTs seem to be needed to obtain a reliable adequate indication of 6MWD%. Thereafter, repeating the 6MWT seems to have little added value.

In adult heart failure literature, data on the use of serial 6MWT is scarce, but a number of studies have shown that repeating 6MWT is useful to evaluate effectiveness of therapeutic interventions. A short term improvement in 6 MWD after a drug intervention in hospitalized patients with chronic heart failure, was a significant independent predictor of survival (2). Likewise, in patients with moderate to severe heart failure, cardiac resynchronization therapy results in significant improvement in 6MWD and in survival (3). In contrast, in the majority of trials that were reviewed, the 6MWD did not increase after a pharmacological intervention in patients with chronic stable heart failure (13). Combining both findings would indicate that repeating the 6MWT is helpful to evaluate the effect of a therapeutic intervention on survival in patients with moderate to severe heart failure.

Upfront, we expected that 6MWD% would decrease in the patients who reached an endpoint. Also, we hypothesized that 6MWD% might increase in the combined group of patients who recover or have ongoing disease. However, our results did not confirm these expectations as 6MWD% was stable from early after diagnosis onward, both in children who reached an endpoint and those who did not. Moreover, it was significantly lower in those reaching an endpoint throughout the time of observation (Fig. 3). Notably, 6MWTs were only obtained in patients who were relatively stable. In two potentially unstable phases of the disease, directly after diagnosis and shortly before death or HTx, a substantial number of patients did not perform a 6MWT. Sixteen percent of potentially eligible patients never performed a 6MWT, because they were too ill and reached an endpoint before a 6MWT could be done. In that respect, not being able to perform a 6MWT can be considered an ominous sign. On the other end of the spectrum, most patients who did perform one or more 6MWT and reached an endpoint within the study period, did not perform a 6 MWT in the last phase of the disease: 8 of the 14 children with a study endpoint were listed for HTx after the last available 6MWT, and were hospitalized awaiting transplantation.

There are several limitations to this study. Firstly, we could not obtain a 6MWT in a number of patients due to logistical difficulties that come with a multicenter study. However, the 14 patients we missed were not significantly different from the study cohort in terms of age, gender, cause of DCM, NYPHFI and percentage of study endpoints (results not shown). We believe therefore that the study cohort was an unbiased selection and that missing 6MWTs have not led to an important change in the results and conclusions of our study. Secondly, the studied cohort is relatively small, though compared to other pediatric studies using 6MWT to predict outcome comparable or larger in the number of children

that we studied and the number of 6MWTs that were obtained. REFS Moreover, it is the first study on serial measurements of 6MWD% in children with DCM. Nevertheless, one could hypothesize that in a study with a larger cohort of children with DCM patients, we would have been able to demonstrate that 6MWD% significantly changes over time and that this change would identify patients with a higher risk of reaching an endpoint. The graph in Figure 3, however does not provide much support for that idea as it depicts that there is no change at all over time in 6MWD%, in both groups. We pose that expanding the study cohort would not lead to a fundamental change in the results we found. Thirdly, the great majority of study endpoints reached in this study was HTx, the number of deaths was low. This is in line with other studies reporting on outcomes in children with DCM, showing that in general, deaths occur relatively early after diagnosis and that HTx takes place later after diagnosis (14-16). In this respect it is fair to state that 6MWTs identify the patients at a high risk for HTx rather than the patients at a high risk to die. Fourthly, a similar amount of 6MWTs were performed in children who did and did not reach a study endpoint, but the 6MWT were performed in a shorter time frame. This reflects the clinical practice in which the most seriously affected children are seen more often at the outpatient clinic. Lastly, the patient cohort we studied contains a low number of children that recover compared to our previous reports and large cohorts of children with DCM. The fact that children under 6 years were not included in this study is probably an important explanation as recovery in this age group is relatively common (14-17).

We conclude, that based on the current study, 6MWT is a useful tool to identify children with DCM at a high risk of death or heart transplantation. In children who are able to perform a 6MWT, 6MWD% remains constant over time, early after diagnosis and in the years thereafter. In those reaching an endpoint, 6MWD% is significantly lower throughout time, than in those not reaching an endpoint. Initially, at least 2 6MWT are needed to reliably estimate 6MWD%, thereafter repeating 6 MWT has little added value.

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Predicting outcome in children with dilated cardiomyopathy: the use of repeated measurements of known risk factors for adverse outcome

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Submitted

ABSTRACT

AIMS We aimed to determine whether in children with dilated cardiomyopathy (DCM) repeated measurement of known risk factors for death or heart transplantation (HTx) can identify children at the highest risk for adverse outcome.

METHODS AND RESULTS Of 137 children we included in a prospective cohort, 36 (26%) reached the study endpoint (SE: all-cause death or HTx), 15 (11%) died and 21 (15%) underwent HTx. Median follow-up was 2.1 years [IQR 0.8-4.3]. Children who reached the SE can be distinguished from those who do not, based on the temporal evolution of 4 risk factors: stunting of length growth (-0.29 vs -0.04 length Z-score/year), less decrease in N-terminal pro-B-type natriuretic peptide (NT-proBNP) (-0.19 vs -0.41 2log pg/mL/year), no decrease in Left Ventricular Internal Diastolic Dimension (0.14 vs -0.23 Boston Z-score/year) and increase in New York University Pediatric Heart Failure Index (0.36 vs -0.49/year). When we compared children who reached the SE to those with ongoing disease (leaving out the children who recovered) we found similar results, though the effect was smaller. In multivariate models, NT-proBNP appeared the only independent predictor for adverse outcome, a twofold higher NT-proBNP was associated with an 2.8 times higher risk of the SE (HR 2.78, 95% CI 1.81 to 3.94, $p<0.001$).

CONCLUSION The evolution over time of NT-proBNP, LVIDd, length growth and NYU PHFI identified a subgroup of children with DCM at high risk for adverse outcome. In this sample, with a limited number of endpoints, NT-proBNP was the strongest independent predictor for adverse outcome.

INTRODUCTION

In children with dilated cardiomyopathy (DCM), up to 50% of children die or undergo heart transplantation (HTx) within 5 years after diagnosis (1-3).

To recognize the children at the highest risk for adverse outcome is essential as these are the patients who should be monitored closely and, importantly, be listed for heart transplantation at a timely stage.

Worldwide, several large pediatric DCM cohorts have reported risk factors for adverse outcome and identified older age (>6 year), worse left ventricular fractional shortening, congestive heart failure at presentation, idiopathic DCM and higher N-terminal pro-B-type natriuretic peptide (NT-proBNP) as risk factors for death or transplantation (1-4). Previously, we reported on similar risk factors in our national cohort of children with DCM (5-8), which we determined at the time of inclusion. However, changes in these risk factors during the course of the disease, such as the level of NT-proBNP or echo-derived fractional shortening, might be predictive of adverse outcomes as well (2, 6, 9).

In this present study we prospectively and repeatedly collected established clinical and echocardiographic risk factors for death and transplantation in a national cohort of children with DCM. We explored the temporal evolution of these factors, as these potentially hold relevant information for clinical decision making.

METHODS

Patients

Data were collected in a multi-center, prospective study design. Patients from eight tertiary cardiac centers were included from October 2010 to July 2017. We enrolled children with prior (until 2010) or new diagnosis (from 2010 onward). DCM was defined as the presence of impaired systolic function based on echocardiography: a fractional shortening (FS) ≤25% in combination with a left ventricular (LV) end-diastolic dimension (LVEDD)> +2 Z-score for body surface area. Patients with structural heart disease or neuromuscular disease were excluded. Data were collected during routine outpatient clinic visits or coincided with hospital admissions. In the first year after diagnosis patients were evaluated 1 to 4 times per year, and 1 to 2 times per year thereafter, depending on the frequency of outpatient visits. Subjects were followed until death or HTx, until the age of 18 years, or until the last outpatient visit within the study window. This study was approved by the Medical Ethical Committee of the Erasmus MC (MEC 2014-062) and was performed in accordance with the declaration of Helsinki. All parents as well as children ≥12 years gave written informed consent.

Study variables

In this study we focused on 6 known risk factors for adverse outcome.
NT-proBNP: NT-proBNP values were measured using the Roche® assay in all participating centers.

New York University Pediatric Heart Failure Index (NYU PHFI): pediatric heart failure score that quantifies the degree of heart failure in terms of symptoms and medication use and ranges from 0-30 points (10). The treating pediatric cardiologist completed the NYU PHFI.

Length Z-score: length was normalized to Z- scores using growth analyser©, which is based on reference values for Dutch children(11).

Left Ventricular Internal Diastolic diameter (LVIDd): a standardized echocardiogram was performed and data was analyzed using the mean value of three consecutive cardiac cycles. Echocardiograms were analyzed by study personnel who were blinded to the patient's name, previous echocardiograms, and other study results. The LVIDd was normalized to a Boston Z-score for BSA, age and gender (12).

Global longitudinal Strain (GLS): GLS was calculated as the mean of the peak strain of all left ventricular segments in a longitudinal 6-segment model as previously described (8).

6-minute walking distance (6MWD%): The distance walked within 6 minutes on a 8-meter track was recorded and expressed as percentage of predicted (6MWD%) according to Geiger et al, accounting for height, gender and age (7, 13).

The clinical team was blinded to the results of the 6MWD% and GLS, the other study variables were readily available. In addition to the above mentioned study variables, DCM etiology, age at diagnosis, weight (normalized to Z-score), and current heart failure medication was collected as well. Genetic etiology was defined as the presence of a mutation in a known pathogenic DCM gene. Myocarditis was accepted as etiology if diagnosis was definite or probable (14).

Study endpoint

The study endpoint (SE) was all cause death or HTx. We also recorded the status of the surviving patients at the end of the study period: ongoing disease or recovered. Recovery was defined as 2 consecutive echocardiograms with normalized LVIDd and FS, the date of the first normalized echocardiogram was considered as date of recovery.

Statistical analysis

Continuous variables with normal distribution are described as mean (standard deviation, SD), or as median (interquartile range, IQR) otherwise. Categorical variables are described as numbers and percentages. Differences in characteristics between patients with and without the study endpoint were compared by Student's t-tests (normal distribution) or Mann-Whitney tests. We applied the method of Kaplan-Meier to study survival and transplant-free survival.

To explore the temporal evolution of the study variables, while accounting for the correlation between measurements in the individual patient, we applied a linear mixed effects model (LMEM) for longitudinal data. To study the association between study variables (repeatedly measured) and the study endpoint, we subsequently combined the LMEM with a Cox proportional hazard regression model in a so-called joint model (JM).

In the multivariable joint model, we included stepwise the studied risk factors starting with NT-proBNP based on the fact that NT-proBNP has shown to be a robust marker for outcome. To further characterize the children who reached the study endpoint, we compared them with the patients who remained endpoint free, but still had ongoing disease at the end of the follow-up period, thus leaving out the children who recovered. Temporal evolution of the study variables was studied, in the same way as described above. The level of statistical significance for all analyses was set at p=0.05. Analyses were performed using IBM SPSS statistics 24 (IBM, New York, USA) and R statistical software version 3.5.1 (package JMbayes).

RESULTS

Patients and clinical outcome

A total of 137 children with DCM were included, 3 patients were lost to follow up. Of these 137 children, 96 were included directly after diagnosis, whereas 41 children had a prior diagnosis. Thirty-six children (26%) reached the study endpoint (SE), 15 children (11%) died and 21 children (15%) underwent transplantation. Seventeen children reached a study endpoint within 1 year after diagnosis (12 died and 5 underwent transplantation), and 9 of those 17 early endpoints were reached within 1 month after diagnosis (7 children died and 2 were transplanted). The majority of deaths occurred within 1 year after diagnosis, 12 out of 15 (80%). Median time from diagnosis to death was one month (0.09 years [IQR 0.03-0.7]), median time to HTx was 2.9 years [IQR 0.8 to 6.1]. At some stage, during the first year after inclusion, the majority of children was on heart failure medication: 84% of children was on an ACE-inhibitor, and 65% was on a β-blocker and 87% was on one of both. Sixteen of seventeen children who were not on heart failure medication reached a SE before medication could be started. All medication was up-titrated to the maximum tolerated dose at the discretion of the treating physician.

At the end of the study, after a median follow-up of 3.0 years [IQR 1.5-4.7], 23 children (24% of newly diagnosed patients) had recovered and 78 children (57%) had ongoing disease. Time from diagnosis to recovery was 0.6 years [IQR 0.5 to 1.4]. When comparing children who reached a SE and those who did not, we found no important differences in patient - and clinical characteristics, see Table 1.

One-year survival after first diagnosis was 88% (95% CI 83 to 95%) and 5-year survival of 82% (95% CI 74 to 90%). The transplant-free survival was 82% (95% CI 74 to 89%) after 1 year and 72% (95% CI 62 to 82%) after 5 years (Supplementary Figure 1).

In the children with a previous diagnosis (n=41), 13 patients (32%) reached a study endpoint, 2 children died and 11 patients underwent transplantation. Median time from diagnosis to inclusion was 3.8 years [IQR 2.5 to 8.7] years, median time from diagnosis to SE was 6.0 years [IQR 3.1to 9.7] year. At the end of the study, 28 patients had ongoing disease and no patient had recovered.

Table 1. Patient - and clinical characteristics at the time of diagnosis, comparing children who died or underwent transplantation with children who survived without transplantation

	Study Endpoint N=36	No Study Endpoint N=101
Age, years, IQR)*	5.3 [0.2-11.3]	0.9 [0.1-4.8]
< 1 year, n (%)	14 (39)	50 (49)
1 to < 6 years, n (%)	5 (14)	28 (28)
6 to < 18 years, n (%)	17 (47)	23 (23)
Female**	23 (64)	45 (45)
Time Dx to study inclusion, years, [IQR]*	0.01[0.0-2.5]	0.05 [0.0-2.3]
Time inclusion to last FU, years [IQR]*	0.15 [0-1.7]†	3.0 [1.5-4.7]
Time diagnosis to last FU	1.3 [0.1-4.3] †	3.5 [2.0-6.1]
Primary diagnosis, n (%)		
Idiopathic	15 (42)	47 (47)
Myocarditis	2 (6)	18 (17)
Other	19 (53	36 (36)
Weight for age, Z-score, mean (SD)***	-0.6 (1.7)	-0.8 (1.3)
Weight for height, Z-score, mean (SD)	-0.4 (1.5)	-0.4 (1.8)
Height for age, Z-score, mean (SD)	-0.4 (1.4)	-0.5 (1.5)
Shortening fraction (%)	13 (9-16)	16 (12-20)

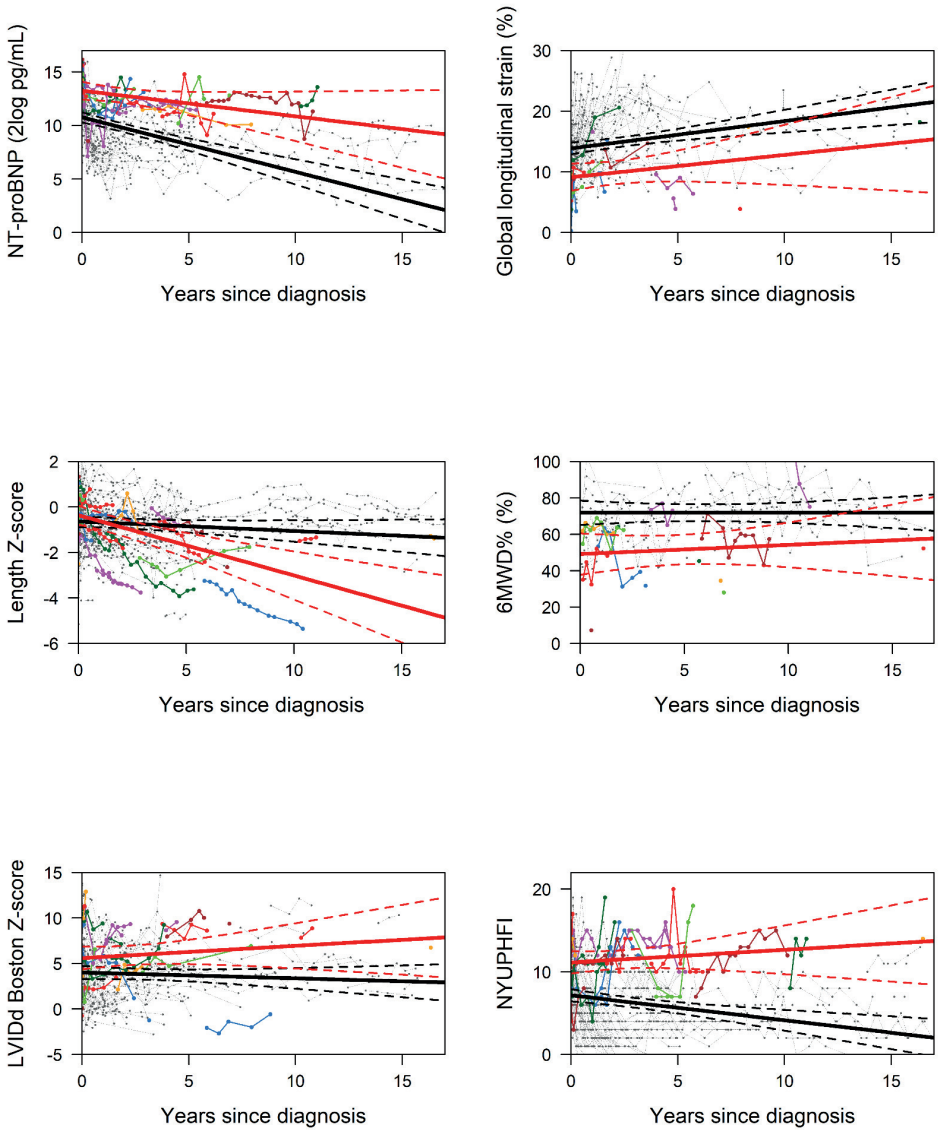
IQR Inter Quartile Range, Dx diagnosis, FU Follow Up
* Mann-Whitney *U* test ** Chi square test
*** Weight for age only in children under 15 months (n=43)
† $p < 0.05$

Differences in risk factors, SE vs no SE

At the moment of first diagnosis, children who ultimately reached the SE had an unfavorable clinical profile compared to those who remained event-free. In particular (mean) NT-proBNP levels and NYU PHFI scores were higher, whereas global longitudinal strain and 6MWD% were lower (Table 2).

The temporal evolution of 4 risk factors was clearly different between children with and without the SE (Table 2; Figure 1). NT-proBNP levels, LVIDd and NYU PHFI score remained high in those who reached the SE, while event-free children showed (steadily) decreasing values. Furthermore, length growth was severely stunted in children with the SE, while a normal length growth was maintained over time in their event-free counterparts.

Figure 1. Serial measurement of risk factors: NT-proBNP (2log pg/mL), Length Z-score, LVIDd Boston Z-score, Global longitudinal strain (%), 6MWD% (%) and NYU PHFI



The average estimates of the longitudinal trajectory risk factors: the black line indicates the patients without a study endpoint, the red line the patients with a study endpoint. The dashed lines depict the 95% confidence interval. The colored lines show the individual patients with a study endpoint.

Table 2. Differences in risk factors between patients who reached the study endpoint of all-cause death or heart transplant and those who remained endpoint-free

Measurement	Mean value (95% CI) at diagnosis			Mean (95% CI) change/year				
	No SE	SE	P-value #	No SE		SE		P-value #
NTproBNP (2log pg/mL)	3.3 (3.2, 3.4)	4.0 (3.8, 4.2)	<0.001	-0.32 (-0.38, -0.25)	†	-0.08 (-0.20, 0.05)		0.001
Length Z-score	-0.67 (-0.89, -0.44)	-0.18 (-0.58, 0.22)	0.040	-0.02 (-0.08, 0.04)		-0.42 (-0.56, -0.27)	†	<0.001
LVIDd Boston Z-score	4.5 (3.8, 5.2)	5.4 (4.1, 6.6)	0.233	-0.60 (-0.82, -0.38)	†	0.24 (-0.28, 0.75)		0.004
Global longitudinal strain (%)	13.3 (12.4, 14.2)	9.8 (7.7, 11.9)	0.003	1.14 (0.75, 1.52)	†	0.55 (-0.51, 1.62)		0.313
6MWD% (%)	73.0 (67.0, 79.0)	50.7 (39.6, 61.7)	0.001	-0.23 (-0.92, 0.45)		0.22 (-1.47, 1.90)		0.627
NYU PHFI *	7.8 (7.1, 8.6)	10.6 (9.2, 11.9)	<0.001	-1.16 (-1.41, -0.91)	†	0.49 (-0.13, 1.10)		<0.001

CI: confidence interval; HR: hazard ratio; SE: study endpoint

* After Box-Cox transformation # P-value for the difference between patients who reached the composite study endpoint of all-cause death or heart transplant and those who survived (without transplantation) † The slope is significantly (P-value <0.05) different from 0

Results are derived from linear mixed effect models with risk factor as dependent variable, and time, classification (SE versus still diseased) and time*classification as independent variables

Table 3. Differences in risk factors between patients who reached the study endpoint of all-cause death or heart transplant and those who survived (without transplantation) but remained diseased

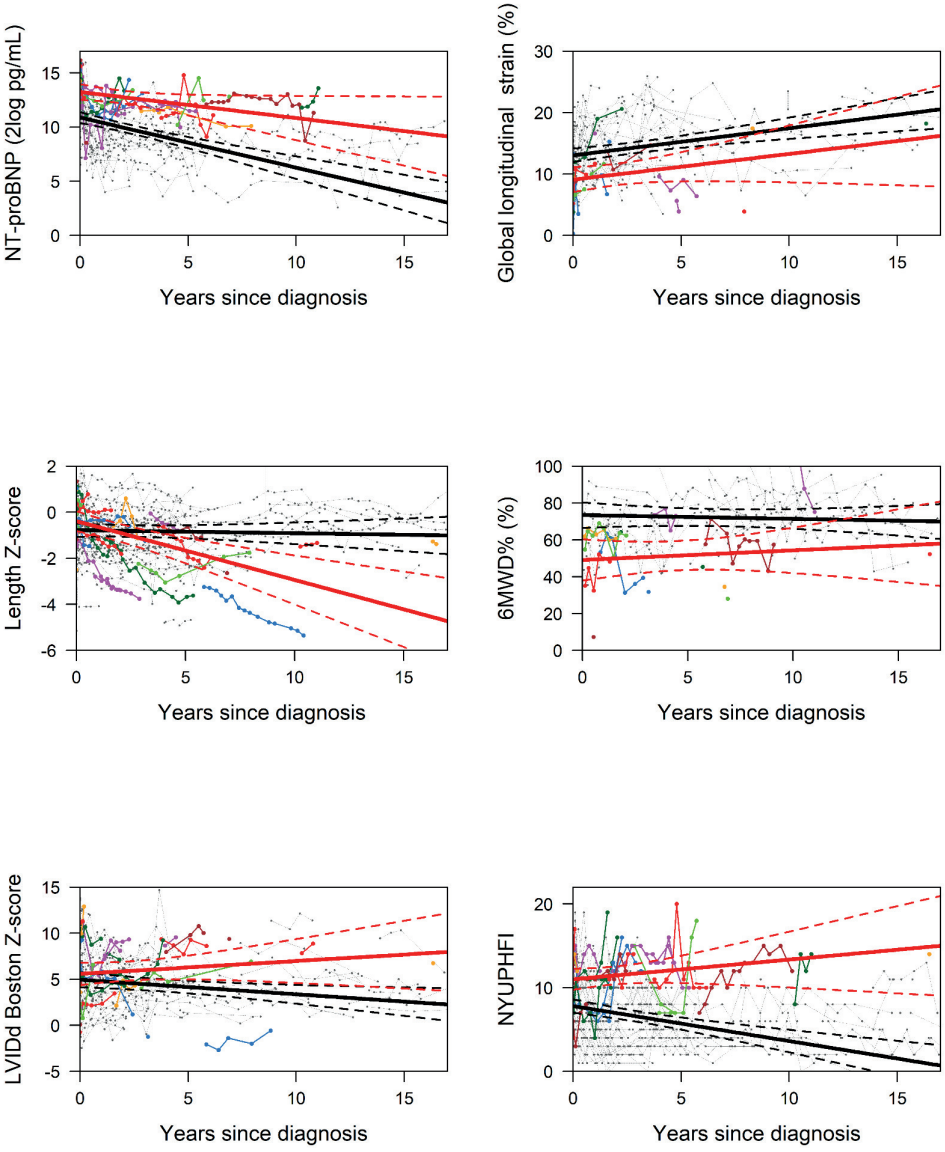
Measurement	Mean value (95% CI) at diagnosis			Mean (95% CI) change/year				
	Still diseased	SE	P-value #	Still diseased		SE		P-value #
NTproBNP (2log pg/mL)	10.82 (10.29, 11.35)	13.22 (12.45, 13.99)	<0.001	-0.41 (-0.49, -0.33)	†	-0.19 (-0.38, -0.01)	†	0.037
Length Z-score	-0.67 (-0.95, -0.39)	-0.20 (-0.63, 0.23)	0.076	-0.04 (-0.06, -0.01)	†	-0.29 (-0.37, -0.22)	†	<0.001
LVIDd Boston Z-score	5.24 (4.43, 6.05)	5.57 (4.33, 6.82)	0.658	-0.23 (-0.33, -0.12)	†	0.14 (-0.12, 0.41)		0.010
Global longitudinal strain (%)	13.01 (11.90, 14.12)	9.03 (6.97, 11.09)	0.001	0.42 (0.24, 0.60)	†	0.43 (-0.04, 0.91)		0.948
6MWD% (%)	74.13 (67.64, 80.63)	50.69 (39.55, 61.82)	0.001	-0.34 (-1.05, 0.37)		0.20 (-1.49, 1.90)		0.560
NYU PHFI *	8.35 (7.51, 9.18)	10.58 (9.23, 11.93)	0.006	-0.49 (-0.60, -0.38)	†	0.36 (0.07, 0.66)	†	<0.001

CI: confidence interval; HR: hazard ratio

* After Box-Cox transformation # P-value for the difference between patients who reached the composite study endpoint of all-cause death or heart transplant 'Study Endpoint') and those who survived (without transplantation) but remained diseased ('Still diseased')

† The slope is significantly (P-value <0.05) different from 0 Results are derived from linear mixed effect models with risk factor as dependent variable, and time, classification (SE versus still diseased) and time*classification as independent variables

Figure 2. Serial measurement of risk factors: NT-proBNP (2log pg/mL), Length Z-score, LVIDd Boston Z-score, Global longitudinal strain (%), 6MWD% (%) and NYU PHFI



The average estimates of the longitudinal trajectory risk factors: the black line indicates the patients with ongoing disease, the red line the patients with a study endpoint. The dashed lines depict the 95% confidence interval. The colored lines show the individual patients with a study endpoint.

