ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN

SCIENZE CARDIO-NEFRO-TORACICHE

Ciclo XXXVI

Settore Concorsuale: 06/E1 Chirurgia cardio-toraco-vascolare

Settore Scientifico Disciplinare: MED/23 Chirurgia Cardiaca

NON-INVASIVE DETECTION OF ACUTE CELL-MEDIATED GRAFT REJECTION IN PAEDIATRIC HEART TRANSPLANT RECIPIENTS: THE ROLE OF CARDIOVASCULAR MAGNETIC RESONANCE

Presentata da: ***Dr.ssa Valentina Gesuete***

Coordinatore Dottorato
Chiar.mo Prof.
Niccolò Daddi

Supervisore
Chiar.mo Prof.
Gaetano Domenico Gargiulo

Co-Supervisore
Dr. Luca Ragni

Esame finale anno 2024

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ABSTRACT

Background and Aim: Acute cardiac rejection is currently diagnosed by endomyocardial biopsy (EMB), but multiparametric cardiac magnetic resonance (CMR) may be a non-invasive alternative by its capacity for myocardial structure and function characterization. Our primary aim was to determine the utility of multiparametric CMR in identifying acute graft rejection in paediatric heart transplant recipients. The second aim was to compare textural features of parametric maps in cases of rejection versus those without rejection.

Methods: Fifteen patients were prospectively enrolled for contrast-enhanced CMR followed by EMB and right heart catheterization.

Images were acquired on a 1,5 Tesla scanner including T1 mapping (modified Look-Locker inversion recovery sequence – MOLLI) and T2 mapping (modified GraSE sequence). The extracellular volume (ECV) was calculated using pre- and post-gadolinium T1 times of blood and myocardium and the patient's hematocrit. Markers of graft dysfunction including hemodynamic measurements from echocardiography, catheterization and CMR were collated. Patients were divided into two groups based on degree of rejection at EMB: no rejection with no change in treatment (Group A) and acute rejection requiring new therapy (Group B). Statistical analysis included student't t test and Pearson correlation.

Results: Acute rejection was diagnosed in five patients. Mean T1 values were significantly associated with acute rejection. A monotonic, increasing trend was noted in both mean and peak T1 values, with increasing degree of rejection. ECV was significantly higher in Group B. There was no difference in T2 signal between two groups.

Conclusion: Multiparametric CMR serves as a noninvasive screening tool during surveillance encounters and may be used to identify those patients that may be at higher risk of rejection and therefore require further evaluation. Future and multicenter studies are necessary to confirm these results and explore whether multiparametric CMR can decrease the number of surveillance EMBs in paediatric heart transplant recipients.

Keywords: acute cardiac rejection, paediatric heart transplant, endomyocardial biopsy, multiparametric cardiac magnetic resonance

BACKGROUND

Heart transplantation (HTx) in infants and children is now a treatment option for selected paediatric patients with end-stage heart failure or inoperable congenital cardiac defects [1].

Since the first orthotopic heart transplantation over 50 years ago, the field has grown tremendously, including many advancements in the application of this therapy to children. Today, heart transplantation is performed routinely in many paediatric centers throughout the world with over 100 centers [2].

Nevertheless, several questions remain unsolved such as the shortage of donors, waitlist survival and the impact of long-term immunosuppression therapy.

Acute allograft rejection remains the third leading cause of post-transplant mortality [3]. The Pediatric Heart Transplant Society database demonstrated that although there has been a decline in the rates of early rejection, the incidence of rejection with haemodynamic compromise or associated mortality has remained unchanged [4].

Similarly, despite a decline in rate of late rejection, affected patients continue to be at significant risk for coronary vasculopathy, need for re-transplantation, and mortality [5].

Periodic endomyocardial biopsy is the current gold standard test for rejection surveillance. However, its utility and diagnostic accuracy have been debated [6] and because of risks associated with this invasive test, there is significant interest in non-invasive testing modalities to monitor graft function and for evidence of acute rejection [7].

HISTORY

More than fifty years ago there was a race to perform the first human heart transplant. Those involved included the visionaries Norman Shumway, Christiaan Barnard and Adrian Kantrowitz. On December 3, 1967, Christiaan Barnard and his team at Groote Schuur Hospital in Cape Town South Africa performed the first human heart transplant in a 53-year old man, making world history [8]. The recipient survived only 18 days, dying of pneumonia.