Differences in risk factors, SE vs ongoing disease

Comparable differences between patients with and without SE were observed in a sensitivity analysis that excluded the patients who clinically recovered, although effect sizes were smaller (Table 3; Figure 2). Thus, in children with the SE the mean value at the time of diagnosis of NT-proBNP and NYU PHFI were significantly higher, and GLS and 6MWD% were significantly lower. Furthermore, in children with the SE, NT-proBNP, LVIDd and NYU PHFI all remained high, while in the children without the SE all decreased over time. Length Z-core indicated that in children who reached an endpoint length growth was severely stunted, while in those with ongoing disease growth was less severely impaired.

Risk factors for death or heart transplantation (Table 4)

Factors that were associated with an increased risk of death or heart transplantation included age older than 6 years at diagnosis and at any time during follow up: NT-proBNP, length Z-score, LVIDd Z-score, global longitudinal strain, 6MWD% and NYU PHFI. In multivariable analyses, 6MWD% appeared the only risk factor that was independent of NT-proBNP.

Table 4. Risk factors for the composite study endpoint of death or heart transplantation

	No. patients	No. repeated observations	No. endpoints	HR	95%CI	P-value
Age over 6 years	137	--	36	3.97	(1.94, 8.15)	<0.001
Idiopathic DCM	137	--	36	0.79	(0.41, 1.55)	0.502
NT-proBNP (2log pg/mL)	113	605	32	2.78	(1.81, 3.94)	<0.001
Length Z-score	133	880	36	0.77	(0.56, 1.06)	<0.001
LVIDd Boston Z-score	126	540	33	1.29	(1.12, 1.52)	<0.001
Global longitudinal strain (%)	110	384	22	0.45	(0.34, 0.77)	<0.001
6MWD% (%)	55	249	14	0.91	(0.86, 0.95)	<0.001
NYU PHFI *	129	755	33	3.16	(2.25, 4.52)	<0.001
Age over 6 years	113	605	32	2.96	(0.65, 12.5)	0.113
NT-proBNP (2log pg/mL)				2.86	(2.01, 3.71)	<0.001
Idiopathic DCM	113	605	32	1.78	(0.36, 11.9)	0.391
NT-proBNP (2log pg/mL)				2.89	(1.76, 3.99)	<0.001
Length Z-score	112	605	32	1.09	(0.65, 1.81)	0.712
NT-proBNP (2log pg/mL)				6.96	(3.74, 1.30)	<0.001
LVIDd Boston Z-score	109	602	30	0.92	(0.74, 1.10)	0.376
NT-proBNP (2log pg/mL)				7.74	(4.03, 16.5)	<0.001
Global longitudinal strain (%)	95	549	19	0.89	(0.74, 1.06)	0.234
NT-proBNP (2log pg/mL)				8.33	(3.76, 20.5)	<0.001
6MWD% (%)	44	286	13	0.95	(0.89, 1.00)	0.046
NT-proBNP (2log pg/mL)				8.34	(3.76, 20.5)	<0.001
NYU PHFI *	106	508	29	0.78	(0.29, 2.63)	0.618
NT-proBNP (2log pg/mL)				10.9	(3.65, 35.6)	<0.001

DCM: dilated cardiomyopathy; CI: confidence interval; HR: hazard ratio

* After Box-Cox transformation

DISCUSSION

In this study we found that the temporal evolution of 4 risk factors distinguished the children who died or underwent transplantation from those who have ongoing disease or recover: no decrease in NT-proBNP or LVIDd, a severe decrease in length Z-score, and an increase in NYU PHFI. Similarly, when comparing children who reached an endpoint with children with ongoing disease solely, excluding those who recovered, we found comparable differences in the evolution of risk factors, although the effect size was smaller. In the multivariate analysis, NT-proBNP remained the only independent risk factor for adverse outcome.

We performed 2 analyses and excluded the children who recover in the secondary analysis because, in general, children who recover tend do clinically well at a relatively early stage after diagnosis. The clinical challenge is to identify within the group of children those who do not recover, who are at the highest risk for adverse outcome and should be considered for HTx. In this study, 23% of the children, who newly presented, recovered and median time from diagnosis to recovery was 0.6 years and confirmed that recovery tends to be an early event (1, 3). This the first study to our knowledge to analyze this subgroup of children who remain ill but do not reach the SE. The finding that the abovementioned risk factors also discern these children from those who reached a SE supports outpatient management and the decision to suspend listing for HTx.

In the adult counterpart of pediatric dilated cardiomyopathy, non-ischemic cardiomyopathy, studies of risk factors for outcome have focused on the prevention of sudden cardiac death (15). However, in children with dilated cardiomyopathy the risk of sudden cardiac death is considerably lower than in adults, and most children die or undergo HTx because of pump failure (16). Studies on risk factors for adverse outcome in adult non-ischemic heart failure secondary to pump failure are, therefore, potentially better comparable to the results of the pediatric studies. In parallel with the risk factors we noted in pediatric DCM, natriuretic peptides, including NT-proBNP, have been shown robust prognostic markers for outcome in adult heart failure, including also in the non-ischemic subgroup (17-19). Longitudinal strain on CMR imaging has documented incremental predictive value for adverse events in addition to LGE and indexed LV end-diastolic volume (20). In contrast, LV dimension has not been identified as a solid marker for adverse events (21). However, in patients demonstrating reverse remodeling, LV dimensions at presentation were lower and significantly decreased over time (22). The six-minute walking test at presentation and the change during follow-up has been associated with heart failure outcome (23). However, the application of the test in adults has been limited, but may be useful in those with severe heart failure (24).

When comparing the results of the above-mentioned adult reports to the results of our study, NT-proBNP also remains a strong predictor of outcome in pediatric DCM. Even after exclusion of the children who recovered, the differences in temporal evolution of NT-proBNP remained significant in the univariate model. In this study we reported a difference in NT-proBNP at diagnosis between children who reached the SE versus those who did not. This seems to contradict our previous publication in which we reported no

difference in NT-proBNP at diagnosis (6). A likely explanation is the steep decline of NT-proBNP in children without the SE in the first 30 days after diagnosis, as shown by den Boer et al (6). In this study we analyzed the data in one timeframe, and the linear model underestimated NT-proBNP at diagnosis. We could confirm this as median NT-proBNP at diagnosis were not different in standard statistical tests (results not shown). Our results further corroborate the association of NT-proBNP and outcome in children and adults with heart (6, 17, 25-27). Whether the evolution of NT-proBNP truly is the only predictor of outcome or whether the other risk factors also hold independent predictive information remains to be studied in a larger cohort or with longer follow-up. Moreover, our findings apply to the mid-term outcome with a median time after diagnosis to last follow up of 3.5 years. In addition, we found that the 6MWD% was independently associated with outcome, but this should be interpreted with some constraint as the number of children (> 6 years of age) who performed a 6MWD test was limited.

Although in the multivariate analysis all risk factors but NT-pro BNP lost their association with the SE, the temporal evolution of the other 3 risk factors still holds important information. First, LVIDd in children who reached an endpoint did not change over time in contrast to children not reaching an endpoint where LVIDd decreased, even after exclusion of children who recovered (Table 2 and 3). Temporal evolution of global longitudinal strain cannot be used to predict outcome because the slopes are not different in the two analyses. However, GLS at the time of diagnosis still might be useful. In the model in the intercepts of global longitudinal strain were significantly different between the two groups (Table 2 and 3), suggesting that early after presentation it may be an indicator for outcome. That observation is compatible with our previous report that lower global longitudinal strain was associated with an increased risk of death or heart transplantation in a cross-sectional sample and similar to observations in adults with chronic heart failure (8, 28). The second and the third risk factor specifically pertain to the pediatric population: length Z-score and a pediatric heart failure score (NYU PHFI). Length growth is considered a solid proxy for overall health in children and provides a long-term reflection of disease severity. The development of length Z-score distinguished children who reached an endpoint from those with ongoing disease (Table 3). In clinical practice, length growth virtually ceased in children reaching an endpoint. Similarly, Alvarez et al reported that small height-for-age was associated with a risk of death, but not for being listed for transplantation, also suggesting that length growth may be a useful indicator for disease severity in these children (3). Previously we reported that NYU PHFI independently predicts death and HTx in children with DCM in a cross-sectional sample (29). Now we have demonstrated that children who reach the SE were characterized by an increase in NYU PHFI opposed to those with ongoing disease.

In the light of the interpretation and generalization of our findings it is important that after the first year after diagnosis the (transplant free) survival curves of the PCMR and the NACCS are similar to ours (1, 2). We have previously reported a low HTx rate in children with DCM in the first year after diagnosis, without an increase in mortality (5). The data presented in this study confirm that finding and support the idea that a low early HTx rate does not lead to an increase in mortality or Htx rate in the years following diagnosis as a result of postponing HTx. This implies that the children with DCM in both cohorts are

comparable in disease severity. The results we found would therefore be applicable in other populations of children with DCM, after the first year after diagnosis.

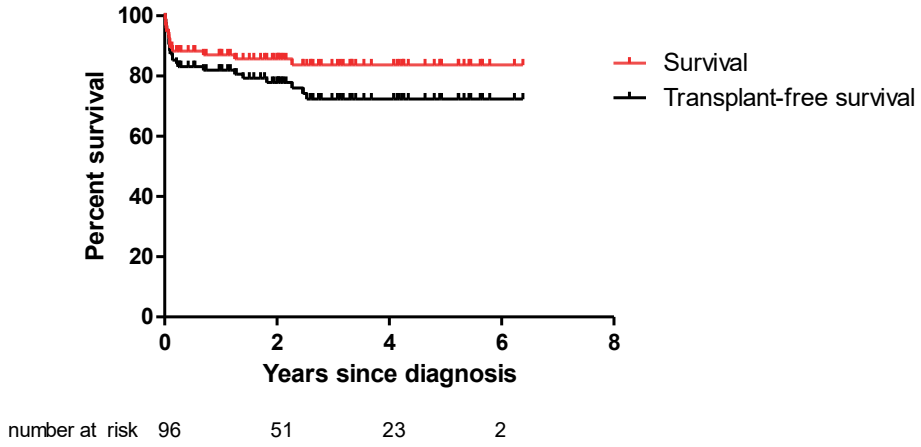
Study limitations

This study has limitations. First, our study cohort is a combination of 2 cohorts and consists of children with a prior diagnosis before inclusion in this study, as well as children who are included directly after diagnosis. This may have induced a selection bias. However, the outcome of the cohort of newly diagnosed children that we included (Suppl. Figure 1) is the same as the outcome of the children that we previously reported that presented between 2005-2010 indicating that the large majority of children that we included with a prior diagnosis were obtained from a cohort with the same characteristics (5). Second, missing data was an issue. In particular, 6MWD% could only be obtained in outpatient children older than 6 years. Thirdly, the number of endpoints was limited which hampered multivariate analysis. Lastly, the decision to list a patient for HTx is based on multiple clinical factors. It could be that the reported risk factors predict decision-making by clinicians rather than outcome. However, the children that underwent HTx were in such a condition that they most likely would have died otherwise (30).

Conclusion

In summary, we conclude that in children with DCM, change over time in known risk factors for death or HTx is predictive for outcome. In children who do not recover, persistent high levels of NT-proBNP, no decrease in LVIDd, severe stunting of growth and an increase in the heart failure score identifies those at the highest risk for death or HTx. In this study, with limited study endpoints, we identified NT-proBNP as the strongest independent predictor for adverse outcome.

Supplementary figure 1. Kaplan-Meier plot showing survival and transplant-free survival since diagnosis of 96 newly diagnosed children with DCM



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How safe are ACE inhibitors for heart failure in children?

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Arch Dis Child. 2018;103(1):106-9.

6

SCENARIO

A 1-year-old boy, recently diagnosed with dilated cardiomyopathy, has to be treated with Enalapril according to the heart failure protocol. The physician wants to know which adverse events she can expect.

Structured Clinical Question

What are the potential adverse events related to administration of an ACE-inhibitor to children with heart failure, what is the prevalence and what are the possible risk factors.

Search

Secondary sources – nil

Electronic databases (Pubmed/Medline and Embase) and reference lists of relevant articles were searched with the following strategy: 'angiotensin-converting enzyme inhibitors' and 'heart failure' and 'children'. The search yielded 415 individual articles, of which 14 were identified as relevant.

SUMMARY

Table 1. Summary of study results

Citation	Study group	Study type	Outcome	Key Result	Comments
Scammell *et al* Int J Cardiol 1987;16(3): 295-301(1)	18 infants, 4 - 41 weeks, CHD and HF	Retrospective Cohort (4)	Hypotension	'Mild' in 11% (Mild defined as 'no resuscitation needed") A*	Captopril considered safe.
			Neutropenia	nil	
			Skin rash	nil	
			proteinuria	nil	
Loyd *et al* The Journal of Pediatrics 1989 114 (4):650-654 (2)	10 infants, 6 weeks - 8 months, CHD with HF, effect of different doses on enalprilat concentrations and AEs, on enalapril	Clinical Trial (3b)	Incidence of hypotension	nil	Enalapril in 3 relatively low doses: 0.02 ; 0.04 and 0.08 mg/kg.
			Rise in Serum creatinin	nil	
			Incidence of proteinuria	nil	
			Decrease in urine volume	nil	
Dutertre *et al* Br J Clin Pharmacol 1993; 35(5): 528-30 (3)	18 infants, 3 weeks - 6 months, CHD with HF, comparing baseline ACE activity in 8 children on enalapril and 10 controls,	Clinical Trial (3b)	Incidence of hypotension	nil	AEs including renal dysfunction or hyperkalemia were not evaluated.
			Incidence of vomiting	6%, E*	
Leversha *et al* Arch Dis Child 1994 70(1):35-39 (4)	63 children, 1 week -17 years, CHD with HF, on enalapril	Clinical Trial (3b)	Incidence of renal failure	8 of 63 (13%) 6 rise in creat and 2 oliguria, all D*	Renal failure in eight patients was related to young age, low weight, and left-to-right shunt group. Three patients died in congestive heart failure with renal failure.
			Incidence of hypotension	9 of 63 (14%), 8 (89%) in C* and 1 (11%) in D*	
			Other SAE's	1 cough and 1 neutropenia	
				Both A*	
Gantenbein *et al* J Perinat Med 2008; 36 (5): 448-52 (5)	43 infants of 1-44 weeks, CHD with HF, on captopril	Retrospective cohort (4)	Incidence of renal failure	6 of 43 (14%) renal impairment or failure, 4 (67%) in C* or D*	Renal side effects occurred more often in smaller infants.
			Incidence of hypotension	8 of 43 (19%)1 (13%) in C* or D*	

Hsu *et al* Circulation 2010; 122(4):333-340 (6)	185 infants, 1-6 weeks with single ventricle physiology, on enalapril	Randomized controlled trial (3b)	Incidence of renal failure	1 of 185 (0.5%) E*	No difference in the number of adverse events between the treatment groups (423 in the enalapril group and 389 in the placebo group; P=0.23).
Orchard *et al* Arch Dis Child 2010; 95(7):566-7 (7)	66 infants, 6 weeks-8 months, heart failure (cause unknown), on enalapril	Clinical trial (3b)	Incidence of renal failure Incidence of hyperkalemia Incidence of hypotension	nil nil 10 of 66 (15%) showed a > 25% drop in blood pressure, 2 of which (20%) in D*	Hypotension was well tolerated and only two patients discontinued captopril.
Lindle *et al* Pediatr Cardiol 2013; (8)	206 children, 16–18 days (20% preterm), 95 % CHD with HF, on enalapril or captopril	Retrospective cohort (2b)	Incidence of renal failure Incidence of hyperkalemia	29% failure according to ipRIFLE definition, E* rise of K from 4.2 to 5.7 mEq/L), no hyperkalemia, E*	Premature neonates were more likely to experience ACE-i-related renal failure (55%) than their term counterparts (23%; p < 0.001).
Terano *et al* Eur J Pediatr 2016; 175(5):631-7 (9)	312 children, 2 months - 13 years, 86% CHD with HF and 8% myoarditis with HF, on enalapril, captopril or lisinopril	Retrospective cohort (2b)	Incidence of AKI Incidence of hyperkalemia	45 of 312 (14%) AKI 2-3, E* 14/312 (5%), K≥ 5.5 mmol/L, E*	Younger age, myocardial disease, cyanotic congenital heart disease, concomitant use of spironolactone, and cardiac surgery were risk factors for AKI.
Roche *et al* J Am Heart Assoc. 2016;5:e003230 (10)	46 infants, 2.3 ± 4.7 years, (82% HF, 13% pulmonary overflow) comparing a rapid (RU) versus prolonged uptitration (PU) protocol of ACE-i	Clinical trial (3b)	Symptomati-chypotension Serum creat increase > 2x baseline Serum potassium > 5 mmol/L	RU: 28%, E* PU: 10%, E* RU: 0%, E* PU:5%, E* RU:8%, E* PU:5%, E*	No significant difference between incidence of adverse events between the RU and PU group.

Ku *et al* Pediatr Cardiolo 2017; 38:155–161 (11)	662 neonates, < 120 days postnatal age, exposed to enalapril, indications unknown, CHD excluded	Retrospective Cohort (2b)	Incidence of adverse events	142 (21%), E*	Significant risk factors for adverse events were postnatal age < 30 days at first exposure and longer exposure duration.
			Incidence of hyperkalemia	Serum potassium > 6 mmol/L in 13%, E*	
			Incidence of renal failure	Serum creat > 1.3 mg/dL in 2%, E*	
			Incidence of hypotension requiring inotropes	4%, E*	
			Incidence of death	0.5%, E*	

In bold, the studies with adverse effects as primary outcome
CHD: congenital heart disease; HF: heart failure; AKI: acute kidney injury
* In key results, categories of measures taken following AEs: A. no dose change, B. dose interruption, C. lowering of dose, D. discontinuation of ACE-i, E. unknown or not applicable

Commentary

Heart failure is an important cause of morbidity and mortality in children (12).
It is commonly treated with ACE-inhibitors (ACE-i) despite the lack of evidence on its efficacy (13). This treatment is based on the assumption that blocking of the renin-angiotensin-aldosterone system has a positive effect on morbidity and mortality, as it does in adults with heart failure (14-17). In adults, the most prevalent adverse events (AEs) related to the use of ACER-i are renal failure (18, 19), hypotension (20, 21), hyperkalemia (20, 22), cough (23) and angioedema (24). It is assumed that the safety profile of ACE-i differs for children and differs from that for adults, but also for children of different ages, as growth and development contribute to variation in the disposition and effect of most drugs administered to children (25).

Our search yielded seven studies (1, 5, 7-11) in which the primary outcome was AEs of ACE-i. The other seven studies included, either described the actual AEs, or did not report whether they specifically looked into the AEs. One of the studies was a randomized placebo-controlled trial, one a randomized clinical trial, 4 were clinical trials, and 5 were retrospective cohort studies (Table 1). The total number of patients in these studies was 1629, in the age range from 1 week to 17 years. The prescribed ACE-inhibitors were enalapril (n=1050 patients), captopril (n=409), lisinopril (n=4), and 34 patients switched from captopril to enalapril. The dose of enalapril ranged from 0.02 mg/kg/day to 1.8 mg/kg/day; that of captopril from 0.07 to 2.5 mg/kg/day. The treatment duration ranged from 1 day to 3 years.

ACE-i related adverse events

Two studies reported death as an AE possibly related to ACE-i use: Three in the study of Leversha et al (4) and three in the study of Ku et al (11) Leversha et al do not draw a conclusion on the relationship between ACE-I use and death, however, Ku et al state that it is unlikely that these three deaths can be attributed to the use of ACE-i.

Renal impairment is a descriptive term, and was defined differently in the presented studies: anuria, oliguria with creatinine rise, creatinine rise only, creatinine clearance, stage 2-3 acute kidney injury (AKI) based on the KDIGO guidelines (26) and renal risk, renal injury or renal failure by modified pRIFLE criteria (27). From 0 to 29% of all patients suffered from a form of renal impairment during exposure to ACE-i. In most cases, normal renal function was regained after interruption of ACE-i and/or lowering of the ACE-i dose. None of the studies made mention of kidney replacing therapy. Terano et al described seven cases of stage 2 chronic kidney disease (9). In the remaining studies, long-term renal sequelae were either not reported or not observed.

Hypotensive episodes during ACE-i use were described in eight studies. Only Ku et al reported severe hypotension that required inotropes administration. Whether hypotension in milder cases led to ACE-i dose adjustments is not clear. Hypotension was reported in a range of 0 to 19% of patients on ACE-i.

Hyperkalemia was described in 3 studies. We found no reports of hyperkalemia resulting in cardiac arrhythmias or requirement of dialysis. Hyperkalemia was reported in a range of 0-13% of patients on ACE-i.

Several other adverse effects were reported, including vomiting once in one child (3), cough in one and neutropenia in one (4). The cough and neutropenia resolved after reduction or stop of enalapril. No cases of angioedema were reported.

Risk factors for ACE-i related AE

Children are possibly at an increased risk for ACE-i related adverse events compared to adults (28). Pathophysiologically this could be explained by several mechanisms: reduced GFR at young age (29), impaired autoregulation of renal blood flow in newborns (30), and increased ACE-i levels due to immature renal clearance.

In general, we found no clear relationship between dose level and risk of AEs. Regarding renal failure, multiple studies suggest that young age and low weight predispose to renal failure. In the study of Leversha et al, young age (< 4 months) and low weight were risk factors for renal failure (4). Lindle et al reported that nearly one third of the term neonates and more than half of the preterm neonates were in the pRIFLE kidney failure category (8). Furthermore, Gantenbein et al noted that the six children who developed renal impairment or failure weighed on average 500 g less than the other children (5) Terano et al denoted younger age as an independent risk factor for AKI (9). Ku et al also identified a postnatal age of less than 30 days as a risk factor for AEs.

Concomitant medication should also be considered a risk factor for AEs. Diuretic-mediated lowering of renal perfusion may increase the risk of ACE-i-mediated nephrotoxicity (10). Terano et al identified the use of spironolactone as an independent risk factor for AKI (9). Lindle et al noted trends towards significant decrease in creatinine clearance in patients on chlorothiazide or spironolactone (8).

Lastly, conditions which may lead to hypovolemia, (e.g. acute gastroenteritis or inadequate food/water intake), are risk factors for AEs. Terano et al reported that 37% of all AKI cases occurred under such circumstances (9).

A major limitation of this review is the heterogeneity among the included studies in terms of patient characteristics and outcome parameters. Most importantly, patients differed in disease severity or severity of heart failure was unknown; dosages of ACE-i differed, and clear and consistent definitions of adverse events were lacking.

Clinical bottom lines

1. ACE-i are considered relatively safe for children with heart failure, but renal impairment, hypotension and hyperkalemia are common adverse reactions (grade of recommendation: B)

2. Low weight, young age, and being prone to dehydration, (e.g. an account of gastroenteritis or high doses of diuretics) are factors carrying an increased risk for adverse reactions related to ACE-1 consumption (grade of recommendation: B)

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Emotional and behavioral problems in children with dilated cardiomyopathy

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ABSTRACT

BACKGROUND Dilated cardiomyopathy (DCM) in children is an important cause of severe heart failure and carries a poor prognosis. Adults with heart failure are at increased risk of anxiety and depression and such symptoms predict adverse clinical outcomes such as mortality. In children with DCM, studies examining these associations are scarce.

AIMS We studied whether in children with DCM: (1) the level of emotional and behavioral problems was increased as compared to normative data, and (2) depressive and anxiety problems were associated with the combined risk of death or cardiac transplantation.

METHODS To assess emotional and behavioral problems in children with DCM, parents of 68 children, aged 1.5-18 years (6.9±5.7 years) completed the Child Behavior Checklist.

RESULTS Compared to normative data, more young children (1.5-5 years) with DCM had somatic complaints (24.3% vs. 8.0%; $p < .001$), but fewer had externalizing problems (5.4% vs. 17.0%; $p = .049$). Overall internalizing problems did not reach significance. Compared to normative data, more older children (6-18 years) showed internalizing problems (38.7% vs. 17.0%; $p = .001$), including depressive (29.0% vs. 8.0%; $p < .001$) and anxiety problems (19.4% vs. 8.0%; $p = .023$), and somatic complaints (29.0% vs. 8.0%; $p < .001$). Anxiety and depressive problems, corrected for heart failure severity, did not predict the risk of death or cardiac transplantation.

CONCLUSION Children of 6 years and older showed more depressive and anxiety problems than the normative population. Moreover, in both age groups, somatic problems were common. No association with outcome could be demonstrated.

INTRODUCTION

Cardiomyopathies are disorders characterized by structural and functional abnormalities of the heart. The most common subtype in children is dilated cardiomyopathy (DCM), accounting for approximately 60% of pediatric cardiomyopathies (1, 2). DCM, which is characterized by impaired systolic function and dilation of the left ventricle (3), is estimated to affect 0.57 per 100,000 children annually (4). Though disease presentation can vary greatly, 80% of patients show symptoms related to heart failure, such as fatigue, orthopnea, edema, and excessive sweating. Symptoms can also include circulatory collapse, arrhythmias, thromboembolic events, and sudden death (5). Although some children recover (6), the prognosis of DCM generally is poor: within 2 years after diagnosis, approximately 40% of children die or undergo cardiac transplantation (4, 6-8), making DCM the leading indication for cardiac transplantation worldwide (9-11). Considering the symptoms and prognosis of DCM, substantial effects on psychosocial wellbeing can be expected (12).

Compelling evidence from two meta-analyses shows that adults with heart failure are at increased risk of anxiety and depression (13, 14). Few studies have examined the psychosocial wellbeing of children with DCM, but, indeed, it has been found that children with DCM have a lower health-related quality of life (HRQoL) than healthy children (12, 15-18). However, studies examining emotional and behavioral problems in children with DCM are scarce. Moreover, the currently available studies have small sample sizes ($n \leq 15$) and show contradictory results. In a cross-sectional study, half ($n = 6$ out of 12) of children with cardiomyopathy listed for cardiac transplantation showed clinically significant overall emotional and behavioral problems (19). In contrast, a study examining depressive symptoms in children with DCM ($n = 15$) did not find higher rates of symptoms compared with healthy children (20). However, it should be noted that these studies used different questionnaires.

Regarding the impact of impaired HRQoL on cardiac outcomes, two studies have shown that children's physical HRQoL (reported by parents) predicts mortality and cardiac transplantation, independent from heart failure severity (15, 17). Also, a meta-analysis (13) and reviews (21, 22) have consistently found that depressive and anxiety symptoms in adults with heart failure predict mortality and other adverse clinical outcomes, such as hospitalization and arrhythmias. However, to the best of our knowledge, the predictive value of depressive and anxiety symptoms in children with DCM has not been previously studied. Information on the predictive value of depressive and anxiety symptoms may be valuable for clinical management strategies. Depressive and anxiety problems may lead to poorer self-care and, in turn, to disease progression (22).

The aim of the present study was twofold: firstly, we evaluated the level of parent-reported emotional and behavioral problems in children with DCM compared with the general population. Secondly, we exploratively examined whether the level of parent-reported anxiety and depressive problems predicted the combined risk of death and cardiac transplantation whilst controlling for heart failure severity. Based on the aforementioned adult studies, we hypothesized that children with DCM would show more anxiety and

depressive problems than children in the general population. Moreover, we hypothesized that anxiety and depressive problems would predict mortality in children with DCM independent from heart failure severity.

METHODS

All data used in this observational, cross-sectional study were derived from a larger multicenter longitudinal study in children with heart failure secondary to cardiomyopathy (15). The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center (protocol number NL45663.078) and by the institutional review boards of all participating centers. The study performed conformed to the ethical guidelines of the Declaration of Helsinki (23) and reported following the STROBE statement. Before participation, written informed consent was obtained from all patients' parents or legal guardians and from all patients aged 12 years or above.

Participants

Participants were recruited from 1 October 2010 to 1 November 2015 through seven tertiary centers for pediatric cardiology in the Netherlands. The database was closed on 1 July 2017. Children were eligible to participate if they had heart failure secondary to DCM. DCM was defined as fractional shortening ≤25% and left ventricular end-diastolic dimension z-score >2 for body surface area. DCM could be idiopathic or secondary to other causes. Exclusion criteria were known mental retardation, congenital heart disease, neuromuscular disease, and insufficient mastery of the Dutch language by parents. In the current study, we only included 1.5-18-year-old children due to age restrictions of the used questionnaire. Since age and gender-matched normative data on emotional and behavioral problems is available, we did not recruit a healthy control group (24).

Procedure

Children were either included at DCM diagnosis or were included at an outpatient appointment for a previously diagnosed DCM in one of the participating tertiary pediatric cardiology centers. Demographic variables were obtained at inclusion. Socioeconomic status was based on the highest of both parents' occupations and categorized into low, low to middle, middle, or high according to the international classification system (25). Parents were asked to complete a questionnaire assessing their child's emotional and behavioral problems during an outpatient clinic visit. During the same visit, a pediatric cardiologist completed the New York University Pediatric Heart Failure Index (NYU PHFI) (26). This validated index assesses heart failure severity based on symptoms and medication use. Scores range from 0 to 30. A higher score indicates more severe heart failure.

Emotional and behavioral problems

One of each participant's parents completed the problem section of the Child Behavior Checklist (CBCL) (27). Depending on the child's age, the CBCL 1½-5 (100 items; children aged 1.5-5 years) or the CBCL/6-18 (120 items; children aged 6-18 years) was completed. For both versions, response categories range from 0 (not true) to 2 (very true or often true). The CBCL assesses overall emotional and behavioral problems and specific aspects

of mental health and problem behavior. In addition to an overall total problem score, broadband scale scores can be calculated for Externalizing Problems (i.e., externally directed problems affecting the environment, such as aggression and delinquency) and Internalizing Problems (i.e., internally directed problems such as depression, anxiety, and somatic complaints). Furthermore, the CBCL 1½-5 consists of five scales based on the fifth edition of the Diagnostic and Statistical Manual Of Mental Disorders (DSM-5; i.e., Depressive Problems, Anxiety Problems, Attention Deficit/Hyperactivity Problems, Oppositional Defiant Problems, and Autism Spectrum Problems). In addition, seven empirical scales can be calculated (i.e., Anxious/Depressed, Somatic Complaints, Attention Problems, Aggressive Behavior, Emotionally Reactive, Withdrawn, and Sleep Problems). The CBCL/6-18 consists of six DSM-5 based scales (i.e., Depressive Symptoms, Anxiety Problems, Somatic Problems, Attention Deficit/Hyperactivity Problems, Oppositional Defiant Problems, and Conduct Problems) and 8 empirical scales (i.e., Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Rule-Breaking Behavior, and Aggressive Behavior). On all scales, higher scores indicate more problems. For each scale, scores can be interpreted as falling in the normal, borderline, or clinical range by comparing scale scores with norm data. Scores in the borderline or clinical range indicate psychopathological problems with a need for clinical follow-up and/or intervention. The CBCL has adequate psychometric properties and normative data from the Dutch general population are available (24).

Endpoint

We used a combined endpoint of death and cardiac transplantation. Information on mortality and cardiac transplantation was retrieved from patient records. Follow-up was censored at July 1, 2017.

Statistical analyses

Firstly, we examined whether the proportion of children scoring in the borderline or clinical range of emotional and behavioral problems was larger in our DCM study population than in the general population. All raw scale scores were converted to percentiles using the Achenbach System of Empirically Based Assessment Standard norm data, which is based on data of Dutch children from the general population and accounts for age and gender (27). Conforming with the CBCL manual (27), for the Total Problems scale, Internalizing Problems scale, and Externalizing Problems scale, percentile scores of 83 or lower were defined as non-clinical and percentile scores of 84 or higher were defined as borderline/clinical. For the DSM scales and empirical scales, percentile scores of 92 or lower were defined as non-clinical and percentile scores of 93 or higher were defined as borderline/clinical. One sample binomial tests were conducted for each scale of the CBCL 1½-5 and the CBCL/6-18 to test whether the proportion of children with DCM scoring in the borderline/clinical range was higher than the proportion in the norm group.

Secondly, we conducted a Cox regression analysis to examine whether anxiety and depressive problems predicted the combined endpoint of death and cardiac transplantation whilst controlling for heart failure severity. The covariates entered into the model were the CBCL scale scores for Anxiety Problems and Depressive Problems and the NYU PHFI. We used t-scores to account for differences between the two versions of the

CBCL (i.e., CBCL/1½-5 and CBCL/6-18) Anxiety Problems and Depressive Problems scale scores. The NYU PHFI was added to the model because heart failure severity is a known predictor of mortality (15). All analyses were performed using SPSS Statistics version 24.0 (28).

RESULTS

In total, 144 children with DCM participated in the larger multicenter longitudinal study in children with heart failure secondary to cardiomyopathy from which data for the current study was derived. Of this group, 52 children were excluded from participation in the current study (N = 26 had a too short follow-up period to fill out the CBCL, N = 14 were younger than 1.5 years, N = 7 had a neuromuscular disease, N = 5 did not master the Dutch language sufficiently). Therefore, 92 children met the eligibility criteria for the current study, 68 of whom consented to participate in the current study (see Figure 1). Participant characteristics are presented in Table 1.

Emotional and behavioral problems compared with the general population

1.5- to 5-year-old children (CBCL 1½-5)

The proportion of parent-reported emotional and behavioral problems in 1.5- to 5-year-old children with DCM and children in the norm group is shown in Table 2. The CBCL 1½-5 was completed for 37 participants (by N = 9 fathers, N = 25 mothers, N = 3 parents together) at a median time of 19.0 months after DCM diagnosis (range 10.0-65.0 months; see Table 1). Compared with the normative data of same-aged children (8.0% borderline or clinical score), a significantly larger proportion of children with DCM (24.0% borderline or clinical score) showed somatic complaints in the borderline or clinical range, $p < .001$. In contrast, the proportion of children showing a borderline or clinical level of externalizing problems was significantly smaller in the DCM study group (5.4% clinical or borderline score) than in the general population (17.0% borderline or clinical score), $p = .049$.

For the other scales, the proportions of borderline and clinical problems in children with DCM and children from the general population did not significantly differ. However, trends towards significance were found for more emotionally reactive ($p = .062$) and depressive problems ($p = .062$), and less attention deficit/hyperactivity problems ($p = .068$).

Figure 1 Participation flowchart.

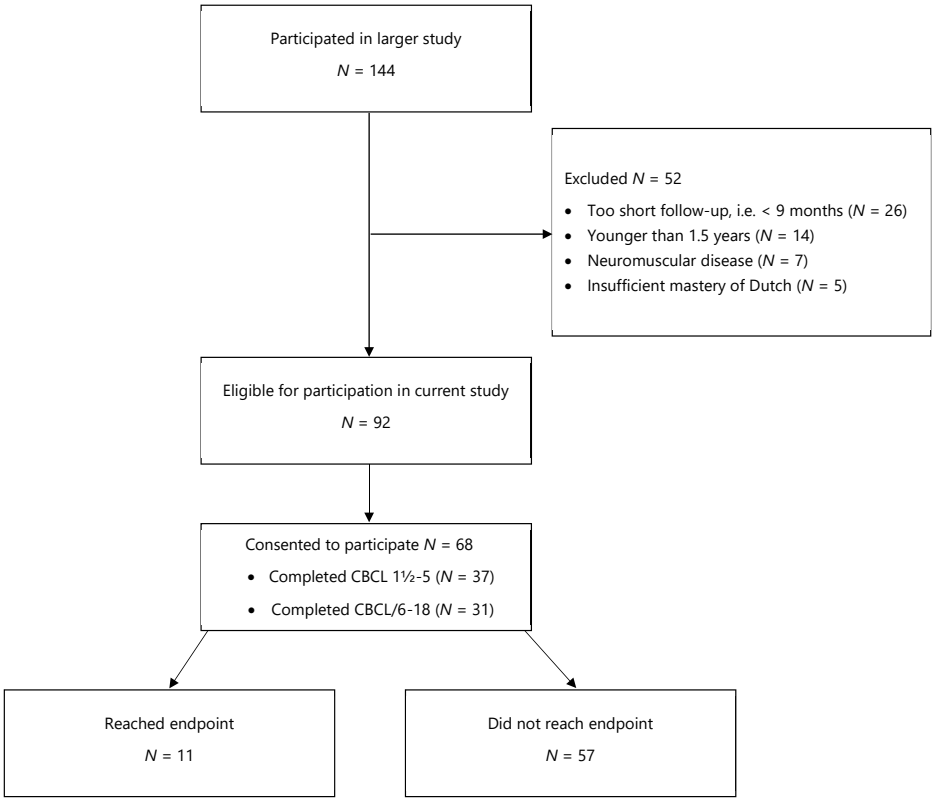

Table 1 Participant characteristics.

Characteristic	Overall group (N=68)		Did not reach endpoint (*N*=57)		Reached endpoint (*N*=11)	
	1.5-5 years (*N*=37)	6-18 years (*N*=31)	1.5-5 years (*N*=31)	6-18 years (*N*=26)	1.5-5 years (*N*=6)	6-18 years (*N*=5)
Male gender, *N* (%)	20 (54.1%)	17 (54.8%)	18 (58.1%)	16 (61.5%)	2 (33.3%)	1 (20.0%)
Age in years, *M* (*SD*)	2.2 (1.3)	12.4 (3.5)	2.0 (0.5)	12.5 (3.6)	3.3 (1.2)	11.8 (3.4)
Time since DCM diagnosis in months, median (IQR*)	19.0 (12.0-36.0)	57.0 (24.0-107.0)	18.0 (12.0-24.0)	58.5 (27.0-110.0)	40.5 (24.5-59.0)	24.0 (17.0-73.5)
NYU PHFI†, *M* (*SD*)	6.3 (4.8)	7.2 (3.8)	4.8 (3.2)	5.5 (3.1)	14.2 (3.9)	12.6 (2.1)
Socioeconomic status‡, N (%)						
Low	1 (2.7%)	2 (6.5%)	1 (3.2%)	2 (7.7%)	0 (0.0%)	0 (0.0%)
Low to middle	10 (27.0%)	10 (32.3%)	8 (25.8%)	10 (38.5%)	2 (33.3%)	0 (0.0%)
Middle	3 (8.1%)	6 (19.4%)	3 (9.7%)	6 (23.1%)	0 (0.0%)	0 (0.0%)
High	16 (43.2%)	10 (32.3%)	13 (41.9%)	5 (19.2%)	3 (50.0%)	5 (100.0%)
Missing	7 (18.9%)	3 (9.7%)	6 (19.4%)	3 (11.5%)	1 (16.7%)	0 (0.0%)

* IQR = Interquartile range.
† NYU PHFI = New York University Pediatric Heart Failure Index.
‡ SES was determined by parents' occupation level(25).

Six- to 18-year-old children (CBCL/6-18)

The distribution of parent-reported emotional and behavioral problems in 6- to 18-year-old children with DCM and children from the general population is shown in Table 3. The CBCL/6-18 was completed for 31 children (by N = 5 fathers, N = 23 mothers, N = 3 parents together) at a median time of 39.0 months after DCM diagnosis (range 12.0-177.0 months; see Table 1). Compared with normative data of same-aged peers, significantly larger proportions of children with DCM showed problems in the borderline or clinical range on the following scales: Internalizing Problems (p = .001; 17.0% vs. 38.7%), anxious/depressed problems (p = .023; 8.0% vs. 19.4%), somatic complaints (p < .001; 8.0% vs. 29.0%), depressive problems (p < .001; 8.0% vs. 29.0%), anxiety problems (p = .023; 8.0% vs. 19.4%), and somatic problems (p < .001; 8.0% vs. 25.8%). For the other scales, the proportion of borderline and clinical problems did not significantly differ between children with DCM and children from the general population.

Table 2 Distribution of non-clinical versus borderline/clinical emotional and behavioral problems reported by parents of 1.5- to 5-year-old children (CBCL 1½-5).

CBCL 1½-5 scale	DCM patients (*N* = 37) *		General population		
	Non-clinical, *N* (%)	Borderline/ clinical, *N* (%)	Non-clinical %	Borderline/ clinical %	*p*-value
Broadband scales					
Internalizing Problems	29 (78.4%)	8 (21.6%)	83%	17%	.298
Externalizing Problems	35 (94.6%)	2 (5.4%)	83%	17%	.049
Total Problems	31 (83.8%)	6 (16.2%)	83%	17%	.500
Syndrome scales					
Anxious/Depressed	36 (97.3%)	1 (2.7%)	92%	8%	.188
Somatic Complaints	28 (75.7%)	9 (24.3%)	92%	8%	< .001
Attention Problems	36 (97.3%)	1 (2.7%)	92%	8%	.188
Aggressive Behavior	35 (94.6%)	2 (5.4%)	92%	8%	.390
Emotionally Reactive	31 (83.8%)	6 (16.2%)	92%	8%	.062
Withdrawn	33 (89.2%)	4 (10.8%)	92%	8%	.372
Sleep Problems	34 (91.9%)	3 (8.1%)	92%	8%	.500
DSM-oriented scales					
Depressive Problems	31 (83.8%)	6 (16.2%)	92%	8%	.062
Anxiety Problems	32 (86.5%)	5 (13.5%)	92%	8%	.175
Attention Deficit/ Hyperactivity Problems	37 (100%)	0 (0%)	92%	8%	.068
Oppositional Defiant Problems	36 (97.3%)	1 (2.7%)	92%	8%	.188
Autism Spectrum Problems	34 (91.9%)	3 (8.1%)	92%	8%	.500

* Reported by fathers (*N*=9), mothers (*N*=25), or both parents together (*N*=3).

Predictive value of anxiety and depressive problems on endpoint

We examined whether anxiety and depressive symptoms predicted the combined risk of death or cardiac transplantation whilst controlling for NYU PHFI. The proportional hazard assumptions were not violated. Before July 1, 2017, 11 participants (16.2%) had reached an endpoint. One had died and 10 had undergone cardiac transplantation. The results of

the Cox regression analysis are presented in Table 4. Anxiety problems and depressive problems did not significantly predict death or cardiac transplantation. However, the NYU PHFI did significantly predict the risk of death or cardiac transplantation, p < .001. A one unit increase in the NYU PHFI resulted in a 42% higher risk of death or cardiac transplantation (hazard ratio 1.42, 95% confidence interval 1.19-1.69).

Table 3 Distribution of non-clinical versus borderline/clinical emotional and behavioral problems reported by parents of 6- to 18-year-old children (CBCL/6-18).

CBCL/6-18 scale	DCM patients (*N* = 31)[a]		General population		
	Non-clinical, *N* (%)	Borderline/ clinical, *N* (%)	Non-clinical %	Borderline/ clinical %	*p*-value
Broadband scales					
Internalizing Problems	19 (61.3%)	12 (38.7%)	83%	17%	.001
Externalizing Problems	28 (90.3%)	3 (9.7%)	83%	17%	.199
Total Problems	26 (83.8%)	5 (16.1%)	83%	17%	.500
Syndrome scales					
Anxious/Depressed	25 (80.6%)	6 (19.4%)	92%	8%	.023
Withdrawn/Depressed	28 (90.3%)	3 (9.7%)	92%	8%	.495
Somatic Complaints	22 (71.0%)	9 (29.0%)	92%	8%	< .001
Social Problems	28 (90.3%)	3 (9.7%)	92%	8%	.495
Thought Problems	26 (83.9%)	5 (16.1%)	92%	8%	.091
Attention Problems	27 (87.1%)	4 (12.9%)	92%	8%	.250
Rule Breaking Behavior	31 (100%)	0 (0%)	92%	8%	.095
Aggressive Behavior	29 (93.5%)	2 (6.5%)	92%	8%	.500
DSM-oriented scales					
Depressive Problems	22 (71.0%)	9 (29.0%)	92%	8%	< .001
Anxiety Problems	25 (80.6%)	6 (19.4%)	92%	8%	.023
Somatic Problems	23 (74.2%)	8 (25.8%)	92%	8%	< .001
Attention Deficit/ Hyperactivity Problems	28 (90.3%)	3 (9.7%)	92%	8%	.495
Oppositional defiant problems	28 (90.3%)	3 (9.7%)	92%	8%	.495
Conduct problems	29 (93.5%)	2 (6.5%)	92%	8%	.500

[a] Reported by fathers (*N*=5), mothers (*N*=23), or both parents together (*N*=3).

Table 4 Results of Cox regression analysis.