A year and a half prior to Barnard' s historic transplant, Kantrowitz and his group had planned to perform the first human transplant in a paediatric patient but had to abandon the transplant because of problems with the donor heart.

Three days after the first human-to-human heart transplant in 1967, Adrian Kantrowitz performed the world's second heart transplant at Maimonides Medical Center in New York in a 19-day old infant with Ebstein's anomaly and pulmonary atresia [9]. The family of the child who underwent the transplant had actually signed the surgical consent form more than 1 week prior to Barnard's historic first transplant. At the initiation of the transplant, both the donor and recipient were immersed in iced water to achieve topical cooling (Fig. 1). Donor cardiac activity ceased at a temperature of 27° C, and a bilateral, transverse thoracosternotomy incision was then performed in order to proceed (Fig.2). Once harvesting was complete, the donor heart was stored in 5° C saline solution. The recipient developed ventricular fibrillation as the chest was opened. As implantation of the heart was to be performed under circulatory arrest, open cardiac massage was required until the recipient could be cooled to 17° C. The implantation technique was the biatrial anastomoses method. Rewarming of the implanted heart and the recipient was achieved by placing warmed saline within both the recipient's chest and in the tub containing the infant. The baby survived only 6 hours and Kantrowitz never pursued clinical heart transplantation. It would be 16 years before neonatal heart transplantation was again attempted.

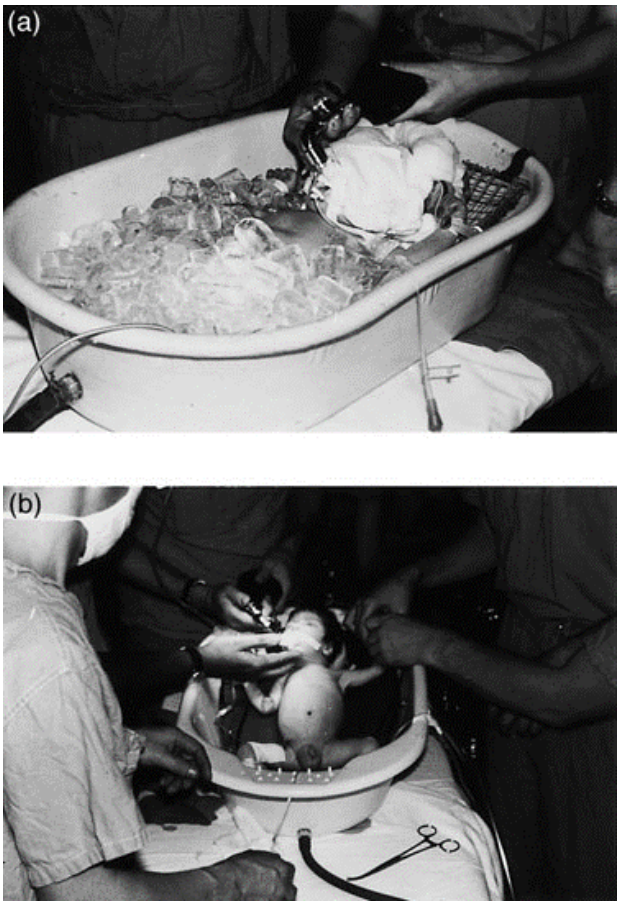


Fig.1 The first paediatric heart transplant was performed on December 6th, 1967 by Adrian Kantrowitz [9]