Variable	HR†	95% CI*		p-value
		Lower	Upper	
Anxiety Problems (t-score)	0.98	0.89	1.09	.72
Depressive Problems (t-score)	0.98	0.88	1.08	.64
NYU PHFI‡ (per unit)	1.42	1.19	1.69	< .001

*CI = confidence interval

† HR = hazard ratio

‡ NYU PHFI = New York University Pediatric Heart Failure Index

DISCUSSION

The current study is the first to investigate emotional and behavioral problems in a substantial cohort of children with DCM. Some results are in line with our expectations. Importantly, we found that, compared with normative data of same-aged peers, larger percentages of older children (6-18 years old) with DCM showed overall internalizing problems, anxiety problems, and depressive problems. Also, we found trends towards significance suggesting that, compared with normative data of same-aged peers, larger percentages of younger children (1.5-5 years old) with DCM showed emotionally reactive problems and depressive problems. These results are in line with meta-analyses in adult heart failure populations, which demonstrate an increased risk of anxiety and depression (13, 14).

Until now, only two studies have examined emotional and behavioral problems in children with DCM. The first study was conducted by Wray and Radley-Smith(19) who found that 50% of the children with cardiomyopathy in their study (N=19, age 3½-17 years) showed a clinical level of overall emotional and behavioral problems on the CBCL questionnaire. In our study, this percentage was markedly lower (i.e., 10.8% in younger children and 16.1% in older children). This difference may be due to the fact that all children with cardiomyopathy in Wray and Radley-Smith's study were listed for cardiac transplantation, whereas in our study this was not the case.

In the second study, Menteer and colleagues (20) compared the level of depressive symptoms in children (aged 7-21 years) with DCM (N = 15), children who had successfully undergone cardiac transplantation for heart failure (N = 23), and healthy children (N = 24). In contrast to our results, they found similar levels of depressive symptoms in all groups. That is, the level of depressive symptoms in children with DCM did not significantly differ from the level of depressive symptoms in healthy children and children who had undergone cardiac transplantation. However, it should be noted that Menteer and colleagues used small sample sizes, which limits the statistical power to detect differences between groups. Moreover, this discordance in results may be explained by the fact that we assessed depressive problems through the CBCL questionnaire whereas Menteer and colleagues used the Children's Depression Inventory (CDI) (29). Although both instruments assess depressive symptoms, previously, moderate correlations between CDI total scores and CBCL depressive problems scores have been found(30).

Depressive and anxiety problems in children with DCM may be caused by factors directly or indirectly related to the illness. For example, in other chronic illnesses, it has been shown that the symptoms of the illness itself (31, 32) and side effects of medical treatments (33) can provoke anxiety and depressive symptoms. More indirectly, illness uncertainty (i.e., uncertainty regarding prognosis, disease course, and treatment) can increase symptoms of depression and anxiety (31, 34). This can be explained by the cognitive coping theory (35), which states that children interpret situations based on previous knowledge and experiences. When such information is lacking, a situation may be interpreted as a threat, which consequently increases symptoms of depression and anxiety (31, 36-39). Similarly, medical treatments such as injections may be experienced as distressing and threatening, thereby increasing children's anxiety levels (31). Furthermore, it is known that parental

overprotectiveness can promote anxiety and depressive symptoms in children with a chronic illness (31, 34, 40). Depressive and anxiety problems may also have a biological cause. In adult (41, 42) and pediatric (43) heart failure populations, reduced brain tissue volumes have been found in brain areas which regulate mood. Future research is needed to draw definite conclusions as to biological causes of mood problems in DCM.

Besides increased anxiety and depressive problems, we demonstrated that, compared with normative data, a larger percentage of young and older children with DCM showed a borderline or clinical level of somatic problems. This is not surprising considering all children had heart failure problems secondary to DCM. Furthermore, previous studies have reported reduced levels of physical health related quality of life in this population (15).

Other results of the current study were unexpected or contrary to our hypotheses. Firstly, we found that, compared with normative data, a smaller percentage of young children with DCM showed a borderline or clinical level of externalizing problems. In line with this result, we found a trend towards significance suggesting that, compared with normative data, a smaller percentage of young children with DCM showed attention deficit/hyperactivity problems. This might be explained by increased levels of fatigue reported in DCM (5), which may contribute to children showing less hyperactive behavior.

Secondly, contrary to our hypothesis, we found that anxiety and depressive problems in children with DCM did not predict the risk of death and cardiac transplantation whilst controlling for heart failure severity. However, in line with the results of a previous DCM study, heart failure severity (NYU PHFI) did predict the risk of death and cardiac transplantation (15). In contrast with our findings, in adult heart failure populations, a multitude of studies have shown that depressive problems predict mortality and other adverse clinical outcomes (e.g., 13, 21, 44, 45-48). Furthermore, increasing evidence shows that anxiety problems predict mortality in adult heart failure (22). An explanation for our different findings is that, in adults, depressive and anxiety problems can lead to poorer self-care (49). In children, however, parents may compensate for children's poorer self-care behaviors which subsequently diminishes the impact of depressive and anxiety problems on their physical health. Also, it should be noted that statistical power to detect associations was limited.

This study has several strengths. Studies exclusively examining pediatric cardiomyopathy patients are scarce (12). As stated, the current study is the first to examine emotional and behavioral problems in a relatively large cohort of children with DCM. We recruited children with DCM through seven tertiary centers for pediatric cardiology. Also, we investigated problems in a broad age range (1.5 to 18 years), using an internationally well-validated questionnaire (CBCL) to assess a wide range of emotional and behavioral problems. The multicenter recruitment and the inclusion of a broad age range improve the generalizability of our results in the pediatric DCM population. Moreover, we examined the predictive value of depressive and anxiety problems on mortality and cardiac transplantation whilst controlling for heart failure severity, which is a known predictor of adverse outcomes. Furthermore, results from our study population were compared to representative normative data matched on age and gender.

The results of this study must also be interpreted in light of a few limitations. Firstly, although the study sample is relatively large considering the prevalence of DCM, the number of events in the study was 11, which limits the statistical power of the prediction analyses. Secondly, we only used proxy-reports completed by parents because most participating children were too young to complete the self-report version of the CBCL (50). Of the children who were old enough to complete the self-report version of the CBCL an insufficient number to analyze completed the questionnaire. The use of proxy-reports has been frequently debated. Studies have found that parent proxy-reports of quality of life in pediatric cardiac populations may differ from child self-reports (51, 52). In another pediatric cardiac population, Patel and colleagues (53) found that parent-child agreement was stronger for more readily observable variables such as physical functioning and externalizing behavior and lower for variables which tend to be less visible, such as anxiety, emotional functioning, and internalizing behavior. In contrast, in a pediatric cardiac population, Marino and colleagues (54) found that parent-proxy reports and child self-reports on quality of life did not differ. Moreover, Wilmot and colleagues (16) reported moderate parent-child agreement on quality of life of children with cardiomyopathy. Considering the scarcity of research into emotional and behavioral problems in children with DCM, further research is needed using well-attuned self-reports as well. Thirdly, we combined both father and mother reports in our analyses. Although this may induce bias (55), it should be noted that the majority of questionnaires were completed by mothers and previous research has found moderately high inter-parent agreement on the CBCL (56). Fourthly, considering the relatively long period of time between DCM diagnosis and participation in the current study (see Table 1), it should be noted that the current cohort represents children with chronic heart failure. Children who reached an endpoint or recovered shortly after diagnosis are likely underrepresented.

In conclusion, this first study specifically examining emotional and behavioral problems of children with DCM showed, compared with normative data, significantly more borderline or clinical levels of anxiety, depressive problems, and somatic problems in 6-18-year-olds and significantly more borderline or clinical somatic problems and less externalizing problems in 1.5-5-year-olds. These findings demonstrate the importance of including routine screening for internalizing problems to the clinical management of children with DCM (10) and of providing psychosocial support attuned to the needs of these children. Considering the previously mentioned influence of parental behavior on anxiety and depressive symptoms in pediatric chronic illness, such psychosocial support should not only focus on the children themselves but also include their parents. Future research should focus on evidence-based psychosocial programs to treat and prevent internalizing problems in pediatric cardiomyopathy. As the available literature on emotional and behavioral wellbeing in pediatric cardiomyopathy is limited, many aspects remain to be studied. Considering previous adult studies and our findings, future research should focus on anxiety and depression in pediatric DCM. Moreover, as the results of our study show that emotional and behavioral problems in DCM seem to differ per age group, it would be useful to examine this in more age groups. Furthermore, a previous study (15), found that HRQoL in children with DCM was more impaired at diagnosis than more than 1 year after diagnosis. Whether this is also the case for emotional and behavioral problems remains to be studied. Also, since little is known about the psychosocial wellbeing of children with cardiomyopathy, future qualitative studies would be valuable.

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Mechanical circulatory support in the Dutch National Pediatric Heart Transplantation Program

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ABSTRACT

OBJECTIVE Mechanical circulatory support (MCS) with a Ventricular Assist Device (VAD) as a bridge to heart transplantation (HTx) or recovery may improve outcome in children with terminal heart failure. We report our experience with mechanical circulatory support in children eligible for HTx and its effect on waiting list mortality.

METHODS Retrospective single center cohort study, national pediatric HTx program including all children eligible for HTx, from introduction of MCS-VAD in 2006.

RESULTS A total of 43 patients were eligible for HTx, median age 11.7 years, (IQR 3.0-14.7). In 18 patients (42 %) a VAD was implanted, 11 (61%) survived to HTx (n=9) or recovery (n=2). Techniques and devices used were LVAD (n=16, 89%), in 4 cases preceded by ECMO, and BiVAD (n=2, 11%), both preceded by ECMO. In the VAD group median time to death (n= 7) was 18 days (IQR 7-75), median time to HTx (n=9) 66 days (IQR 33-223), and 2 patients recovered after 30 and 308 days. The main cause of death on MCS was neurological injury in 4 patients (22%) and systemic thrombo-embolic events in 2 (11%). The most common Serious Adverse Events included confirmed thrombus requiring pump replacement (in 11 patients, 61%) and pericardial effusion leading to rethoracotomy (in 5 patients, 28%). Compared to the era before MCS (1998-2006), waiting list mortality decreased from 44% to 21% and was now mainly related to complications of VAD support.

CONCLUSIONS Since the introduction of MCS-VAD waiting list mortality halved and more children with end-stage heart failure survived to heart transplantation, thus improving outcome. Although there is substantial mortality and morbidity, overall mortality decreases, making MCS-VAD an essential therapeutic tool. The need for donor organs remains critically urgent.

INTRODUCTION

Heart failure is an important cause of morbidity and mortality in children. The majority of children are diagnosed with (idiopathic) dilating cardiomyopathy (iDCM) or congenital heart disease (1). At presentation, 87% of children suffer from severe cardiac decompensation (2). Estimates are that 50% of children with DCM die or undergo heart transplantation within 5 years after diagnosis (3, 4). For children with medically incurable end-stage heart failure, heart transplantation is the only long term therapeutic option. As donor availability is limited, children face a prolonged time on the waiting list. North American studies report a waiting list mortality of 25%, which is the highest in transplantation medicine (5, 6).

In order to improve survival in patients with terminal heart failure who fail medical therapy, much effort has been put into the development of mechanical support of the circulation. Extra Corporeal Membrane Oxygenation (ECMO) has successfully been used to sustain the circulation for several days to weeks, but has serious limitations in providing long term support (7).

Ventricular Assist Devices (VADs) are nowadays a well-accepted, long-term therapeutic option in adult end stage heart failure (8). Implementation of VADs in the pediatric population has been troublesome due to technical problems and difficulties in medical management. However, in recent years, several centers have presented their experience with Berlin Heart Excor VAD as long-term support. They have shown that Berlin Heart Excor VAD is a reliable and relatively safe device in bridging children to heart transplantation or recovery (9-13).

Since 1998 our institute serves as a referral hospital in the Netherlands for end-stage heart failure and heart transplantation in children. Since 2006 the Berlin Heart Excor Pediatric Ventricular Assist Device has become available in our hospital. The primary aim of this study is to describe the outcome of children supported with a VAD. Secondly, we aim to determine the effect of the introduction of the VAD on waiting list mortality.

METHODS

Patients

This study was approved by the local medical ethical committee (MEC 2014 – 525). All children listed for heart transplantation, as well as those who were eligible for heart transplantation at the time of VAD implantation at our centre, from September 1, 2006 to July 31, 2014 were included in the study. Medical records were retrospectively reviewed. In addition, medical records of patients listed for heart transplantation from 1998 to September 1st 2006 were reviewed to serve as historical control group of the era before introduction of VADs. Primary end points for analysis were all cause mortality, heart transplantation and weaning from VAD. Serious Adverse Events (SAE) were assessed in the VAD group only and consisted of: major bleeding, pericardial effusion requiring rethoracotomy, infection-sepsis, neurological dysfunction thrombo-embolic and hemorrhagic), renal dysfunction,

right heart failure, hepatic dysfunction , arterial non-CNS thrombo-embolism, device malfunction, including confirmed pump thrombus requiring pump replacement and others (definitions adapted from INTERMACS) (14). Demographic data were defined at listing for heart transplantation. In the VAD group additional demographics, including INTERMACS profile, were determined at the time of VAD implantation. Glomerular Filtration Rate was estimated using the Schwartz formula (15).

VADs and Surgical procedure

The Berlin Heart Excor Pediatric Ventricular Assist Device (Berlin Heart AG, Berlin, Germany) is a paracorporal, pneumatically driven, polyurethane bloodpump (stroke volumes of 10-25-30-50-60 ml) and can be implanted as left ventricular assist device (LVAD), right ventricular assist device (RVAD) or biventricular assist device (BiVAD).

All operations were performed through a standard median sternotomy using normothermic extra-corporeal circulation. The heart was kept beating throughout the procedure. For LVAD the inflow cannula was inserted at the left ventricular apex, the outflow cannula in the ascending aorta. Additional right atrial to main pulmonary artery cannulation was used in case BiVAD was required. In our hospital it was standard to first connect the Berlin Heart Excor cannulae to a Centrimag Levitronix centrifugal pump (Levitronix GmbH, Zurich, Switzerland). In the initial phase of our pediatric VAD program this approach was primarily based on logistic reasons. The Levitronix pump can be connected to all sizes of Berlin Heart Excor cannulae, thus only the different cannulea sizes had to be in stock. Furthermore, this policy allows for a less strict anti-coagulatory regimen in case of actual or expected bleeding complications (16). In the course of days, when patients were clinically stable and hemostasis was achieved, the Levitronix pump was changed for a matching Berlin Heart Excor ventricle.

Criteria for VAD implantation

In general, patients had to be eligible for heart transplantation (17). Furthermore the following criteria were applied: a) deterioration of the circulation with increasing dosages of inotropes and/or development of metabolic acidosis, b) development of end-organ failure including inability to wean from respiratory support on the ventilator, signs of renal or hepatic damage and altered neurological state e.g. confusion. An individualized decision was made by the clinical team, based on these criteria and the rate of clinical deterioration.

Anticoagulation during VAD support

All children on VAD were treated with a standardized anticoagulation and antiplatelet regimen known as the Edmonton protocol (12, 18). This protocol is developed by pediatric specialists in hematology, mechanical support, heart failure, and surgery, and encompasses 15 years of experience with the Berlin Heart Excor worldwide. In general, children were treated with unfractionated heparin, low-molecular-weight heparin, or warfarin, in combination with anti-platelet agents (dipyridamole and/or acetylsalicylic acid).

Weaning criteria
Children with ongoing improvement of myocardial function, laboratory parameters (declining N-terminal Pro-BNP levels), echocardiographic signs of reverse remodeling and echocardiographic findings matching improvement of systolic function (opening of aortic valve, increasing shortening fraction), were considered for weaning. The pump flow was decreased by decreasing pump rate as described by Stiller et al. (19). Pump stops were evaluated by assessment of clinical hemodynamic parameters (e.g. blood pressure, peripheral perfusion), and the above mentioned echocardiographic parameters, followed by surgical explantation when weaning was tolerated well.

Statistical analysis
Statistical analysis was conducted with IBM SPSS statistics 21 (IBM, NY, US). Continuous variables were expressed as medians (Inter Quartile Range) or numbers (percentage). The Independent median test was used to compare continues variables, categorical analysis was conducted by chi-square and Fisher's exact test. Mortality was compared between groups by Cox regression analysis. The level of significance was 0.05.

RESULTS

Patients
Data of 15 patients were collected over a study period from 1998 to 2006, the pre-VAD era (cohort I), and data of 43 patients were collected from September 2006 to July 2014 (cohort II). In cohort II, 18 patients underwent implantation of a VAD (VAD group). Baseline clinical characteristics are described in Table 1. There was no difference in age or weight between patients on VAD and non-VAD patients. VAD patients were more often diagnosed with iDCM. A GFR < 30% of predicted was found in 2 patients (11%), 1 patient died while on VAD and the in the other renal function fully recovered after 7 days on VAD. A bilirubin above the upper level of normal was found in 65% of VAD patients. In the VAD group, 94% of patients were on inotropic support at the time of VAD implantation and 89% had an INTERMACS profile of I or II.

VAD
Of the 18 implanted VADs, there were 16 LVADs and 2 BiVADs. In 6 cases implantation of a VAD was preceded by ECMO followed by LVAD in 4 patients and BiVAD in 2 patients. Duration of ECMO in these 6 cases was 2.5 days median (IQR 1.0-10.3 days). In 3 cases the rapidly deteriorating clinical condition precluded a suitable timeframe for direct implantation of a VAD, in 2 cases patients were supported by ECMO in a 'crash' condition, and 1 patient was referred to our hospital while already on ECMO. Median duration of treatment on Levitronix pump prior to placement of a Berlin Heart Ventricle was 6 days (IQR 1.0-9.3 days). Three children were treated with a Levitronix system only. The first child died after 6 days in ongoing multi-organ failure . The other two were older children in whom we expected a short waiting time as they were waiting for larger donors. These two patients underwent successful transplantation after 10 and 13 days on MCS, respectively.

Primary end points

In cohort I, the pre-VAD era, 7/15 patients died (47%) and 8/15 patients were transplanted (53%), see Figure 1. In cohort II, after the introduction of VADs, 9/43 patients died (21%) and 31/43 patients were transplanted (72%). Cox regression analysis showed a trend towards a significant reduction in waiting list mortality after the introduction of VAD (p = 0.08). In the VAD-group of cohort II, 9/18 patients were transplanted (50%), 7/18 patients died (38%; 2/2 BiVAD and 5/16 LVAD) and 2/18 patients were successfully weaned from VAD, one diagnosed with myocarditis and one with iDCM, after 30 and 308 days respectively. Median time to death (n=7) was 18 days (IQR 7-75), median time to HTx (n=9) was 66 days (IQR 33-223). Of the 9 VAD patients undergoing heart transplantation, 1 died 3 years after transplantation, due to non-compliancy to medical treatment. The most important causes of death for children who died on VAD were thrombo-embolic stroke (43%) and systemic thrombo-embolic events (29%), see Table 2. One patient in the VAD group died while he was still on a Levitronix system.

Table 1. Characteristics of patients listed for heart transplantation.

Demographic	total (n=43)	VAD (n=18)	non VAD (n=25)	p value
Age, median (IQR), y*	12 (3-15)	11 (2-13)	12 (4-12)	0.30[1]
Weight, median (IQR), kg*	33 (12-45)	20 (9-44)	36 (13-51)	0.32[1]
Female sex, n (%)	23 (54)	10 (56)	13 (52)	0.81[2]
Diagnosis n (%)				0.006[3]
(i)DCM	23 (54)	13 (72)	9 (36)	
myocarditis	1 (2)	1 (6)	0	
other**	19 (44)	4 (22)	16 (64)	
At VAD implantation:				
INTERMACS status				
I/II n (% of 18)		16 (89%)		
I/IV n (% of 18)		2 (11%)		
Serum creatinine µmol/L, median (IQR)		58 (36-91)		
GFR categories (age adjusted), n (% of 18)				
< 30% predicted		2 (11%)		
30-99% predicted		10 (56%)		
> 99% predicted		6 (33%)		
Total Bilirubin µmol/L, median, (IQR), n (% of 17)		16 (8-34%)		
≤ 16 µmol/L		6 (35%)		
> 16 µmol/L		11 (65%)		
Preoperative inotropic support no. (%)		17/18 (94%)		

* At listing for HTx

** Others consist of congenital heart diseases (n=3) restrictive cmp (n=6), hypertrofic cmp (n=2) anthracycline induced cmp (n=4), Beckers muscular dystropy (n=1) and rythm disturbances induced cmp (n=3)

p value: differences between VAD and non-VAD patients

[1]Non parametric test, independent samples

[2]Chi square test

[3]Fisher's exact

Table 2. Cause of death for children who died on a VAD.

Factor	n	(% of 7)
Neurological	4	57%
Thromboembolic stroke	3	43%
Intracranial bleeding	1	14%
Systemic	3	43%
Thromboembolic	2	29%
Bleeding	1	14%
Total	**7**	

In the non-VAD group of cohort II, 22/25 patients were transplanted (88%), 2/25 patients died (8%) and 1 patient is still listed, no patients were delisted, see Figure 1. Median time to transplantation was 137 days (IQR 18-417). Regarding the 2 deaths in the non-VAD group: the parents of one patient refused implantation of a VAD, the parents of the other patient postponed their consent for VAD implantation until continued cardiopulmonary resuscitation was needed and implantation of a VAD was not achievable anymore. Comparing the VAD-group with the non-VAD patients, we found no difference in median time to death or transplantation.

Figure 1. Flow diagram of Dutch National Pediatric Heart transplantation Program before and after availability of VAD.

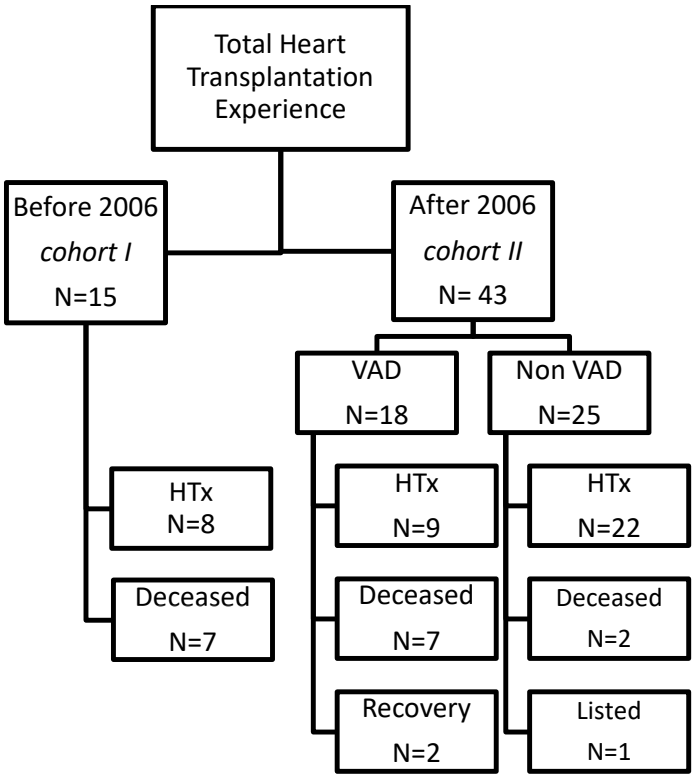
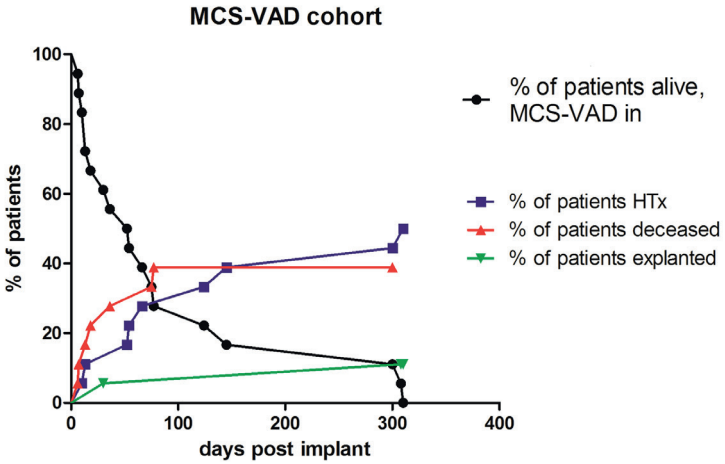
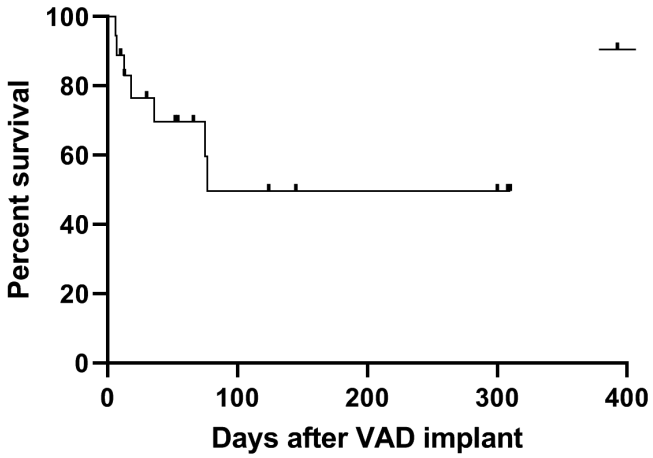


Figure 2. Competing outcomes (HTx, deceased, explanted) for children on waiting list supported with VAD



The primary endpoints are shown as competing events (Figure 2), depicting that the majority of end points were reached in the first 100 days after VAD implantation. In Figure 3 patient survival on VAD is shown.

Figure 3. Survival of VAD patients



number at risk 18 5 3

Serious Adverse Events

The most common SAEs that occurred in the VAD patients are described in Table 3. In 16/18 (89%) of patients, at least one SAE occurred. The most prevalent SAEs were confirmed thrombus requiring pump replacement in 11 patients (61%), neurological dysfunction in 7 patients (39%) and pericardial effusion requiring rethoracotomy in 5 patients (28%). Pump replacements occurred in 4/5 (80%) of children who died of a thromboembolic event. The median number of pump replacements was 1 per patient, in 4 patients the pump was replaced 3 times or more. In those 4 patients median time on VAD was 306 days, they all survived. The range of Berlin Heart Excor ventricle sizes was 10 to 60 ml: 1 ventricle of 10 ml, 6 ventricles of 25 ml, 1 ventricle of 30 ml, 5 ventricles of 50 ml, 2 ventricles of 60 ml and 3 children were assisted with a Levitronix system only. Considering children below 15 kg as "small patients", our series included 5 small patients (4 with 25 ml ventricle, 1 with a 10 ml ventricle) of which 3 patients developed a thrombo-embolic stroke.

Of the 7 patients with thrombo-embolic stroke or intracranial bleeding, 4 patients died and 3 patients suffered from neurologic disability. In the latter 3, the degree of neurological damage did not preclude heart transplantation. Follow up data of 8, 3 and 2,5 years respectively after VAD implantation were available. One patient had a right sided hemiplegia, disabling dystonia of the right arm and mild problems with the study of (foreign) language and calculus. A second patient suffered from a left sided hemiplegia, but fully recovered. The third patient has recovered completely from a right sided hemiplegia.

Table 3. Serious Adverse Events during VAD support, summary.*

Serious Adverse Event	events	patients with event n (% of 18)
Any SAE	40	16 (89%)
Major Bleeding	2	2 (11%)
Pericardial effusion requiring rethoracotomy	5	5 (28%)
Infection-Sepsis	1	1 (6%)
Neurological Dysfunction	7	7 (39%)
Thromboembolic	6	6 (33%)
Hemorrhagic	1	1 (6%)
Renal Dysfunction	2	2(11%)
Right Heart Failure	2	2 (11%)
Hepatic Dysfunction	1	1 (6%)
Arterial Non-CNS Thromboembolism	1	1 (6%)
Device Malfunction		
Confirmed thrombus requiring pump replacement	16	11 (61%)
Major Device Malfunction	1	1 (6%)
Other**	2	2 (11%)

*SAE's are defined according to the INTERMACS criteria

** including possible airembolism and Posterior Reversible Encephalopathy Syndrome

DISCUSSION

In this study we found that since 2006, 18 patients were supported on VAD and 61% of these children survived to heart transplantation or recovery. The majority of children who died on VAD were struck by thrombo-embolic events. Similarly, lasting neurological damage was caused by thrombo-embolic events as well. Furthermore we found that, compared to the era before VADs, waiting list mortality decreased from 44% to 21% and mortality was largely related to complications of VAD support. Our study points out that the overall survival of children with end stage heart failure increases with the availability of VADs, but this comes at a high prize. A significant number of children die on a VAD or have lasting neurological disability. This urges to critically consider the results and to aim for an improvement of outcome in these patients.

In this regard a reflection on the mortality in our VAD patients is relevant. Data from prior studies demonstrated a wide range of mortality in pediatric patients on a VAD, varying from 38% to 6% (9-13, 20-22), placing our outcome on the unfavourable end of the spectrum. Several factors might contribute to mortality in our patient population, of which patient selection may play an important role. The studies with the lowest mortality are those with the most strict inclusion criteria for implantation of a VAD. For example, in the North American IDE-trial, children with significant end-organ dysfunction were excluded. In this study they found a low mortality rate (10). Clinical centers who used VADs on a 'compassionate use' basis presented mortality data of 37%, which is more comparable to our findings. They found that the degree of end-organ dysfunction, in particular the severity of renal failure, had an important effect on outcome (12). Patient profiles of the 'compassionate use' group were much like our patient group in terms of INTERMACS profile and percentage of significant end-organ failure. Other single center studies reporting on pediatric VAD outcome, either showed quite similar mortality (21), were difficult to compare with regard to patient characteristics at implantation of VAD (22), or showed an encouraging learning curve in demonstrating that mortality decreased to 18% in the last 4 years of their experience (23). The relatively low mortality found in the IDE-trial suggests that favorable patient selection leads to improvement of outcome. Our mortality data may reflect our somewhat prudent policy in implanting VADs in children, resulting in a severely ill patient group with (beginning) end-organ failure. However, we also found that mortality in the non-VAD patients was very low (2/25, 8%) and in the 2 children who died, parents did not consent to VAD implantation. This suggests that although we implant VADs relatively late in the disease process, we do not lose patients as a result of this conservative policy.

With regard to SAE, we found a significant number of these events during VAD treatment. The most common SAE was formation of thrombus in the system leading to VAD replacement, occurring in 61% of patients. Brancaccio et al report pump replacement in 52% of patients (23), similar to Malaisrie et al who found 50% (21). The comprehensive study performed by Almond et al and the single center study by Rockett et al are not clear on this matter (12, 22).

In the days following VAD implantation 5 patients (28%) developed pericardial effusion necessitating rethoracotomy. This figure is comparable to the rethoracotomy percentages in other studies (22).

The most alarming Serious Adverse Events are the thrombo-embolic events, which are even more disconcerting in the face of our relative high number of pump replacements. The limited number of observations did not allow a formal analysis with regard to a possible association between Berlin Heart Excor ventricle size and thrombo-embolic events. Considering children below 15 kg as "small patients", our series included 5 small patients (4 with a ventricle of 25 ml, 1 with a ventricle of 10 ml), 3 patients developed a thrombo-embolic event. In the whole VAD group we found 7/18 (38%) patients with a thrombo-embolic event, 4/13(31%) in the patients over 15 kg versus 3/5 (60%) in the small patients. This suggests that in smaller children more (lethal) thrombo-embolisms may occur. In the present study we found thrombo-embolic stroke in 33% of children and thrombo-embolic stroke accounted for 43% of all deaths on VAD. The number of thrombo-embolic strokes in our study is similar to other single centre outcomes (21, 22), but higher than the earlier mentioned compassionate use cohort (12). This might suggest that the high stroke rate cannot be fully explained by patient selection only. Preventing thrombo-embolic events is quite challenging. In our hospital we follow the well accepted Edmonton protocol (16,17). Closer monitoring of coagulation and possibly exploration of other anti-coagulatory strategies (18) might lead to reduction of thrombo-embolic events.

In spite of a considerable mortality and morbidity in VAD patients, we still believe that a VAD is an acceptable therapeutic tool. Taken into account that survival of heart transplantation patients is unaffected by previous VAD implantation (24), we are inclined to believe that the positive effect of VAD support on survival in end-stage pediatric heart failure outweighs the disconcerting mortality and morbidity rate. We conclude that optimal timing in placement of a VAD is to be determined for every individual patient and antithrombotic monitoring deserves highest priority. It is clear that we need to continue to closely monitor our results and aim for improvement of outcome.

This study has several limitations. First of all, the study is prone to selection bias as a result of its retrospective design. We expect however that selection bias was minimal as we serve as referral hospital, and were to our knowledge, able to evaluate all Dutch pediatric VAD patients. Secondly, the number of patients is small preventing robust statistical analysis. Nevertheless, the trends that we observed in our study are comparable to other reports on the use of VADs in the pediatric age group. Thirdly, we present the cumulative experience over the course of 8 years, reflecting a learning curve of selecting patients as well as surgical and medical management of patients on VAD. Lastly, we reported the outcome of patients from 2 consecutive eras, inherently resulting in a historical comparison. Indeed, our data reflect a growing heart transplantation program and over the course of years the number of patients referred to our hospital and listed for heart transplantation increased accordingly. Nevertheless patient profiles in the 2 cohorts were quite similar. In cohort 1 all patients were on the waiting list. In this era, we did not use any form of mechanical support (including ECMO) as a bridge to transplantation. In cohort 2, all patients were on the waiting list or were eligible for heart transplantation at the time of

admission for mechanical support. Patients with expected short time mechanical support, for example patients with myocarditis, were excluded as they were not considered eligible for heart transplantation. Except for one patient with a protracted disease course (> 6 weeks). Therefore, we believe that the data presented in this study reliably describe *all* patients who were referred to our hospital and who were considered eligible for heart transplantation. This implies that cohort 1 and 2 are a fair representation of children requiring transplantation in the respective eras, and patient profiles were sufficiently similar to meaningfully compare the two cohorts.

Conclusion

We found that in our hands 61% of children with end-stage heart failure, supported on VAD, survive to heart transplantation or recovery. This modest survival rate most likely reflects a selection of a severely ill patient population. Although thrombo-embolic events on VAD lead to considerable mortality and morbidity, overall mortality in the children on the waiting list halved since introduction of VADs. This supports the growing evidence that VADs are an acceptable therapeutic tool to bridge children with end-stage heart failure to transplantation or recovery. Improvement of outcome in these children is to be sought in optimal timing of VAD implantation for every individual patient, and in improvement of anti-coagulatory strategies. We expect that in future years, an increasing number of children with end-stage heart failure will survive and will be possible candidates for heart transplantation, leaving the need for donor organs critically urgent.

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Favorable outcome after heart transplantation in children: 18 years' evaluation of the Dutch program

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ABSTRACT

BACKGROUND Previously, we reported a low heart transplantation (HTx) rate (3%) in children with dilated cardiomyopathy in the first year after diagnosis as compared to other registries (18%), without increased mortality. This low early transplantation rate might reflect our reluctance to list children early after diagnosis which might have led to a selection of children with an unfavorable risk profile. Therefore, we evaluated outcomes on the waiting list and after HTx.

METHODS A retrospective, descriptive study of all Dutch children listed for HTx between 1998 and 2017.

RESULTS 69 children were listed for HTx, at median age of 10.3 years [IQR 2.5 to 13.1]. Median time from diagnosis to HTx listing was 1.3 years [IQR 0.3 to 3.9]. The majority of children was diagnosed with DCM (n=51, 74%). Sixteen children (23%) died on the waiting list, 8 of whom on mechanical circulatory support.

Forty-seven (68%) children underwent successful transplantation at the age of 11.7 years, [IQR 6.3 to 13.9]. Five-years' survival after HTx was 95% (95% CI 88 to 100). Three patients died, 2 at adolescent age after severe rejections related to non-compliance. The functional outcome after HTx was favorable: the majority of patients were able to carry on normal activity. The most important transplantation-related complications were post-transplant lymphoproliferative disease (n=6, 13%) and cardiac allograft vasculopathy (n=3, 6%).

CONCLUSION Our low early transplantation rate does not seem to have a negative impact on waiting list and post HTx outcomes, which are favorable and comparable to published international experience.

INTRODUCTION

Heart transplantation (HTx) is a widely accepted treatment for both adults and children with end-stage heart failure without alternative therapeutic options (1, 2).

In the Netherlands, the paediatric heart failure- and heart transplantation program is centralized in one center and covers a population of 3.8 million children.

In our center, patient volume is small- to medium-sized and our patient population predominantly consists of children with dilated cardiomyopathy and only a small minority of children have a congenital heart disease (CHD). Our program can thus be characterized as a typical European HTx program based on the yearly ISHLT reports (2).

Previously, we reported a low transplantation rate in children with dilated cardiomyopathy in the first year after diagnosis (3%), as compared to the large PCMR registry which reported a 18% transplantation rate in the 1st year after diagnosis. Similar data were shown by the Australian NACCS (3,4). Also, median time to listing was considerably longer in our cohort, 18 months versus 1.4 months. We did not find differences in clinical characteristics of our cohort as compared to other (large) registries and importantly, we did not find an increased mortality (5). Our low early transplantation rate might reflect a policy that defers listing patients for HTx as long as possible, and that pursues stabilizing patients on oral heart failure therapy before listing. This listing strategy may, however, select children with more severe heart failure at listing, having an unfavorable risk profile that could affect outcome on the waiting list, and after HTx.

It is against this background of a small- to medium- sized program mainly consisting of children with DCM, and a low early transplantation rate that we evaluate the outcome of 18 years of paediatric HTx in the Netherlands. We report waiting list - and heart transplantation outcome, and compare our results to published international experience.

PATIENTS AND METHODS

Patients

We included all patients < 18 years old listed for HTx from 1998 to April 2017. Two independent researchers (MM, SR) reviewed the medical records. Patients were listed for HTx in accordance with the ISHLT guidelines (1). Use and duration of mechanical circulatory support (MCS) was recorded. We used either ventricular assist device (VAD, Berlin Heart EXCOR) or extra corporal membrane oxygenation (ECMO) which were available as of 2007 in our center. The primary endpoint of the study was waiting list outcome (HTx or death) and survival after HTx. Secondary endpoints were transplantation-related complications, categorized as (acute) rejection, cardiac allograft vasculopathy (CAV), post-transplant lymphoproliferative disorders (PTLD), renal failure (estimated glomerular filtration rate (eGFR), using the Schwartz formula (6), psychological and psychiatric disorders, and infections. PTLD was considered a continuum of: early lesions (i.e. infectious mononucleosis-like PTLD), polymorphic - and monomorphic PTLD(7). EBV infection

was defined as a viral load >1000 copies/ml with or without clinical symptoms, leading to an adjustment of medication. Functional assessment included 2D echocardiography (shortening fraction (SF)) and cardiopulmonary exercise testing (CPET) (8). Functional status of the patient was determined by the 100-point Karnofsky and Lansky scale (9). Growth data were collected at HTx, 1 year after HTx and at the last follow-up visit and expressed as SD scores using Growth Analyser©. The study was approved by the local medical ethical committee (MEC 2018-1348).

Donors
Donor characteristics were collected and included donor age, body weight, country of origin, blood type and total ischemia time.

HTx and post-HTx regimen

HTx procedure
All transplants were ABO- compatible. The standard surgical procedure was a biatrial anastomose, in 1 patient with congenital heart disease (CHD) a bicaval anastomosis technique was used.

Immunosuppression
The immunosuppression consisted of induction therapy with anti-thymocyte globulin, followed by triple therapy: before 2000 patients were treated with 1. steroids and successive tapering, 2. cyclosporin and 3. Azathioprine. After 2000, tacrolimus replaced cyclosporin and mycophenolate mofetil (MMF) replaced azathioprine. Rejection therapy consisted of pulse dose of methylprednisolone (10-15 mg/kg), incidentally followed by ATG.

Graft surveillance
Routine surveillance endomyocardial biopsies were taken at: week 1-2, 3-4, 6 and 12, month 4-5, 6, 9 and 12 and also when rejection was suspected on clinical grounds. Rejection was graded according to ISHLT classification, grade 2R and higher were recorded. Classifications before 2005 were revised according to ISHLT guidelines (10). CAV was graded according to the ISHLT guidelines and evaluated 1 and 2 years post-HTx with coronary angiography and subsequently every 2 years by CT angiography or by coronary angiography (11).

Statistical analysis
Categorical variables were reported as numbers and percentages. Continuous variables were reported as means with standard deviation (SD) when normally distributed, and medians with interquartile range (IQR) when distribution was not normal. Differences in characteristics between patient groups were compared by Student's T-tests (normal distribution) or Mann-Whitney U tests. Categorical analysis was conducted by $\chi 2$ and Fisher's exact test. Differences in length SD score at HTx and at the last follow-up after HTx were compared using Wilcoxon signed rank test. Survival after HTx was estimated using Kaplan-Meier curves and the log-rank test was used to compare survival curves.

All analyses were performed using IBM SPSS Statistics for Windows, version 24 (IMB Corp, Armonk, NY).

RESULTS

Patients

Sixty-nine children were listed for HTx at a median age of 10.3 years [IQR 2.5 to 13.1], 51 children (74%) were diagnosed with DCM. Median time from diagnosis to HTx listing was 1.3 years [IQR 0.3 to 3.9]. Median time on the waiting list was 53 days [IQR 21 to 188], Table 1.

Table 1. Characteristics of patients listed for HTx (n=69)

Diagnosis, n (%)	
Cardiomyopathy	
Dilated	51 (74)
Hypertrophic	3 (4)
Restrictive	8 (12)
Congenital heart disease	3 (4)
Other*	4 (6)
Female, n (%)	39 (56)
Time from diagnosis to HTx listing years, median [IQR]	1.3 [0.3 to 3.9]
Age at HTx listing, years, median [IQR]	10.3 [2.5 to 13.1]
Age at HTx, years, median [IQR]	10.4 [6.3 to 13.9]
< 1 year, n (%)	1 (1)
1-10 years, n (%)	17 (25)
11-18 years, n (%)	29 (42)
Length SDS at HTx, median [IQR]	-1.2 [-2.5 to 0.6]
ET classification at HTx listing, n (%)	
Hospitalized	52 (75)
At home	17 (25)
Days at WL of HTx patients, median [IQR]	53 [21 to 188]
Pre HTx MCS, n	22 (21 VAD, 1 ECMO)
Post HTx follow-up, months, median [IQR]	63 [35 to 106]

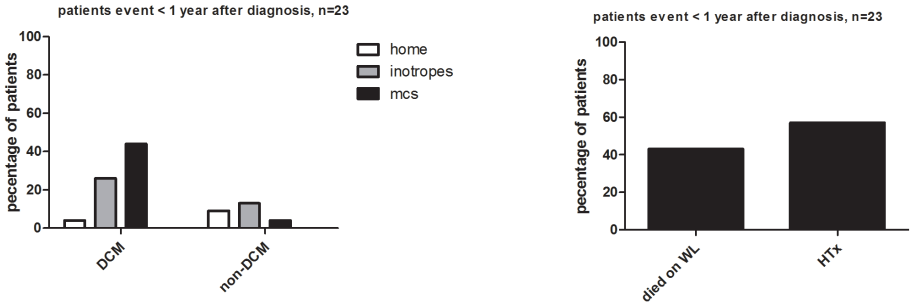
* Other consists of chemo induced cardiomyopathy (n=3) and low cardiac output syndrome after VSD surgery (n=1)

ECMO, Extra Corporal Membrane Oxygenation; HTx heart transplantation; IQR, Inter Quartile Range; MCS, Mechanical Support of the Circulation; SDS standard deviation score; VAD, Ventricular Assist Device

Waiting list outcome

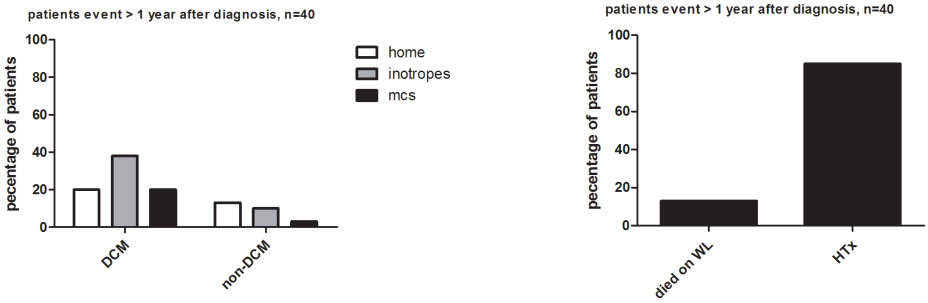
The level of support and mortality on the waiting list was considerably higher in children who died or underwent transplantation within 1 year of diagnosis as compared to those who died or underwent HTx more than 1 year after diagnosis. In children with DCM, 59% was on MCS versus 23% (p=0.02), and waiting list mortality was 47% versus 7% (p=0.002), Fig 1a and 1b.

Figure 1a. Patients with HTx or death within 1 year after diagnosis, percentage of n=23



DCM Dilated Cardiomyopathy, HTx heart transplantation, WL waiting list

Figure 1b. Patients with HTx or death more than 1 year after diagnosis, percentage of n=40

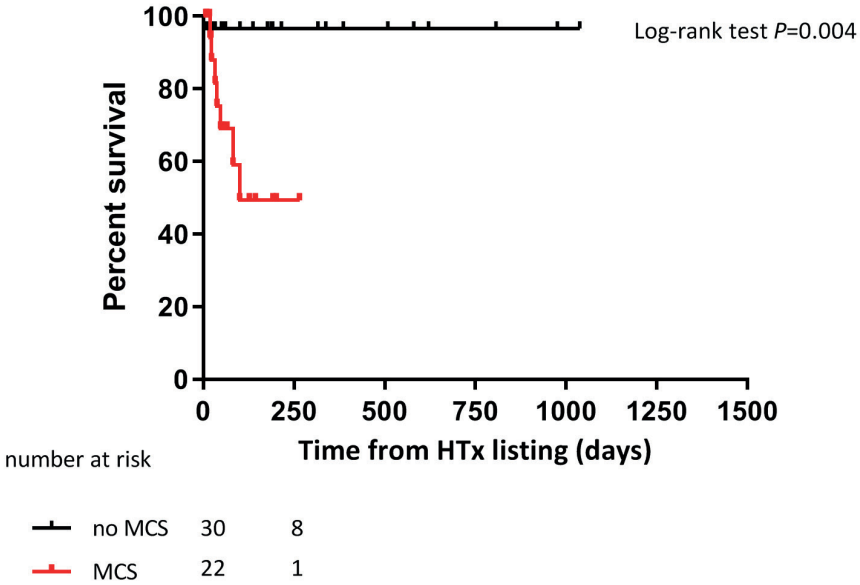


DCM Dilated Cardiomyopathy, HTx heart transplantation, WL waiting list

Forty-seven (68%) children were successfully transplanted at median age of 11.7 years, [IQR 6.3 to 13.9]. Median time from listing to Htx was 0.14 years [IQR 0.1 to 0.5] and 41 children (59%) underwent HTx within one year after listing.

Since the introduction of VADs in our program, 22 children (41%) were on MCS (21 patients on VAD, 1 patient on ECMO), and 12 of them (54%) underwent successful transplantation. Survival was significantly higher in patients without MCS. (Log rank test p=0.001, Figure 2).