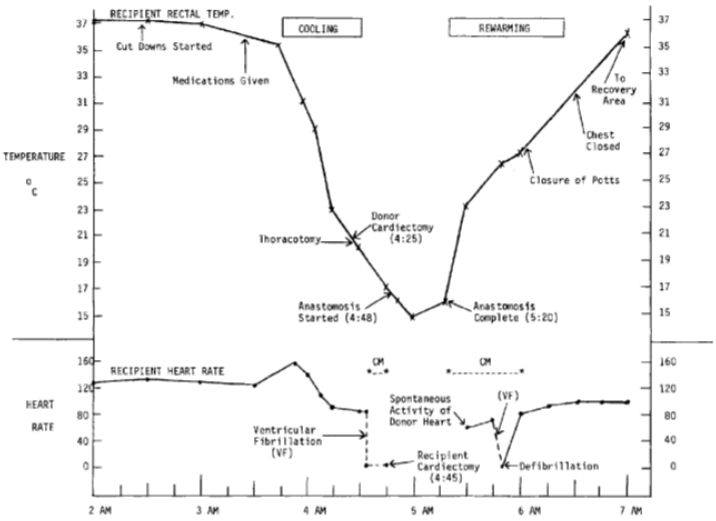


Fig. 2. Hypothermia was induced by placing both the donor (upper panel) and the recipient (lower panel) in baths of iced water

The following year, Cooley et al. performed a heart-lung transplant in a 3-month-old child with an atrioventricular canal defect and pulmonary hypertension [10].

Use of anencephalic donors, as well as recipient survival that could be measured only in terms of hours, were characteristics of each of these first two pioneering paediatric cardiac

transplants. It was not until 1984 that Cooley et al. performed the first clinically successful infant heart transplant in an 8-month-old child with sub-endocardial fibroelastosis [11]. Neonatal and small infant heart donors were unheard of in the early 1980s. Then, in late July of 1984, Magdi Yacoub and his team at the National Heart Hospital in London reported, via the lay press, having transplanted an 11-day-old newborn with hypoplastic left heart syndrome (HLHS) [12]. An allograft donor had been identified in Holland. The infant recipient, Holly Roffey, had a complex postoperative course and died of respiratory failure on the 18th postoperative day.

In 1984, in a desperate attempt to save the life of a baby girl, Bailey performed the first cross-species infant heart transplant at Loma Linda University.

Born three weeks early, Stephanie Fae (known as Baby Fae) was diagnosed with hypoplastic left heart syndrome. For years, Bailey had been performing research through experimental transplantation in sheep, goats, and baboons. By the time Baby Fae was born, he had performed over 200 of these procedures. Baby Fae survived the transplant, which provided her a new, healthy and, against all odds, beating baboon heart. Through the transplant gave her a little extratime, Baby Fae passed away 21 days later. She became the longest-living recipient of a cross-species heart (Fig. 3).

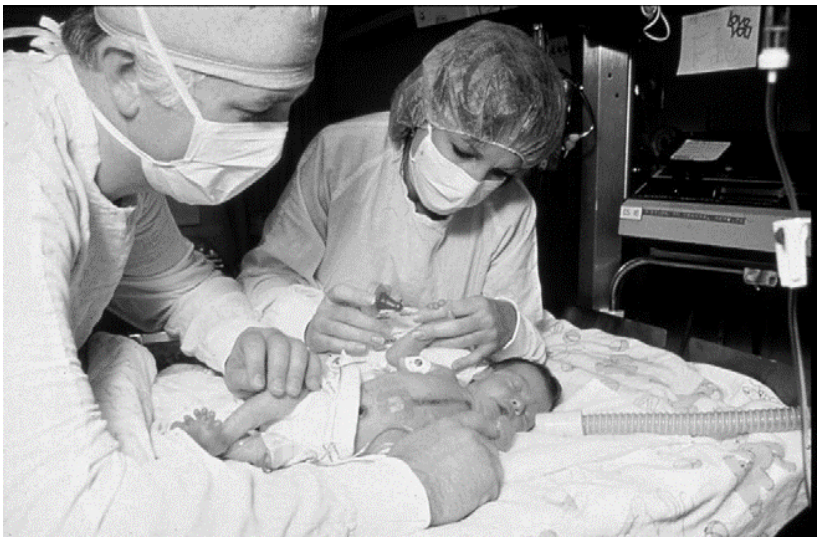


Fig. 3 “Baby Fae” with HLHS, here approximately 3 days after cardiac xenotransplantation using an infant baboon donor. She is flanked by her surgeon and principal immunologist [13]

Baby Fae helped absolutely transform the landscape of paediatric heart transplants, generating unprecedented levels of public awareness. People every when learned of the pressing need for infant organ donation.The next year, in 1985, Bailey performed the first

successful human-to-human heart transplant on an infant. Little Eddie Anguiano (known as Baby Moses) not only survived, but continues to thrive to this day at 38 years old. Eddie is the longest-living recipient of an infant heart transplant – that some heart still beats in his chest (Fig. 4-5)[13].

Fig. 4 Eddie Anguiano, here several weeks of age following heart allotransplantation on his fourth day of life as therapy for HLHS [13]

Fig. 5 Off to school, Eddie Anguiano is the first neonatal recipient of successful cardiac allotransplantation [13]

CURRENT LANDSCAPE

The International Society of Heart and Lung Transplant (ISHLT) Thoracic Registry was created in 1983 to capture multicenter paediatric and adult transplant data with data collection throughout the life of the transplant patient/graft. The registry collects a

multitude of information. Using the data set, there are extensive analyses which result in slide sets publicly available for review. The latest data from 2017 shows that there are 117 centers that perform paediatric heart transplants, 56 of which are in the United States [2] (Fig. 6).

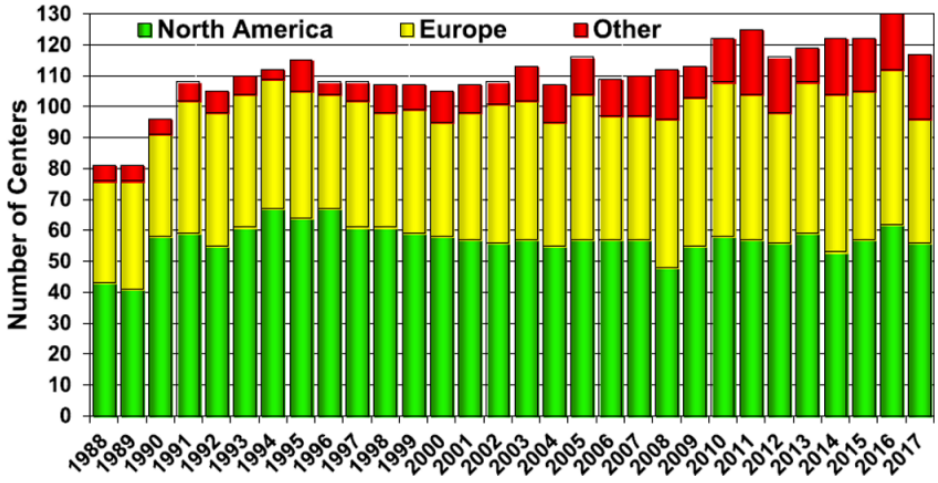


Fig. 6 Number of centers reporting paediatric heart transplants [2]

Among the centers, the frequency of the heart transplants per year varies with a lot of centers being small programs that do < 4 transplants a year. There are 154 centers that average 1-4 transplants a year, 35 centers that average 5-9 transplants a year and 21 centers that average more than 10 transplants a year (Fig. 7).

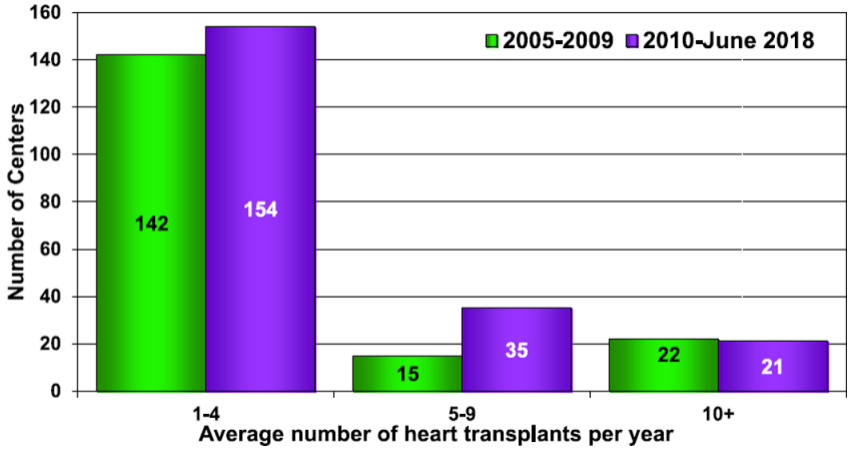


Fig. 7 Number of centers by center volume (transplants: January 2005-June 2018) [2]

Between 2010 and 2018, 210 paediatric cardiac transplants were performed with 45.5% being done at centers who average > 10 transplants a year (Fig. 8).