Figure 2. Kaplan-Meier survival curve of children on the waiting list, children on MCS compared to children without MCS



MCS Mechanical support of the circulation

Twenty-two children (32%) on the waiting list did not undergo transplantation. Sixteen children (23%) died, median time on the waiting list until death was 65 days [IQR 21 to 161]. Six of 16 children died on inotropic support as a result of intractable heart failure in the era before VAD-support was available. One patient developed irreversible pulmonary hypertension and was delisted, and subsequently died. Seven patients died of VAD-related complications, and 2 died as parents withheld their consent for VAD placement. Of the remaining 6 children, 1 patient was delisted with improved cardiac function after 9 months on VAD, 1 patient was delisted on parents request and is still alive after 8 years, and 4 patients were still listed at the end of the study.

Survival after HTx

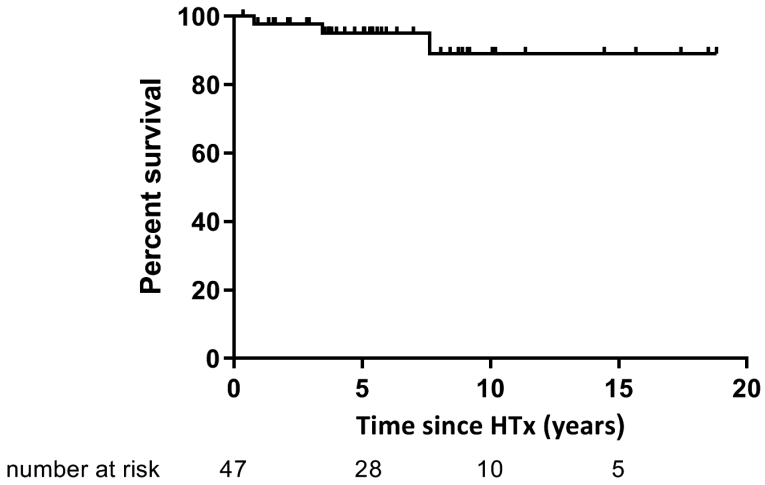
All transplantations, except one, were performed in children 1 to 18 years. Median follow-up was 5.3 years IQR [2.9 to 8.9], nine patients had a follow-up longer than 10 years. Five-year survival was 95% (95% CI 88 to 100) and 10 -year survival was 88% (95% CI 76 to 100), Figure 3. One patient required MCS directly after HTx due to primary graft failure, within 2 weeks cardiac function recovered completely. Three patients died at 0.8, 3.5 and 7.6 years after HTx. The first died of infectious complications after intensification of immunosuppression for uncontrollable cellular rejection. Two adolescents died after graft failure secondary to severe (grade 3R) rejection related to non-compliance.

Donor characteristics

Donors were allocated through Euro transplant. The majority (78%) of the donors originated from outside the Netherlands, the median age was 15 years [IQR 8 to 25]. Median donor/recipient weight ratio was 1.6 [IQR 1.4 to 1.8], ranging from 1.0 to 2.5. Mean

total ischemia time was 218 minutes (± 51). Fourteen grafts (30%) had a total ischemia time longer than 4 hours.

Figure 3. Kaplan-Meier survival curve of all HTx patients (n=47)



HTx, heart transplantation

Immunosuppressive regimens and graft rejection
Induction therapy with ATG and corticosteroids was started in all patients. One year after HTx, 49% of the patients were still treated with steroids. Maintenance therapy consisted of cyclosporin, after 2000 replaced by tacrolimus in all patients. At discharge, 94% of patients were treated with MMF. Everolimus was used in 3 patients (6%), and sirolimus in 2 patients (4%). Biopsy proven graft rejection was present in 21 patients (45%), in 20 of them rejection occurred in the first year after HTx. Median time from HTx to first rejection was 1.8 months [IQR 0.6 to 6.3]. The majority of patients with rejection encountered only 1 episode (62%) and in 88% of cases rejection was graded as 2R. Figure 4a and 4b.

Cardiac Allograft Vasculopathy
CAV was diagnosed in 3 patients (6%), all in patients > 18 years, grade 3 in two patients and grade 2 in one. Time from HTx to CAV was 3.4, 9.1 and 12.8 years.

Malignancy
Six (13%) patients were diagnosed with PTLD and one patient with a melanoma. The PTLD cases all occurred within 1 year after HTx, and all cases were EBV-related. Treatment consisted of Rituximab in 4 patients, lowering of immunosuppression in 1 and chemotherapy in 1 patient who was diagnosed with monomorphic PTLD and central nervous system involvement. None of the patients died as a result of malignancy.

Figure 4a. Frequency of rejection episodes per patient

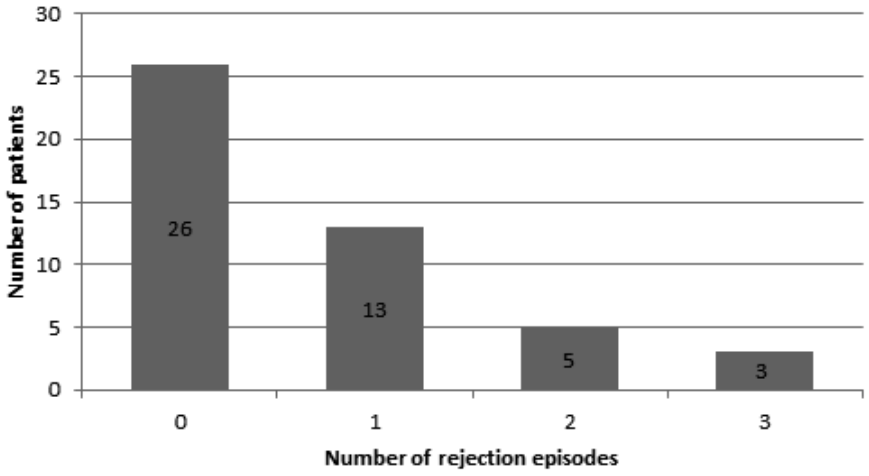
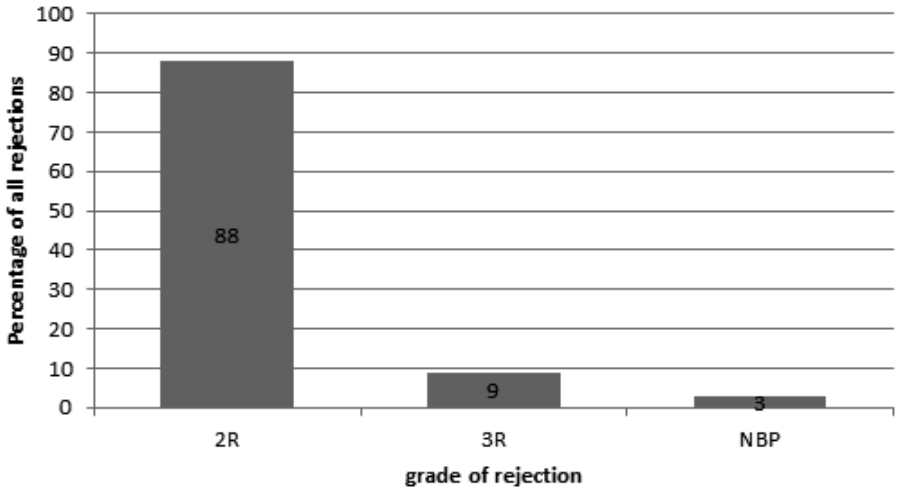


Figure 4b. Grades of rejection, as percentages of total amount of treated rejections



2R and 3R grading based on the ISHLT classification, NBP: non-biopsy proven graft rejection

Infectious complications

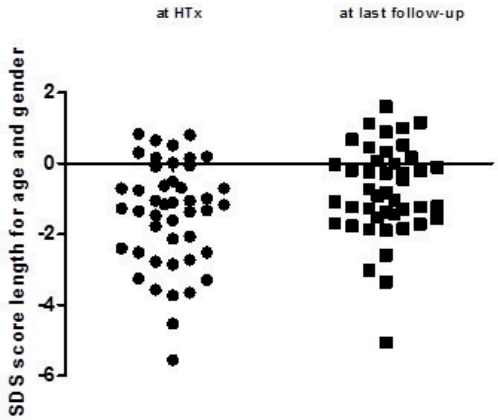
Fifteen (32%) patients had an EBV infection, 11 patients (23%) a CMV infection, and 7 patients (15%) a varicella zoster infection. Four bacterial infections were found. Other infections consisted of Candida Albicans infections (1 spondylodiscitis, 1 sepsis), 1 pulmonary Aspergillus infection, 1 Cryptosporidium diarrhea, 1 Pneumocystis Jerovici Pneumonia infection, 1 clostridium difficile colitis and 1 paronychium with lymphangitis.

Psychiatric disorders
Nineteen (40%) patients were newly diagnosed with some sort of psychiatric disorder. The most common disorders were an anxiety disorder (n=7, 14%), depression (n=4, 9%), sleeping disorder (n=2, 4%) and eating disorder (n=2, 4%).

Other complications
Neurological complications were found in 7 patients (15%) and consisted of Posterior Reversible Encephalopathy Syndrome (n=3), epileptic insult (n=2), peripheral neuropathy (n=1) and a transient ischemic attack (n=1), all without long-term sequelae. Temporary insulin dependent diabetes was diagnosed in 5 patients (10%). Kidney function was reduced in 21 patients (45%), GFR was 30-90 ml/min/1.73 m2, none required dialysis or kidney transplantation.

Long-term outcome
On the Karnovsky Lansky scale, at the last follow to up visit, patients scored median 100 points, [IQR 80 to 100], indicating that the majority of patients was able to carry on normal activity. Mean shortening fraction on echocardiography was 39% (SD: 6). Twenty patients performed a CPET on the last follow -up visit, median peak VO2 was 29 ml/kg/min [IQR 23 to 37], 78% [IQR 63 to 89] of predicted. At last follow -up visit median SDS length was higher than at HTx, -0.9 [IQR -1.6 to 0.9] versus -1.2 [IQR -2.5 to 0.6] at HTx, (p=0.007), indicating catch-up growth after HTx, Figure 5.

Figure 5. SDS score of length, at HTx and at last follow-up visit, (n=47, p=0.007)



HTx, heart transplantation

COMMENT

In this study we found that 68% of patients listed for HTx underwent transplantation, and mortality on the waiting list was 23%. Five- and 10-year survival was 95% and 88%. Causes of death after HTx were dominated by graft failure due to non-compliance. The most important transplantation related complications were PTLD and CAV.

Characteristics of the Dutch Htx program

Seventy-four percent of listed children were diagnosed with DCM, while only a few had congenital heart disease. The characteristics of our program are in line with ISHLT reports demonstrating important differences between the case mix and the number of transplantations between North American and European pediatric HTx centers. In North America 2/3 of the centers perform more than 10 transplantations per year and more than 40% of transplants are in (young) children with mostly failed palliation of univentricular hearts. In contrast, in Europe 60% of the centers perform less than 5 transplantations per year and congenital heart disease represents only 20% of the case mix (2,12). Recent reports indicate that in children with single ventricle physiology the transplantation rate is only around 1% and mainly confined to young children with (severe) systemic ventricular dysfunction (13). This is a group of children we have not typically evaluated for heart transplantation. In summary, ours would be a typical small- to medium sized European pediatric HTx program, the number of CHD transplants being at the low side of the spectrum.

Waiting list outcome

We review our results against a background of a low HTx rate within one year of diagnosis. Our previous report on a low early HTx was derived from a nation-wide retrospective cohort study covering all children with DCM that presented between 2005 and the end of 2010 (5). In a recent analysis of children with DCM presenting between 2010 and 2017 we found the same outcome (unpublished data), indicating that the low transplantation rate in the 1st year after diagnosis is consistent. The question is whether, compared to international literature, our low early HTx rate is associated with more severely ill patients on the waiting list, which could negatively affect waiting list outcome. The SCTR reports on a cohort of 3098 children and show that 56% of children with DCM are UNOS 1A at listing, indicating continuous mechanical ventilation and/or MCS. Overall 1-year waiting list mortality was 17% (14). The ISHLT database shows that 50% of children with DCM over 1 year of age, are bridged to HTx on a VAD. Pietra et al report a waiting list mortality of 11% in children with DCM specifically (15). And lastly, Eurotransplant reported an overall 1-year waiting list mortality between 13% and 30% (12).

In this study, 74% of children were diagnosed with dilated cardiomyopathy and 41% of children were on MCS on the waiting list. We tend to reserve listing early after diagnosis to patients who require MCS or who cannot be weaned of inotropes as shown in Figure 1a and b. The group of children who underwent transplantation or died while waiting within 1 year of diagnosis, was more often supported on MCS and waitlist mortality was higher as compared to the children who died or underwent HTx more than one year after diagnosis. However, overall percentage of MCS and waiting list mortality was within the

range of international literature. We therefore conclude that our listing strategy and a low early transplantation rate are not associated with an overall negative effect on waiting list outcome.

Survival after HTx
Given the low number of children with CHD in our cohort, the fairest approach would be to compare our results to conditional 1-year survival which leaves out early post-HTx mortality which is relatively high in CHD patients and infants (2). The ISHLT reports 5- and 10-year conditional survival of 86% and 74% respectively, and up to 90% and 78% in the most recent era 2004-2016. Survival in our cohort was 95% (95% CI 88 to 100) after 5 year and 88% (95% CI 76 to 100) after 10 years (2). This puts our results in a comparable range, although numbers are limited and median follow-up was only 5.3 years. Overall, we conclude that short- and intermediate term survival after HTx is favorable and comparable with international data.

Morbidity after HTx
We found rejection in 43% of patients in the first year after HTx which is in line with other single center studies who report rejection in a range of 34% to 76% (16,17). The ISHLT database reports treated rejection in only 15% of patients in the first year after discharge. However, the early in-hospital rejections are not taken into account, which may explain part of this difference (2).

PTLD is reported in approximately 10% of patients after 10 years (18,19). In our cohort, the incidence of PTLD was 13%, which is in the same range as the 4% - 12% reported in other single center studies (16,17,20-22).

CAV was present in 6% of our patients. The rate of CAV was low compared to international data, in which 33% of patients were affected 10 years after HTx (2). This is probably explained by our limited number of patients with follow-up > 10 years.

In our cohort, one patient died as a result of a pneumocystis jerovici pneumonia. No patients died of bacterial infections. One study reported bacterial infections in 164 HTx patients (60%) and mortality as a result of infection was 5% (23). Similar results were reported by the PHTS (24). Bacterial infection rate is low in our population, which may be related to the low number of children younger than 6 months who are predominantly affected by bacterial infections. Underreporting cannot be ruled out.

The proportion of patients who develop a psychiatric disorder is relatively high in our cohort. Literature on psychosocial outcome after HTx in children is scarce, but several studies indicate that this is an important issue. Whether psychiatric disorders predict post-transplant non-compliance is not clear (25).

Functional outcome

Functional outcome seems to be excellent, and in line with recent analysis of ISHLT data (9). Furthermore, length growth shows a significant trend to catch-up, which is an encouraging finding. Although only a subset of patients performed a CPET, median VO2 max was 78% of predicted VO2 max.

Limitations

A limitation of our study is the relatively small size of the cohort and the limited number of patients who have a post-transplant follow-up longer than 10 years which precludes all too strong conclusions. Also, this is a retrospective study with inherent limitations.

Conclusion

Despite a low early transplantation rate, mortality on the waiting list is in line with international literature, and outcome after HTx is favorable. Heart transplantation is a viable option for a selected group of children with end-stage heart failure without alternative treatment options. Currently, donor shortage remains the critical limitation for HTx.

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General Discussion

10

In this thesis we aimed to obtain better insight into the etiology of childhood DCM; second, we evaluated the contribution of temporal evolution of risk factors in predicting adverse outcome, and third, we evaluated treatment of children across the clinical spectrum of DCM. Here we start with commenting on the CARS cohort as the basis of our study. We will than focus on insights we gained in understanding disease etiology and in particular the role of genetics and cell biology. Furthermore we will discuss the development of feasible prediction models and leading points for improving treatment.

CARS

In the Cardiomyopathy Registry Study (CARS) we prospectively collected longitudinal data of children with DCM in different domains that reflect disease severity, and that are thought to be associated with outcome. During 7 years (2010-2017) we aimed to include all newly diagnosed children and prospectively gathered data of children who were already diagnosed before the start of the study. At the end of the study we had included 144 children, 103 children (72%) were newly diagnosed with DCM and 41 children (28%) were diagnosed before 2010 (the large majority diagnosed between 2005 and 2010). The inclusion rate of 93% was favorable, and loss to follow-up less than 5%. When we critically appraised the included patients, two possible issues arose that might have led to a selection of patients. First, we noticed that during the last years of CARS the yearly number of newly diagnosed children did not match the 20 we expected based on previous incidence rates. In seven years we expected to include 160 children in total, which are 16 more than we actually did include. This might have been caused by a failure to include patients that died within hours or days after presentation to the hospital in whom diagnosis may have been missed or not reported to the study team. We also may have missed patients who recovered quickly after initial presentation and have not been reported to the study team for that reason. Otherwise the incidence rate is truly lower than expected, for reasons not known to the study team. Secondly, the 41 children with a previous diagnosis are most certainly selected as they survived to study inclusion, thus leaving out the children who died early after presentation (approximately 20% in the first year after diagnosis). On the other hand, we cannot rule out the possibility that patients that already had (partly) recovered were not were not included in this group of children with a previous diagnosis as well. The net result of these opposing selection mechanisms is represented by the 41 children we included. At least we know that the survival rate and the HTx- free survival rate of the children that presented between 2005-2010 (which is cohort of all children with DCM we could retrospectively identify) is similar to the cohort of newly diagnosed children. In the 2005-2010 cohort, HTx-free survival was 82% 1 year after diagnosis and 72% 5 year after diagnosis, which is similar to 82% and 72% we found in the newly diagnosed children (1). Thus, the event rate in children with DCM seems consistent over the last 12 years and we concluded that the large majority of the 41 children with a prior diagnosis were obtained from a cohort with the same characteristics. Adding the children with a previous diagnosis provided a relative large number of children we were able to follow for many years (median follow-up was 3.8 years in the children who did not reach a study endpoint). In a rare pediatric disease as DCM, this approach is suboptimal but inevitable. We combined therefore the two cohorts in the analysis of association

between temporal evolution of risk factors and outcome, keeping in mind the limitations of this construction.

ETIOLOGY OF DCM

Why do we need to know the etiology of DCM in children? First of all, because we are asked by parents every time a child presents with DCM, what causes this disease. Also, doctors need to find a definite etiology as it is related to outcome and might have therapeutic consequences. For example DCM caused by myocarditis is known to have a more favorable course over time. When a genetic disease is found that renders the child prone to rhythm disturbances, an implantable cardioverter-defibrillator (ICD) should be considered. In this thesis we focused on 2 aspects of disease etiology that are closely related: genetic origin of DCM and cellular patho-mechanisms that underlie DCM.

Genetics of DCM

Starting point of the genetic study was to find out how many children with DCM underwent genetic testing, and if genetic etiology was related to outcome. First of all we found that only 74% of children were tested (**Chapter 2**). This is an outcome that we should not accept now that guidelines are available and genetic testing is easily accessible (2). Ways to improving the rate of genetic testing might be clear counseling of parents and explaining the pros and cons of genetic evaluation, supported by an interpreter when language barrier is an issue. Also, optimizing collaboration between pediatric cardiologists and clinical geneticists will most likely contribute to an increase in testing, but this will require continuous effort of both disciplines.

Secondly, the yield of testing was 36%, that is, in 39 of 107 children that were genetically evaluated we found a (likely) pathogenic variant. Although this is not a low yield compared to other pediatric DCM cohorts, it certainly leaves room for improvement (3-8). As described by Herkert et al, when copy number variant analysis and whole exome sequencing would be performed in *all* children with DCM, the yield would potentially increase up to 54% (5).

Interestingly, we also found a pathogenic variant (*LMNA*) in a child that was initially diagnosed as DCM related to myocarditis. This finding fits in the mounting evidence that previously silent genetic defects might predispose to DCM early in life when an environmental factor (such as myocarditis or chemotherapy) acts as a second hit (9-12). In the CARS study, 7 of 23 children diagnosed with myocarditis, and 2 of 8 children with anthracycline related DCM, were genetically tested. It would be sensible to start with a genetic evaluation of those 22 children.

Lastly, but perhaps most importantly, we demonstrated that children with DCM who carry a (likely) pathogenic variant were at a 2.8 times increased risk for death or HTx compared to children without a variant.

Up until now, the CARS cohort is the largest cohort of children with DCM that have been genetically tested and that showed this clear association. At this moment, the direct translation of a genetic variant into individual risk prediction is not possible as genetic DCM is a heterogeneous disease and penetrance is variable (3). However, the rationale for offering a genetic test to all children with DCM regardless of a presumed etiology is evident. When a (likely) pathogenic variant is found, children should be monitored with increased vigilance for disease progression, and devices to reduce the risk of sudden cardiac death should be considered when appropriate. Also, identification of at-risk relatives whether affected or clinically unaffected, may also have implications for genetic counseling and reproductive decision making. Most likely, large registries of children with DCM like the Pediatric Cardiomyopathy Registry (PCMR), will report on genetic testing and associations with outcome in the near future (13). Furthermore, improving the next generation sequencing techniques will broaden our knowledge on disease causing variants, but also on genetic modifiers that affect penetrance of DCM (14, 15). A step downstream in genetics, the field of gene expression and translational regulation, is increasingly recognized to play an important role in patho-mechanism of DCM (16). Interestingly, this might be uniquely regulated in children with DCM. Tatman et al recently showed a gene expression profile that affected cytokine signaling, signal transduction, and transcription that was not observed in adult DCM (17). This might be a leading point to develop targeted therapy in children with DCM and again underscores the importance of pediatric-focused investigations.

Hopefully, large cohorts of unselected children with DCM and novel genetic techniques as well as genetic knowledge will provide further insight into the relation between geno- and phenotype that will support personalized care.

Cellular patho-mechanisms of DCM in children

In this study we started with myocardial tissue of 11 hearts we obtained from children with DCM in end-stage heart failure at the time of heart transplantation. We found greatly reduced force generating capacity of single cardiomyocytes that was caused by a significant reduction in myofibril density (**Chapter 3**). Also, the ability to increase contractility upon stretch, which is the cellular basis of the Frank-Starling mechanism, was impaired. Furthermore, we did not find consistent upregulation of the compliant isoform of the giant elastic protein titin (N2BA). The compliance of titin, expressed as the ratio between N2BA/N2B, did not affect contractility. This implies that other factors underlie this impaired length-dependent contractility.

The question that drove this study was whether pediatric DCM differs from adult DCM, which might explain a more aggressive course of the disease in children and no clear improvement on standard heart failure pharmacotherapy. Also, proven differences could be leading points for further research and patho-mechanism guided therapy. We found that pediatric DCM differed from adult DCM in several aspects. First, the decrease in myofibril density seems to be specific for pediatric DCM, at least it has not been found in adult iDCM yet(18). Whether reduced myofibril density is due to an inability to increase myofibril synthesis or is caused by an increased myofibril degradation needs to be investigated first. Second, we found no consistent upregulation of compliant titin (N2BA) as opposed

to adult DCM (18, 19). In the early stages of the disease, increasing compliance might a way of the myocardium to function under overstretched conditions. The observation that children do not seem to upregulate compliant titin could be explained by limited abilities for cardiac remodeling in general, as shown by Patel et al (20). If children with DCM display limited cardiac remodeling and differ from adult DCM in reduced myofibril density, than this might explain that standard therapy that is focused on treating adverse cardiac remodeling is not effective in children with DCM (21, 22). Rather, it would be worthwhile to focus on regeneration of myofibrils instead. However, it should be noted that the tissue that we studied was derived from end-stage DCM. It might also be possible that such compensating mechanisms might (no longer) function at this phase of the disease.