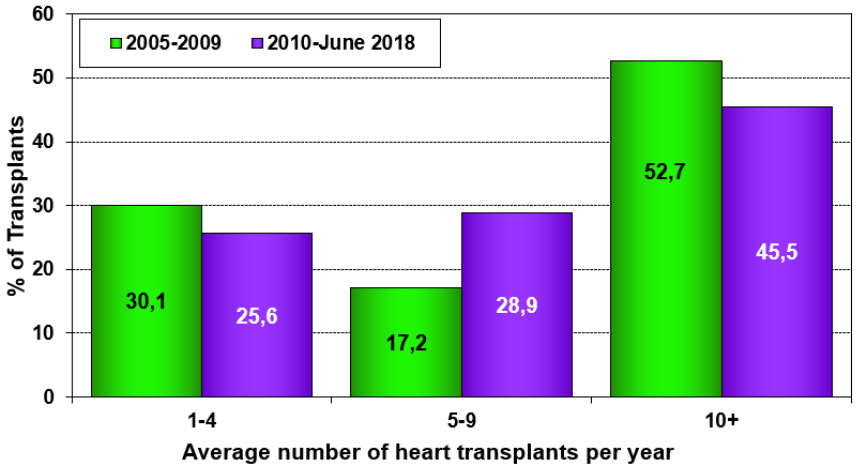


Fig. 8 Distribution of Transplants by Center Volume (transplants: January 2005 – June 2018) [2]

Medium-volume centers (centers averaging 5-9 transplants per year) performed 29% of all transplants since 2010, an increase from 17% from 2005 to 2009. More transplants are performed at small- and medium-volume centers in Europe and in other parts of the world than in North America (Fig. 9).

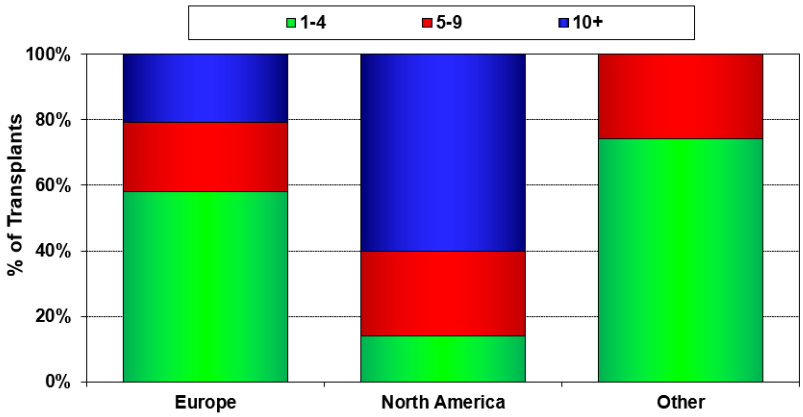


Fig. 9 Distribution of Transplants by Location and Average Center Volume (transplants: January 2005 – June 2018) [2]

Infants account for the greatest number of transplants per 1 year of life with nearly 1,800 transplants from January 2005 to June 2018 (Fig. 10-11).

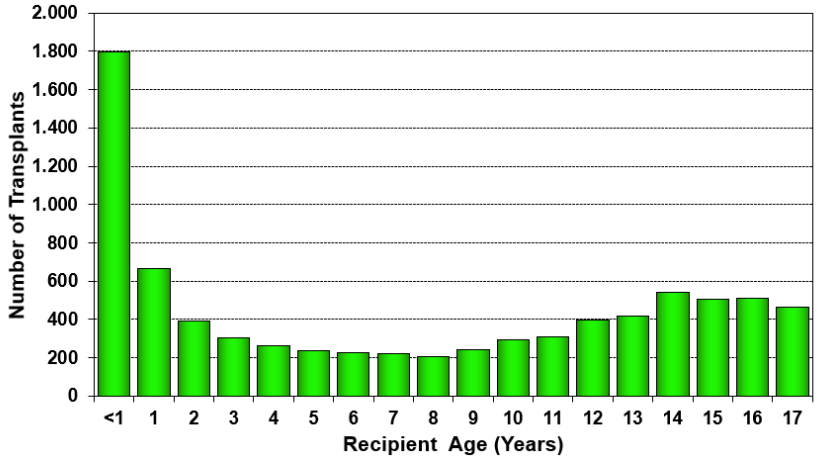


Fig. 10 Recipient age distribution (Transplants: January 2005 – June 2018) [2]

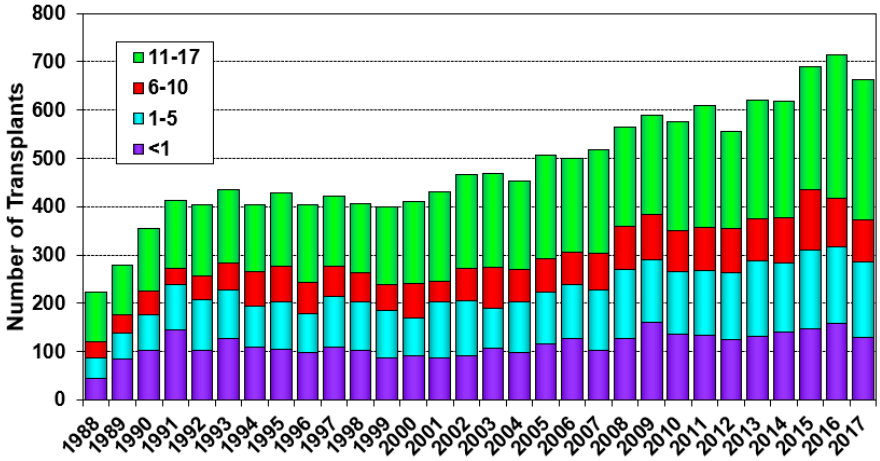
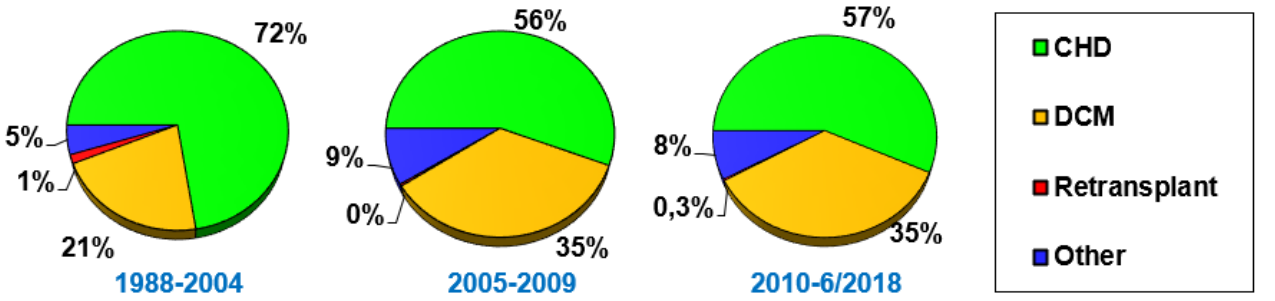
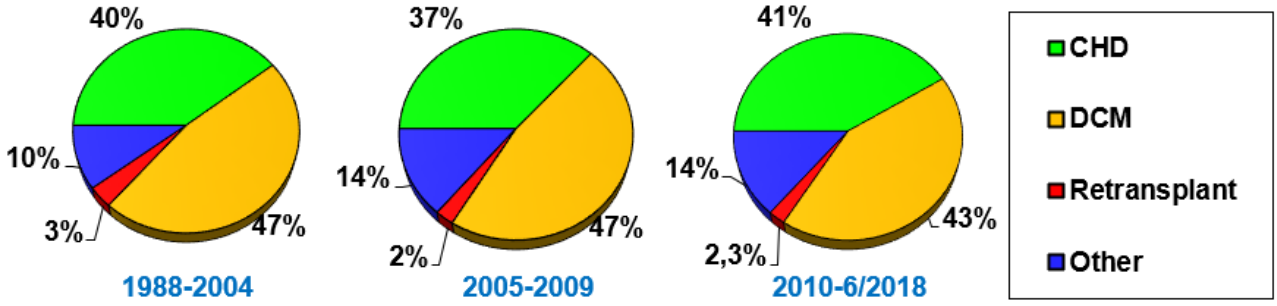


Fig. 11 Recipient age (in years) distribution by year of transplant [2]