Our study is a first step in elucidating cellular patho-mechanisms in children with DCM, but the road to patho-mechanism guided therapy is still long. It proofs to be complex to distinguish primary etiology-specific pathogenic effects like genetic mutations from secondary disease remodeling. However, in adult DCM, recent studies show that different causative genetic mutations lead to different cellular changes (18). If we can link specific genetic mutations to distinct patho-mechanisms than this would be a leading point for targeted therapy in genetic DCM patients. To study the pediatric counterpart is troublesome as the acquisition of cardiac tissue of children with DCM early in the disease is very difficult. The field of research in cardiomyocytes which are derived from patient fibroblasts can overcome the scarcity of pediatric DCM tissue. This principle has recently been demonstrated to work in ALPK3 -deficient cardiomyocytes generated from induced pluripotent stem cells obtained from patient skin tissue. Here abnormal calcium handling was shown (23). Studies like these are essential to understand patho-mechanisms related to genetic disease and to develop therapies that serve the pediatric DCM population.

HOW TO PREDICT OUTCOME – RISK FACTORS FOR ADVERSE OUTCOME

An important part of this thesis is the exploration of the use of serial measurements of known risk factors in the prediction of adverse outcome. In **Chapter 4** and **Chapter 5** the results of these efforts are shown.

First of all, we conclude that modeling the serial measurements of the separate risk factors provides a clear picture of the average evolution over time in children with and without the study endpoint (death or HTx). The majority of the risk factors we studied show a clear association of the evolution over time with the study endpoint. The profile of 4 risk factors in children who reached the study endpoint was clearly distinct from those who did not: no decrease in NT-proBNP or LVIDd, a severe decrease in length Z-score and an increase in NYU PHFI. Furthermore, the temporal evolution of these 4 risk factors also distinguished the children who reached the study endpoint from those with ongoing disease. Most registry based studies compare children with the study endpoint to those without. However, in clinical practice it might be more useful to have tools to discern 3 categories: children who reach the SE, those who remain ill but stable, and those who recover. Based on our own data and other registries we suspect that recovery in general takes place early, the majority within 1 year after diagnosis (1, 24). The clinical challenge

is to identify within the group of children who do not recover, who is at the highest risk for adverse outcome and should be considered for HTx. Ours is the first study to analyze this subgroup of children who remain ill but do not reach the SE. The finding that the abovementioned risk factors also discern these children from those who reached a SE supports outpatient management and the decision to suspend listing for HTx.

Within the field of pediatric DCM, it is unique that we were able to collect prospective data in a relative large cohort which we believe is an adequate representation of the whole spectrum of children with DCM. The data we collected cover multiple relevant domains that are related to disease severity and linked to outcome: exercise tests (6MWD), biomarkers (NT-proBNP), heart failure score (NYUPHFI) and echocardiographic parameters of left ventricular function (LVIDd and GLS). However, a few critical remarks on the CARS study should be made, as these also indicate the possibilities for improvement.

The CARS study cohort consists of children with a diagnosis before inclusion in this study (before 2010), as well as children who are included directly after diagnosis. This may have induced a selection bias as we do not know which children we missed in the group with a prior diagnosis: how many recovered and how many died before 2010. We argued that, by and large, we are informed about the group of children that stems from the time frame 2005 to 2010. This group was well described in a previous publication by den Boer et al (1). Although this covers the large majority of included patient we do not have the complete picture. A way to address this issue is to go back to the eight academic centers and retrospectively collect all DCM diagnoses in children as well as hard endpoints from the date of the diagnosis of the first patient we included. This would be a huge effort but most likely very rewarding.

Furthermore, in the multivariate analysis, NT-proBNP remained the only independent risk factor for adverse outcome. This might be due to an underpowering of the study. Den Boer et al previously calculated that, based on the Dutch population, we would need to collect data for 23 years to obtain enough data to include six risk factors in the analysis. This calculation was based on a 'rule of thumb' that at least 10 endpoints are needed to allow 1 risk factor in the multivariate model. Alternative approaches to calculate sample size for multivariate analyses are conceivable to (25). Either way, we need large data sets to gain sufficient power, and a multi-center study design seems the only sensible way forward. In this way we might create a heart failure score, based on multiple variables, similar to the adult counterpart the Heart Failure Severity Score (26). Levy et al elegantly pointed out that heart failure risk models would improve if: 1) they were properly validated; 2) incremental value of adding new variables to the model would be assessed and 3) online calculators were made to allow for easy provider use (27). A pediatric heart failure score thus composed might be helpful in risk prediction in the global pediatric DCM population, and support counseling of patients and families.

Also, we defined one hard study endpoint: death or HTx, but we also recorded the status of the surviving patients at the end of the study period: ongoing disease or recovered. In clinical practice it would be relevant if these three groups could be characterized and the temporal evolution of risk factors could be determined for all three groups. Because of

the limited number of endpoints we were not able to perform these analyses, but again a multicenter design would allow for such analyses. Than we could explore whether both children who reach an SE, children who have ongoing disease and children who recover display a distinctive profile over time.

For now, we should pursue to maintain this Dutch cohort in long-term follow- up and record data on endpoints of death, HTx and recovery, as well as the risk factors we identified to be most helpful in this thesis. We propose to obtain NT-proBNP, LVIDd, length Z-score and NYU PHFI two times per year in the first year after diagnosis and yearly thereafter. 6MWD% was strongly related to outcome but stable over time, so 2 tests in the first year after diagnosis seem needed to reliably estimate 6MWD%. Thereafter repeating 6MWT seems of no added value. Questions that are to be answered are whether NT-proBNP is also a strong predictor in long term outcome. Long term follow-up is also needed to discern recovery from remission. Everitt et al demonstrated recurrence of heart failure in children with DCM who were recovered (28). In a recent study by Maher et al on children recovered from DCM, subtle cardiac dysfunction was found (measured by left ventricular layer-specific myocardial strain) in patients with recovered DCM. Long-term follow-up is therefore recommended in these patients (29). Also, recovery might take place later on in the disease as well, as shown by Fenton et al so which also argues in favor of long- term follow-up (30).

OPTIMIZING MEDICAL TREATMENT OF CHILDREN WITH DCM

Pharmacotherapy

In daily practice we are confronted with children with DCM who need medical treatment, a large part without a set etiology but all sharing a phenotype of systolic dysfunction and cardiac dilation. In optimizing pharmacotherapy three important issues arise. First, can we demonstrate effectiveness of standard therapy (ACEi and B-blockers). Second, how to optimize the treatment that is already available and third what possible new developments are in medical therapy, taken into consideration the characteristic patho-mechanisms in pediatric DCM.

The first question is notoriously hard to answer. In this era where ACEi en B-blockers are already accepted as standard therapy, randomized placebo controlled trials are neither achievable nor ethical. This leaves the pediatric population with a treatment based on paradigms derived from adult heart failure and the question is whether this is good enough. Recently however, in the field of adult heart failure, a double blind trial compared standard therapy (enalapril) to the new heart failure drug LCZ696 (angiotensin receptor–neprilysin inhibitor). The investigators convincingly showed that LCZ696 was superior to enalapril in reducing the risks of death and of hospitalization for adults with heart failure (31). Such a study design would also be suitable for the pediatric DCM population and currently a study on LCZ696 versus enalapril in children with systemic left ventricular systolic dysfunction is undertaken (32). This PANORAMA-HF study will determine if LCZ696 improves clinical outcome and symptoms as compared to enalapril and will not focus on hard endpoint as death or HTx alone. Choosing such a study end-point allows meaningful

analyses with a substantial lower number of patients and is an important step in drug research in children with a relative rare disease as DCM.

The second question on optimizing current treatment will focus on the ACEi enalapril, but the concept applies to other ACEi's and B-blockers too. A way forward is linking adult data on pharmacokinetics of ACEi to the pediatric population. This approach can be motivated by assuming that, regardless what causes DCM, both adults and children share the same pathophysiology of heart failure. The decrease of systemic blood flow evokes a number of compensatory mechanisms through activation of the sympathetic nervous system and renin-angiotensin system (33). However, this activation is also maladaptive and contributes to myocardial damage, water and salt retention as well as worsening of heart failure. Medical treatment by means of ACEi is primarily based on blocking the effects of the renin-angiotensin system activation. Because efficacy has been demonstrated in adult with heart failure, pharmacokinetic bridging will be sufficient for extrapolation of efficacy of enalapril from the adult population to this pediatric population (34). In this thesis we reviewed available literature on the safety of ACEi in children with heart failure and concluded that ACEi are relatively safe, though low weight, young age and being prone to dehydration, were factors carrying an increased risk for adverse reactions (**Chapter 6**). This was the starting point of a larger research project to develop an age-appropriate pediatric enalapril formulation (EU FP7 LENA project). This study also obtained pediatric pharmacokinetic data to demonstrate the dose-exposure relationship in children with DCM in order to identify the doses that lead to an exposure equivalent to that achieved during the treatment of adults. We participated in this study and included 15 children with DCM. As we are (expectantly) awaiting the analyses at this moment, the results could not be described in this thesis. In the CARS cohort we also obtained pharmacokinetic data of both carvedilol and enalapril. These results too are expected in the near future and will provide further data to bridge the gap between adult and pediatric pharmacokinetic data.

The third issue on new mechanistic leading points for novel pharmacotherapy might be applicable in cases where DCM is diagnosed in a relatively early stage, for instance after screening of siblings of a child with the disease. Here, DCM has not progressed to an end-stage and downstream phase that is no longer amendable to a mechanistic approach (35). An example of this strategy is to target the molecular consequences of a *LMNA* mutation. This has at first been studied in mice where in *LMNA*-mutated mice an increased cardiac activity of p38 MAP kinase was found (36). When the mice were treated with an inhibitor of p38 (ARRY-371797), LV dilation and deterioration of LV function were no longer found. Currently, a double-blind randomized placebo-controlled phase 3 trial is running which studies the benefit of ARRY-371797 on change in 6-minute-walking distance in symptomatic DCM patients caused by *LMNA* mutation (NCT03439514). Outcome of this study will stimulate further studies into the use of targeted therapy, hopefully also in the pediatric population.

Mechanical support of the circulation

After a careful evaluation of our mechanical support of the circulation (MCS) program we concluded that ventricular assist devices (VAD) are an acceptable therapeutic tool to bridge children with end-stage heart failure to transplantation or recovery (**Chapter 8**).

In our hands, 61% of children with end-stage heart failure, supported on VAD, survived to heart transplantation or recovery, and mortality on the waiting list decreased from 44 to 21%. However, improvement of outcome in these children is needed as still a significant number of children die on a VAD, or have lasting neurological disability due to VAD related complications. Here, a critical issue might be optimal timing of VAD implantation in children with acute decompensated heart failure (ADHF). Too early leads to an increase in VAD implantations and increases the risk of serious VAD related complications; too late on the other hand, results in low cardiac output and end-organ failure which is associated with increased mortality on the waiting list and after HTx (37-41). In adults, low systolic blood pressure, high urea and creatinin as well as elevated arterial lactate and hypothermia are strongly related to in-hospital mortality (42-44). Presence of these factors support clinicians in timing of VAD placement, but timing for the individual patient remains a weighed decision. The body of literature on risk factors for imminent death in children with ADHF is limited. Wong et al demonstrated that serial NTpro-BNP levels increased in 24 pediatric ADHF patients who went on to require mechanical support of the circulation (45). Medar et al described similar findings in 16 pediatric ADHF patients (46). Notably, the last two authors report that the trend, rather than the absolute value of NT-pro-BNP should be taken into account in predicting outcome in these patients, which again underscores the importance of repeating measurements. Maybe clinically most applicable is the finding of Singh et al that the hemodynamic profiles cold-dry and cold-wet were associated with mortality in children listed for HTx (40). In this study hemodynamic profile was assessed invasively by pulmonary capillary wedge pressure and cardiac index. In adults with ischemic cardiomyopathy it has been shown that hemodynamic profiles are concordant with clinical evaluation of cardiac output and congestion in 70% of cases (47). Whether clinical evaluation correlates with intracardiac hemodynamics in children with DCM and advanced heart failure, and whether hemodynamic congestion and hypoperfusion can be diagnosed clinically could be subject of future research. Until then, this approach hands on, during the rounds on the PICU, might be equally good. In addition to increase in NT-proBNP, the children who are cold (and not the ones who are most congested (wet)) might be at the highest risk of dying and should be actively considered for VAD implantation. Furthermore, anti-coagulatory strategies sometimes appear more art than a science, and further refining evidence based-strategies is needed to prevent thrombo-embolic events.

Heart transplantation

We aimed to evaluate our outcome after HTx against a background of a transplantation rate that is consistently low (3 to 6%) in the first year after DCM diagnosis as compared to other registries, with data now stemming from 2005 to 2017 (24, 48). Whether in our program we truly are more restrictive in listing children for HTx than other programs is hard to proof. We would need data of *all* children that were evaluated for HTx, including the ones that were not listed. In our study we only had access to data of the listed children which hampered elucidating this issue. However, we did show that the listed children were severely ill (the large majority of children with DCM were supported on a VAD or on inotropes) and otherwise most likely would have died, thus indirectly arguing a restrictive policy (**Chapter 9**). Future research may look into the data of all children that were evaluated for HTx and might find convincing evidence that we are truly restrictive in listing for HTx. The results of our HTx program are favorable. Mortality on the waiting list

and survival after HTx were well within international standards and complications after HTx were relatively low too. Of note, numbers were limited and median follow-up was only 5.3 years.

There are several ways to further improve management of HTx patients and long-term outcome. In this respect, non-compliance is a significant issue, as it accounted for 2 of the 3 deaths in our patient cohort. Non-compliance has been reported in 18% of adolescents and more than 2 episodes resulted in a mortality rate of 56% in the next 2 years(49). Similarly, smaller studies reported non-compliance in 43% up to 65% in teenage transplant recipients (50). Means to improve compliance in adolescents would be peer support groups, smartphone apps, group visits and improved transition to self-management and adult care (51). Whether such an approach would result in better compliance and reduction of post-transplant deaths could be subject of future research.

Also, donor shortage is a critical factor in waiting list mortality. Expanding the donor pool with ABO incompatible (ABOi) donors has been shown a sensible and safe strategy (52-54). Eurotransplant reported that 17% of donor hearts <2 years were not used and that by expanding the ABOi program, a decrease in waiting list mortality can be achieved (55). Another option would be to include donation after circulatory death (DCD) transplants. Several studies showed the potential gain, including a four-fold increase of donor organs (56-59). Recent studies from Australia and the UK report outcomes in adults after DCD HTx that are comparable to that of recipients of donation after brain death (DBD) donors(60). The ISHLT reported DCD transplants in 21 pediatric patients (0.5%) only. DCD 1-year survival was significantly lower than DBD, but probably related to unfavorable acceptor characteristics (61). Potentially, the use of ex-vivo preservation to optimize graft function would enable DCD transplants (62). Recipient characteristics, as well as short- and long-term patient outcome, would have to be monitored with utmost acuity.

Lastly, we should critically evaluate psychological problems and quality of life (QoL) in children after Htx. In a pilot study we performed in 11 children after HTx, we found that QoL only modestly improved after HTx and that parents seemed more content than their children. Also, we found a substantial number of patients who developed a psychiatric disorder (**Chapter 9**). Literature on psychosocial outcome after HTx in children is scarce. Multiple studies however indicate that psychological problems are more prevalent than in healthy children (63-65). Either way, psychosocial care should be standard and liberally available for children after HTx.

FUTURE PERSPECTIVES

The insights we gained in our study call for further research. Future studies in children with DCM should first aim to further elucidate disease etiology and understand pathophysiology. The fruitful collaboration with clinical geneticists and physiologists during this study would certainly allow for new studies. A study proposal is being prepared by a pediatric cardiologist (Dr. M. Dalinghaus, EMC Rotterdam) and a physiologist (Prof J .van der Velden, VUMC, Amsterdam). In this study, translational aspects of DCM will be

addressed by studying protein profiles in blood samples of children with DCM. This will provide further insight into translational regulation which is increasingly recognized as a critical patho-physiological process (16). Furthermore, the future of genetic research might be in large cohorts of children with DCM that are well phenotyped in combination with extensive genotyping to determine geno-phenotype relations. Also, the path of research in cardiomyocytes which are derived from patient fibroblasts is promising. This might be the basis from which we can link specific genetic mutations to distinct patho-mechanisms and this would be a leading point for targeted therapy. With regard to the development of risk prediction tools: multicenter studies are the only rational way forward. As we have seen in a multi-center multi-national drug study (LENA –results not in this thesis) it is possible to collaborate and merge databases of children with DCM. Similar to the PCMR, a registry of all European children with DCM could generate data that would allow for the development of more robust risk stratification than a single center study like ours. Partnerships that already exist (such as LENA) might be used for that end. And lastly, important steps need to be made to bring pharmacotherapy for children with DCM to a next level. A starting point might be to find dose-response relations of standard heart failure drugs for the different age categories. The CARS contributed in this respect by collecting samples of enalapril and carvedilol, the results are expected in the near future. Here also large cohorts of children with DCM in all age ranges are needed to allow for robust pharmacokinetic modeling.

What I would like to accentuate here at the end of the discussion is the importance of close co-operation. We have to pool existing knowledge and skills if we want to make progress in the treatment of children with DCM. This co-operation applies to the laboratory and clinic, centers sharing data, different academic disciplines, experienced senior clinicians and research nurses as well as young investigators. This will speed up progress, and, not unimportant, it makes life more fun.

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Summary and samenvatting

11

SUMMARY

Dilated cardiomyopathy (DCM) is defined as depressed function of the heart muscle and dilation of the left ventricle. Clinical symptoms of the disease vary widely; some children do well with no impairment in daily life, while others develop end-stage heart failure and die or need heart transplantation (HTx) as last resort therapy. Starting point of this thesis is the concept that if we want to improve outcome of children with DCM, we need to know which children are at the highest risk for adverse outcome. Essential in this process is that we need to understand the disease better and gain insight in what marks disease progression. Many questions remain to be answered, for instance, what is the role of genetics in DCM and how is a genetic cause related to outcome? What are the processes by which end-stage heart failure develop, and in what ways is DCM in children different from adult DCM? How can we recognize the children at the highest risk for adverse outcome? And how could this knowledge affect the way children with DCM are treated, either in terms of medical therapy, the institution of mechanical circulatory support or timely offering HTx? Here we provide a summary of the 8 studies included in this thesis, which are preceded by a general introduction and followed by a general discussion.

Chapter 1 provides a general introduction into dilated cardiomyopathy in children. The global contours of current knowledge and leading points for new research that led to this thesis are described.

The etiology of DCM in children is a strong predictor of long-term outcome. For instance, it is known that children with DCM related to myocarditis have a more favorable prognosis than those with idiopathic DCM. It is assumed that in many cases of idiopathic disease yet unrecognized genetic disease may underlie the cardiomyopathy. However, not much is known about the utility of genetic testing for risk stratification in children with DCM. Studies in adult DCM patients have reported a more severe phenotype and earlier onset in those with a pathogenic genetic variant than in variant-negative patients. **Chapter 2** describes the current practice and results of genetic evaluation in our national cohort of children with DCM, and reports the relation between the presence of (likely) pathogenic variants and clinical outcome. We found that 75% of children were tested and 36% of those had a genetic cause of DCM. We also found that the children with a genetic cause had a serious increased risk for death or HTx compared to children without. These findings highlight the importance of early genetic testing in *all* children with DCM. Determination of a genetic etiology can provide a valuable contribution to predict clinical outcome in children with DCM.

In **Chapter 3** we address the similarities and differences between pediatric DCM and adult DCM at a cellular level. Here, we describe how found differences might be a step towards understanding the more aggressive course of the disease in children, and maybe also a clue why no clear improvement on standard heart failure pharmacotherapy is observed. In this study we started with myocardial tissue of 11 hearts we obtained from children with DCM in end-stage heart failure at the time of heart transplantation. We found greatly reduced force generating capacity of single cardiomyocytes that was caused by a significant reduction in myofibril density. Combined with the observation that many

pediatric patients did not show an upregulation of compliant titin, this could imply that the cardiomyocytes do not undergo extensive remodeling and therefore cannot cope with the cardiac stress. The limited adverse remodeling in pediatric CM might have contributed to their early and progressive disease. In future research it might be worthwhile to explore therapies directed at regenerative capacity and myofibrillogenesis of cardiac muscle cells rather than reversal of cellular remodeling.

In **Chapter 4** we evaluate the added value of repeated 6MWT in addition to a single 6MWT in predicting outcome in children with DCM. The 6-minute walk test (6MWT) is a safe, simple and accepted prognostic tool in adults with heart failure. In children, the 6MWT is also feasible and has been shown to be reproducible. The distance walked in a 6MWT can be expressed as a percentage of predicted, taken into account height, gender and age (6MWD%). We have shown that 6MWT is a useful tool to identify children with DCM at a high risk of death or heart transplantation. In children who are able to perform a 6MWT, 6MWD% remains constant over time, early after diagnosis and in the years thereafter. In those reaching an endpoint, 6MWD% is significantly lower throughout time, than in those not reaching an endpoint. We propose that initially at least 2 6MWT are needed to reliably estimate 6MWD%, thereafter repeating 6 MWT has little added value.

In **Chapter 5** we present our study on prospectively and repeatedly collected clinical and echocardiographic risk factors for death and transplantation in a national cohort of children with DCM. We explored the temporal evolution of these factors, as these potentially hold relevant information for clinical decision making. We found that the temporal evolution of 4 risk factors distinguished the children who died or underwent transplantation from those who have ongoing disease or recover: no decrease in NT-proBNP or left ventricular diastolic dimension, a severe decrease in length Z-score, and an increase a pediatric heart failure score. Similarly, when comparing children who reached an endpoint with children with ongoing disease solely, excluding those who recovered, we found comparable differences in the evolution of risk factors, although the effect size was smaller. In the multivariate analysis, NT-proBNP remained the only independent risk factor for adverse outcome. We conclude that in children with DCM, change over time in known risk factors for death or heart transplantation (HTx) is predictive for outcome. In children who do not recover, persistent high levels of NT-proBNP, no decrease in left ventricular diastolic dimension, severe stunting of growth and an increase in the heart failure score identifies those at the highest risk for death or HTx. In this study, with limited study endpoints, we identified NT-proBNP as the strongest independent predictor for adverse outcome.

Chapter 6 reviews the safety of ACE-inhibitors for the treatment of heart failure in children based on an extensive search of all available literature. In adults, the most prevalent adverse events (AEs) related to the use of ACER-i are renal failure, hypotension, hyperkalemia, cough and angioedema. It is assumed that the safety profile of ACE-i differs from that for adults, but also for children of different ages, as growth and development contribute to variation in the disposition and effect of most drugs administered to children. In this study we concluded that first, ACE-i are considered relatively safe for children with heart failure, but renal impairment, hypotension and hyperkalemia are common adverse reactions. Second, low weight, young age, and being prone to dehydration, (e.g. an account of

gastroenteritis or high doses of diuretics) are factors carrying an increased risk for adverse reactions related to ACE-1 consumption.

Considering the symptoms and prognosis of DCM, substantial effects on psychosocial wellbeing can be expected. The predictive value of depressive and anxiety symptoms in children with DCM has not been previously studied. Information on the predictive value of depressive and anxiety symptoms may be valuable for clinical management strategies. Depressive and anxiety problems may lead to poorer self-care and, in turn, to disease progression. In **Chapter 7** we evaluate the level of emotional and behavioral problems and whether depressive and anxiety problems are associated with outcome. We found that, compared with normative data of same-aged peers, larger percentages of older children (6–18 years old) with DCM showed overall internalizing problems, anxiety problems, and depressive problems. These results are in line with meta-analyses in adult heart failure populations, which demonstrate an increased risk of anxiety and depression. We found that anxiety and depressive problems in children with DCM did not predict the risk of death and cardiac transplantation whilst controlling for heart failure severity. This is possibly explained by the fact that in children parents may compensate for children's poorer self-care behaviors. Our findings however do demonstrate the importance of including routine screening for internalizing problems to the clinical management of children with DCM, and of providing psychosocial support attuned to the needs of these children.