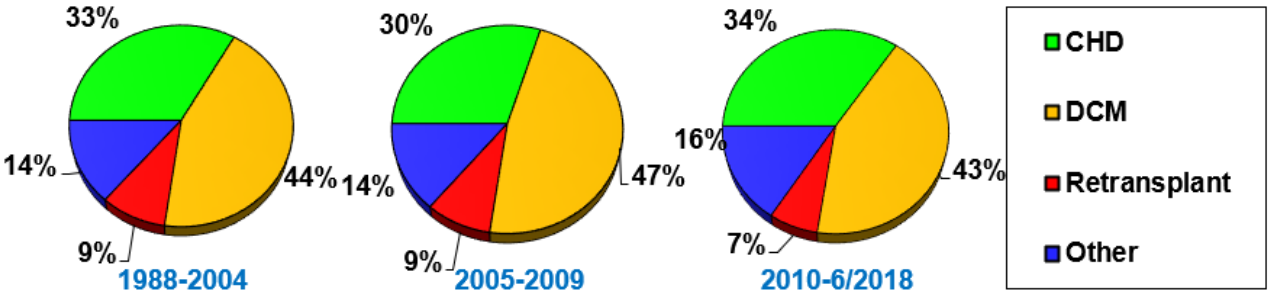
The primary indication for transplant varies by age, with congenital heart disease (CHD) being the most common indication in infants (57%) and cardiomyopathy being the most common indication in older children (43% in children aged 1-10 years and 53% in children aged 11-17 years) (Fig. 12).



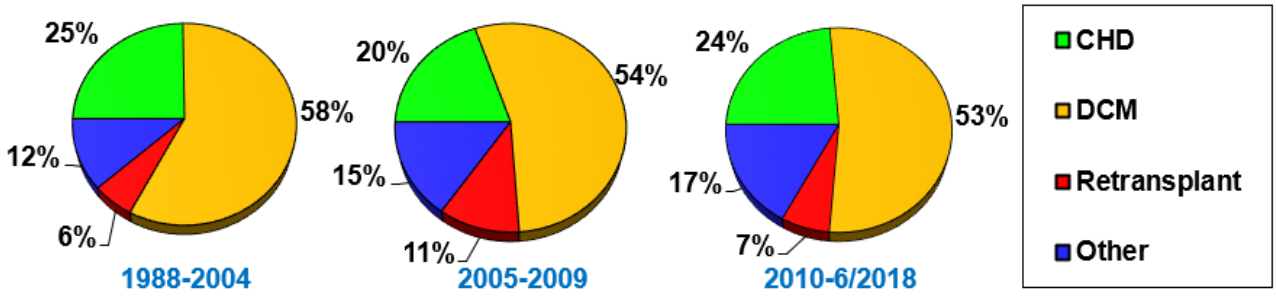
a) Recipient diagnosis (age: < 1 year)



b) Recipient diagnosis (age: 1-5 years)



c) Recipient diagnosis (age: 6-10 years)



d) Recipient diagnosis (age: 11-17 years)

Fig. 12 Recipient diagnosis by age [2]

Mechanical circulatory support (MCS) as a bridge to heart transplantation is being increasingly utilized in children. In 2017, 37% of children were supported to transplant on some form of MCS, with the vast majority supported on a ventricular assist device

(VAD). Fewer patients with CHD were supported with VADs than were children with dilated cardiomyopathy (DCM). Only 12% of infants with CHD were bridged to transplant using some form of MCS, with extracorporeal membrane oxygenation (ECMO) used as commonly as a VAD. This is in contrast to over 50% of older children with DCM supported on MCS before transplant, with ECMO as a bridge in < 3% of patients (Fig. 13-14).