Ventricular assist devices (VADs) are nowadays a well-accepted, long-term therapeutic option in adult end-stage heart failure. Implementation of VADs in the pediatric population has been troublesome due to technical problems and difficulties in medical management. However, in recent years, several centers have presented their experience with Berlin Heart EXCOR VAD as long-term support. They have shown that Berlin Heart EXCOR VAD is a reliable and relatively safe device in bridging children to heart transplantation or recovery. Since 2006, the Berlin Heart EXCOR Pediatric VAD has become available in our hospital. **Chapter 8** describes the outcome of children supported with a VAD and the effect of the introduction of VAD on waiting list mortality is evaluated. We found that, in our hands, 61% of children with end-stage heart failure, supported on VAD, survive to heart transplantation or recovery. This modest survival rate most likely reflects a selection of a severely ill patient population. Although thrombo-embolic events on VAD lead to considerable mortality and morbidity, overall mortality in the children on the waiting list halved since the introduction of VADs. This supports the growing evidence that VADs are an acceptable therapeutic tool to bridge children with end-stage heart failure to transplantation or recovery.

Previously, we reported a low heart transplantation rate (3%) in children with dilated cardiomyopathy in the first year after diagnosis as compared to other registries (18%), without an increase in mortality. Our low early transplantation rate might reflect a policy that defers listing patients for HTx as long as possible, and that pursues stabilizing patients on oral heart failure therapy before listing. This listing strategy may, however, select children with more severe heart failure at listing, having an unfavorable risk profile that could affect outcome on the waiting list, and after HTx. In **Chapter 9** we evaluate the outcome of 18

years of pediatric heart transplantation in the Netherlands against this background of a small- to medium- sized program mainly consisting of children with DCM, and a low early transplantation rate. In this study we found that 68% of patients listed for HTx underwent transplantation, and mortality on the waiting list was 23%. Five- and 10-year survival after transplantation was 95% and 88%, which is favorable compared to international data. Graft failure due to non-compliance was an important cause of death. The most important transplantation related complications were post-transplant lymphoproliferative disorders and cardiac allograft vasculopathy. We concluded that our listing strategy did not result in a selection of more severely ill patients and increased overall waiting list mortality as compared to large pediatric patient cohorts. Heart transplantation is a viable option for a selected group of children with end-stage heart failure without alternative treatment options.

In **Chapter 10** the main findings of the studies in this thesis are discussed in detail and placed in a broader perspective. Furthermore, we made suggestions for future research.

SAMENVATTING

Gedilateerde cardiomyopathie (DCM) is een ziekte van de hartspier die wordt gekenmerkt door een verminderde knijpkracht en een opvallende verwijding van het hart. De verschijnselen van de ziekte lopen sterk uiteen; sommige kinderen ervaren weinig beperkingen in het dagelijks leven, terwijl anderen eindstadium hartfalen ontwikkelen en overlijden of als laatste redmiddel een harttransplantatie nodig hebben.

Dit proefschrift gaat over de vraag, welke kinderen de grootste kans hebben op een slechte afloop van de ziekte. Met een slechte afloop, of uitkomst, bedoelen we dan overlijden of een harttransplantatie. Het is hierbij belangrijk dat we te weten komen wat de kenmerken zijn van een verergering van de ziekte. Er zijn veel vragen die nog moeten worden beantwoord, bijvoorbeeld:

- Wat is de rol van erfelijkheidsonderzoek in DCM en hoe is een erfelijke oorzaak gerelateerd aan een slechte uitkomst?

- Wat zijn de processen waardoor eindstadium hartfalen zich ontwikkelt, en op welke manieren verschilt DCM bij kinderen van DCM bij volwassenen?

- Hoe kunnen we tijdig de kinderen herkennen die het grootste risico lopen op een slechte uitkomst?

- En hoe kan deze kennis van invloed zijn op de manier waarop kinderen met DCM worden behandeld, hetzij door middel van medicijnen, mechanische ondersteuning van de bloedsomloop (steunhart) of het tijdig aanbieden van een harttransplantatie?

In dit hoofdstuk geven we een samenvatting van de 8 studies die in dit proefschrift staan. Het proefschrift begint met een algemene inleiding en wordt afgesloten met een algemene discussie.

In **Hoofdstuk 1** geven we een algemene inleiding op DCM bij kinderen. We schetsen een globaal overzicht van de huidige kennis van dit ziektebeeld. Daarnaast beschrijven we aanknopingspunten in verschillende onderzoeksgebieden van DCM die tot de studies in dit proefschrift hebben geleid.

DCM is eigenlijk een verzamelnaam voor een zieke en verwijde hartspier, maar er zijn vele oorzaken die kunnen leiden tot DCM. Je kunt hierbij denken aan virusinfecties (myocarditis), hoge doses chemotherapie, spierziektes (bv de ziekte van Duchenne) of een erfelijke vorm van DCM. Er is ook een groep kinderen met DCM waar we de oorzaak niet van weten (idiopathische DCM). Uit eerder onderzoek blijkt dat de oorzaak van DCM bij kinderen sterk samenhangt met de uitkomst van de ziekte. We weten bijvoorbeeld dat kinderen met DCM die wordt veroorzaakt door ontsteking van het hart (myocarditis), een veel betere uitkomst hebben dan kinderen met idiopathische DCM. Het is dus heel belangrijk om de oorzaak te weten, omdat dat iets zegt over het verwachte beloop van de ziekte. We weten nog niet zo goed hoe het hebben van een erfelijke/genetische oorzaak

van DCM, van invloed is op het beloop van de ziekte bij kinderen. Uit studies bij volwassen patiënten met DCM hebben we geleerd dat het hebben van een erfelijke oorzaak van DCM leidt tot een ernstiger beloop van de ziekte, en ook dat de ziekte op een jongere leeftijd begint. **Hoofdstuk 2** beschrijft hoe we in Nederland op dit moment omgaan met genetische testen bij kinderen met DCM en wat de resultaten van deze genetische testen zijn. We beschrijven de relatie tussen de aanwezigheid van (waarschijnlijke) erfelijke ziekte en overlijden of harttransplantatie. We vonden dat 75% van de kinderen met DCM genetisch is getest, en dat 36% daarvan een genetische oorzaak van DCM had. We hebben ook vastgesteld dat de kinderen met een genetische oorzaak een sterk verhoogd risico op overlijden of harttransplantatie hadden, in vergelijking met kinderen zonder erfelijke ziekte. Deze bevindingen onderstrepen het belang van vroege erfelijkheidstesten bij alle kinderen met DCM. Het vaststellen van een erfelijke oorzaak van DCM kan een waardevolle bijdrage leveren om de uitkomst bij kinderen met DCM te voorspellen.

In **Hoofdstuk 3** behandelen we de overeenkomsten en verschillen tussen de vorm en functie van hartspiercellen bij kinderen en volwassenen met DCM. In deze studie zijn we begonnen met hartspierweefsel van 11 harten die we hebben verkregen van kinderen met DCM in het eindstadium van de ziekte, op het moment van harttransplantatie. Dit weefsel hebben we onder de microscoop onderzocht en ook hebben we gemeten hoe goed de spiercellen in staat zijn om samen te trekken. We vergeleken het hartspierweefsel van de kinderen met DCM, met hartspierweefsel van gezonde volwassenen. We vonden dat in de hartspiercellen er veel minder samentrekkende onderdelen aanwezig waren. Ook was het hartspierweefsel van de kinderen veel minder goed in staat om krachtig samen te trekken. Bij volwassenen met DCM kunnen de hartspiercellen zich aanpassen aan de verwijding en de stress van het zieke hart. Volwassen hartspiercellen kunnen wat meer mee rekken wat in het begin een gunstige aanpassing is. Later in het ziekte beloop slaat de balans door en leidt deze aanpassing alleen maar tot toename van de verwijding en afname van de pompkracht van het hart. Wij vonden dat kinderen veel minder goed in staat lijken te zijn om hun hartspiercellen op deze manier aan te passen. Dat zou een verklaring kunnen zijn hun snel voortschrijdende ziekte. Ook zou het kunnen verklaren waarom de medicijnen die bij volwassen werkzaam zijn niet zo goed lijken te werken bij kinderen. In toekomstig onderzoek lijkt het nuttig om ons te richten op behandelingen die het hart helpen om nieuwe hartspiercellen aan te maken.

De 6-minuten looptest (6MWT) is een veilig en eenvoudig middel om bij volwassenen met hartziekten de uitkomst van ziekte te voorspellen. Ook bij kinderen is de 6MWT goed uitvoerbaar. Bij andere ziektes, zoals kinderen met de ziekte van Duchenne, wordt de 6MWT al gebruikt om het effect van medicatie te meten. De test meet de afstand die door het kind in 6 minuten gelopen wordt. Deze afstand wordt dan vergeleken dan met de afstand die een gezond kind van dezelfde leeftijd, geslacht en lengte zou lopen. De afstand die gelopen is wordt dan uitgedrukt als een percentage van voorspeld (6MWD%), en waarbij we dus rekening houden met lengte, geslacht en leeftijd. In eerder onderzoek in ons ziekenhuis hadden we al aangetoond, dat 1 enkele 6MWT een goed hulpmiddel is om bij kinderen met DCM diegenen op te sporen met een hoog risico op een slechte uitkomst. Het zou zo kunnen zijn dat niet alleen de uitslag van 1 test maar juist ook het verloop over de tijd van de test extra informatie geeft. Bijvoorbeeld, als er over het verloop

van de tijd een afname is van de gelopen afstand zou dat ook informatie kunnen opleveren. In **Hoofdstuk 4** onderzoeken we of het herhalen van de 6MWT helpt bij het voorspellen van de uitkomst bij kinderen met DCM. We vonden dat bij de kinderen die een 6MWT kunnen uitvoeren, de 6MWD% constant blijft in het verloop van de tijd. Dat geldt voor de periode vroeg na de diagnose DCM, en ook voor de tijd daarna. Bij degenen die overleden of getransplanteerd werden was de 6MWD% wel aanzienlijk lager dan bij degenen die overleefden, maar veranderde dus niet over de tijd. Op basis van ons onderzoek stellen we voor dat er ten minste twee 6MWT worden gedaan om een betrouwbare inschatting te maken van de 6MWD%. Daarna heeft het herhalen van de 6MWT weinig toegevoegde waarde.

Er zijn bij kinderen met DCM risicofactoren bekend die een hogere kans geven op overlijden of transplantatie. Zo weten we bijvoorbeeld dat de grootte van de linkerkamer en het NT-proBNP (een stof die je in het bloed kunt meten en iets vertelt over de mate van oprekking van het hart) iets zeggen over de kans op een slechte uitkomst. De meeste studies die in het verleden zijn gedaan gaan over eenmalige metingen van risicofactoren. In **Hoofdstuk 5** presenteren we onze studie die gaat over het nut van herhaalde metingen van risicofactoren, dus of het beloop over tijd extra informatie geeft. Dit zou ons kunnen helpen om de kinderen met de grootste kans op overlijden of transplantatie te onderscheiden. We vonden dat het beloop over de tijd van 4 risicofactoren de kinderen die stierven of transplantatie ondergingen, onderscheidde van degenen die ziek bleven of herstelden. De kinderen die overleden of een harttransplantatie ondergingen lieten geen afname van het NT-proBNP en de afmeting van de linker hartkamer zien, wel vertoonden ze ernstig achterblijvende lengte groei, en een score die de ernst van de hartspierziekte meet, liep op. Wanneer we naar al die risicofactoren tegelijk keken, bleek het NT-proBNP de enige risicofactor voor slechte uitkomst. We concluderen dat bij kinderen met DCM verandering van bekende risicofactoren voor overlijden of harttransplantatie voorspellend is voor de uitkomst. Aanhoudend hoge niveaus van NT-proBNP, geen afname van de linker hartkamer grootte, ernstige groeivertraging en een toename van de score voor falen van hart, kenmerken de kinderen met het hoogste risico op overlijden of transplantatie.

Hoofdstuk 6 bespreekt de veiligheid van een groep medicijnen, de zo genaamde ACE-remmers, voor de behandeling van hartfalen bij kinderen. Dit hebben we gedaan op basis van alle literatuur die nu beschikbaar is. ACE-remmers worden bij volwassenen veel gebruikt bij onder andere DCM. De meest voorkomende bijwerkingen van ACE-remmers zijn nierfalen, lage bloeddruk, hoog kalium in het bloed, hoest en vasthouden van vocht. Het zou kunnen dat het veiligheidsprofiel van ACE-remmers voor kinderen verschilt van dat voor volwassenen. Dat heeft te maken met het feit dat groei en ontwikkeling bijdragen aan variatie in effect en bijwerkingen van de meeste geneesmiddelen. Op basis van alle beschikbare gegevens in de literatuur concluderen we als eerste dat ACE-remmers als relatief veilig kunnen worden beschouwd voor kinderen met hartfalen. Nierfalen, lage bloeddruk en een hoog kalium in het bloed zijn vaak voorkomende bijwerkingen. Ten tweede zijn een laag gewicht, een jonge leeftijd en kans op uitdroging (bijvoorbeeld bij een buikgriep of een hoge dosis plasmedicijnen) factoren die een verhoogd risico op bijwerkingen geven wanneer kinderen ACE-remmers gebruiken.

DCM is een ernstige ziekte die veel lichamelijke klachten kan geven en waarbij kinderen ook eerder overlijden. Het is daarom heel goed voor te stellen dat er belangrijke gevolgen zijn voor het psychosociaal welzijn bij kinderen met DCM. Je kunt hierbij denken aan angstklachten en depressieve verschijnselen. Het was niet goed duidelijk hoe vaak dat voorkomt bij kinderen met DCM, en of het hebben van angstklachten en depressieve verschijnselen ook voorspelt wat de uitkomst van de ziekte is. Dat is namelijk bij volwassenen met DCM wel het geval, waarschijnlijk omdat angstklachten en depressieve verschijnselen kunnen leiden tot slechtere zelfzorg en daardoor tot verergering van de ziekte. In **Hoofdstuk 7** bekeken we of de mate van emotionele en gedragsproblemen en of angstklachten en depressieve verschijnselen voorspellend zijn voor de uitkomst van de ziekte. We vonden dat, vergeleken met van leeftijdsgenoten, een hoger percentage oudere kinderen (6-18 jaar oud) met DCM angstklachten en depressieve verschijnselen vertoonden. De resultaten die we vonden komen overeen met grote studies bij volwassenen met hartfalen, die een verhoogd risico op angst en depressie aantoonden. We vonden dat angst en depressieve problemen bij kinderen met DCM het risico op overlijden en harttransplantatie niet voorspelden. Dit wordt mogelijk verklaard door het feit dat ouders bij kinderen de slechtere zelf zorg van kinderen kunnen compenseren. Onze bevindingen laten echter wel duidelijk zien dat het van belang is om kinderen met DCM routinematige te screenen op angstklachten en depressieve verschijnselen. Psychosociale ondersteuning afgestemd op de behoeften van deze kinderen is hierbij essentieel.

Een steun-hart (ventricular assist device: VAD) is tegenwoordig een algemeen geaccepteerde behandeling bij volwassenen met eindstadium hartfalen. Het gebruik van een VAD bij kinderen met DCM is ook mogelijk, maar was aanvankelijk lastig, vanwege de technische problemen en complicaties die kunnen optreden in een klein lichaam. De afgelopen jaren hebben verschillende ziekenhuizen hun ervaringen met de Berlin Heart EXCOR VAD gepresenteerd. Er is aangetoond dat de Berlin Heart EXCOR VAD een betrouwbaar en een relatief veilig kunsthart is dat het de tijd die kinderen moeten wachten op een harttransplantatie, kan helpen overbruggen. In een enkel geval overbrugt de VAD zelfs naar herstel. Sinds 2006 is de Berlin Heart EXCOR Pediatric VAD beschikbaar in ons ziekenhuis. **Hoofdstuk 8** beschrijft hoe het gegaan is met de kinderen die een een kunsthart (VAD) kregen. Ook hebben we gekeken naar het effect van de introductie van de VAD op de sterfte op de wachtlijst voor harttransplantatie. We vonden dat 61% van de kinderen die een VAD moeten krijgen vanwege eindstadium hartfalen, overleeft tot harttransplantatie of herstel. Dat betekent dus ook dat bijna 40% van de kinderen overlijdt aan een VAD. Over het algemeen lijkt het zo te zijn dat hoe zieker het kind is wanneer het aan VAD gaat, des te groter de kans is om te overlijden. Dat in onze studie relatief veel kinderen aan een VAD overleden zegt waarschijnlijk dat de kinderen die op de wachtlijst stonden ernstig ziek waren. Aan de andere kant is het zo dat sinds de introductie van VADs in ons ziekenhuis, het sterftecijfer op de wachtlijst is gehalveerd. Dit ondersteunt het groeiende bewijs dat een VAD een acceptabele therapie is om kinderen met eindstadium hartfalen te overbruggen naar transplantatie of herstel.

Wereldwijd is er redelijk veel verschil in het percentage kinderen dat in het eerste jaar na diagnose van DCM getransplanteerd word. Eerder rapporteerden we een relatief laag

harttransplantatiepercentage (3%) bij kinderen met DCM in het eerste jaar na de diagnose in vergelijking met andere grote studies (18%). Belangrijk was, dat we geen toename zagen van het aantal kinderen dat overleed. Het feit dat we zo weinig kinderen vroeg na diagnose transplanteren, zou kunnen komen doordat we harttransplantatie zo lang mogelijk uitstellen en herhaaldelijke pogingen doen om kinderen in een betere conditie te krijgen met medicijnen. Echter, het zou zo kunnen zijn dat deze aanpak leidt tot een selectie van kinderen die erg ziek zijn, tegen de tijd dat ze op de wachtlijst komen. Dit zou weer tot gevolg kunnen hebben dat er meer kinderen op de wachtlijst overlijden, en ook dat de uitkomst na harttransplantatie nadelig wordt beïnvloedt. In **Hoofdstuk 9** bekijken we de uitkomst van 18 jaar harttransplantatie bij kinderen in Nederland. We houden er hierbij rekening mee dat we een klein - tot middelgroot transplantatie programma hebben, dat voornamelijk bestaat uit kinderen met DCM. In deze studie vonden we dat 68% van de kinderen die op de wachtlijst stonden een transplantatie ondergingen. Drieëntwintig procent van de kinderen overleed terwijl ze op de wachtlijst stonden. Vijf jaar na transplantatie was 95% van de getransplanteerde kinderen in leven, 10 jaar na transplantatie was dat nog 88%. Deze percentages zijn gunstig in vergelijking met internationale gegevens. De belangrijkste doodsoorzaak na transplantatie was afstoting, door het niet innemen van medicatie tegen afstoting. De belangrijkste complicaties na transplantatie waren een milde en goed behandelbare vorm van lymfeklier kanker, en ziekte aan de bloedvaten van het nieuwe hart. We concludeerden dat onze behandelstrategie niet resulteerde in een toegenomen sterfte op de wachtlijst. Harttransplantatie is een goed bruikbare behandeling voor een geselecteerde groep kinderen met eindstadium hartfalen.

In **Hoofdstuk 10** worden de belangrijkste bevindingen van de studies in dit proefschrift in detail besproken en in een breder perspectief geplaatst. Verder hebben we suggesties gedaan voor toekomstig onderzoek.

Appendices

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Nederlands Tijdschrift voor Geneeskunde 2004;148:1617-19

PHD PORTFOLIO

Summary of PhD training and teaching activities

Name PhD student:	M.H. van der Meulen
Erasmus MC department:	pediatrics, pediatric cardiology
Research school:	COEUR
PhD period:	2014-2019
Promotor:	Prof. dr. W.A. Helbing and Prof. dr. A.J.J.C. Bogers
Co-promotor:	Dr. M. Dalinghaus

	Year	Workload (ECTS)
General academic skills		
Course Medical Library EMC	2014	0.5
BROK (Basiscursus Regelgeving Klinisch Onderzoek)	2015	1.0
Biomedical English Writing and Communication	2016	3.0
Research Integrity (ID nummer: 212048)	2016	0.7
Research skills		
Coeur Heart failure research	2014	1.5
Exercise Physiology, by Dutch Society of Physiology	2014	0.5
Statistics :CC02	2015	5,7
GCP LENA	2015	1.0
Coeur congenital heart disease	2015	1.0
Coeur inherited cardiomyopathies	2015	0.5
Monthly journal club	2014-2018	1.0
Seminars, workshops and other		
organizer annual CARS multicenter study meeting	2014-2018	1.0
general assembly LENA trial	2014-2016	1.0
Symposium *Gedilateerde cardiomyopathie bij kinderen: 5 jaar CARS, een paar puzzelstukjes verder?*	2016	0.5
Presentations		
EACTS, Mechanical circulatory support in the Dutch National Pediatric Heart Transplantation Program, Milaan, Italie (oral presentation)	2014	1.2
NVK congres, Afbuigende lengte groei is geassocieerd met harttransplantatie of overlijden bij kinderen met gedilateerde cardiomyopathie *(oral presentation)*	2015	1.0
Vergadering sectie kindercardiologie, Utrecht (*oral presentation)*	2015	0.3
AEPC Psychosocial meeting, *Does heart transplantation improve quality of life in pediatric patients (oral presentation)*	2016	1.2
International Invitational Workshop on Coagulation and Extra corporeal assist devices, Rotterdam	2016	0.5
Pediatric heart failure in the Netherlands, COEUR course Congenital Heart Disease, (*oral presentation),* Rotterdam	2017	0.5
AEPC, Athene, Griekenland, 2 poster presentations	2018	1.2
AEPC, Sevilla, Spanje, 2 poster presentations	2019	1.2

International conferences		
EACTS Milaan, Italie	2014	1.0
ESDPPP Belgrado, Servië	2015	1.0
AEPC Psychosocial meeting, Rotterdam	2016	0.5
Teaching activities		
Supervising Master's theses		
Outcome pediatric HTx, submitted article, Stefan Roest,	2017	1.5
Berlin Heart Excor in EMC, submitted article, Sofie Rohde	2018	
Other		
Supervising MD students in writing a review on enalapril in heart failure in Fontan patients	2014	1.0

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Marijke van der Meulen was born on 1st July 1978 in Rotterdam, the Netherlands. She attended pre-university education at the Revius college in Rotterdam. Thereafter, Marijke started medical training at the Erasmus University of Rotterdam. During those 4 year, she worked in a medical students team on the ear-nose-throat ward of the Erasmus MC. She also participated in research in the liver transplantation team of the Erasmus MC, supervised by Prof. H.W. Tilanus. After her last senior internship in the Morgenster Mission Hospital in Kenya, she received her medical degree in 2003. She started working as a pediatric resident at the Reinier de Graaf Gasthuis in Delft under guidance of Dr. N. van der Lely. From 2005 to 2010 she started her formal training in pediatrics, supervised by Prof. M. de Hoog and Dr. A.A.P.H. Vaessen-Verberne. In her first years as a pediatrician she worked a year in general pediatrics and pediatric cardiology, and during 2 years in pediatric intensive care. In 2013 she applied for a research grant at the "Nederlandse Hartstichting" and "Stichting Hartedroom", under supervision of dr. M. Dalinghaus, which was fortunately honored. In 2014 she started the PhD project on dilated cardiomyopathy in children under supervision of Prof. Dr. W.A. Helbing, Prof. A.J.J.C. Bogers and Dr. M. Dalinghaus. She currently works in general pediatrics in the Beatrix ziekenhuis in Gorinchem. Marijke loves art, horses and sports climbing. She lives in Rotterdam with her three children Hannah, Tijn en Abel.

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