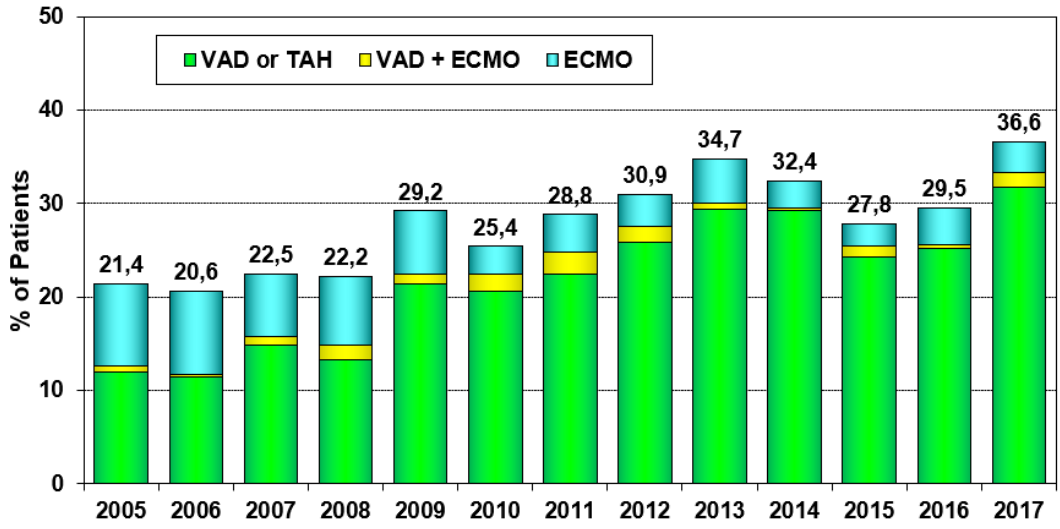


Fig.13 Percentage of patients bridged with mechanical circulatory support by year (Transplants: January 2005-December 2017) [2]

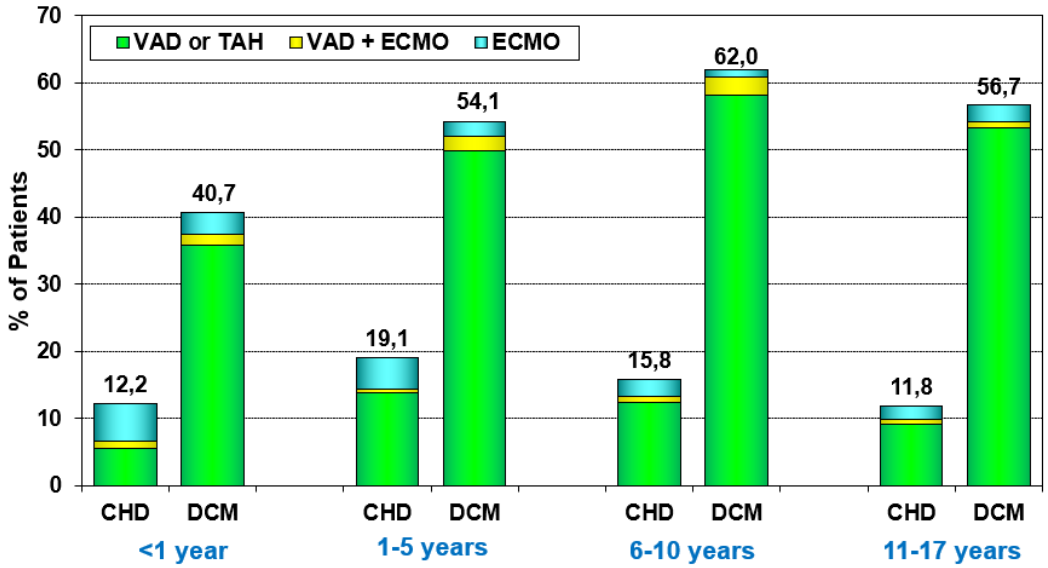


Fig. 14 Percentage of patients bridged with mechanical circulatory support by age group and diagnosis (Transplants: January 2010-June 2018) [2]

Sensitization is the creation of antibodies to foreign proteins exposed to recipient's blood stream. This is another important factor that can affect heart transplant outcomes and is tracked by the registry. During the evaluation process, a recipient's blood is tested for panel reactive antibodies (PRAs) to determine if there are strong antibodies to any

proteins, leading to the inability to take specific donors as rejection would be immediate. The result is expressed in a calculated percentage of the population that cannot be a donor for the patient. PRAs > 10% is considered sensitized. There has been a gradual increase, shown in the registry data [2], of patients with significant panel reactive antibodies over time (Fig. 15). In 2007, only 14% of recipients had allosensitization compared with 32% in 2017.

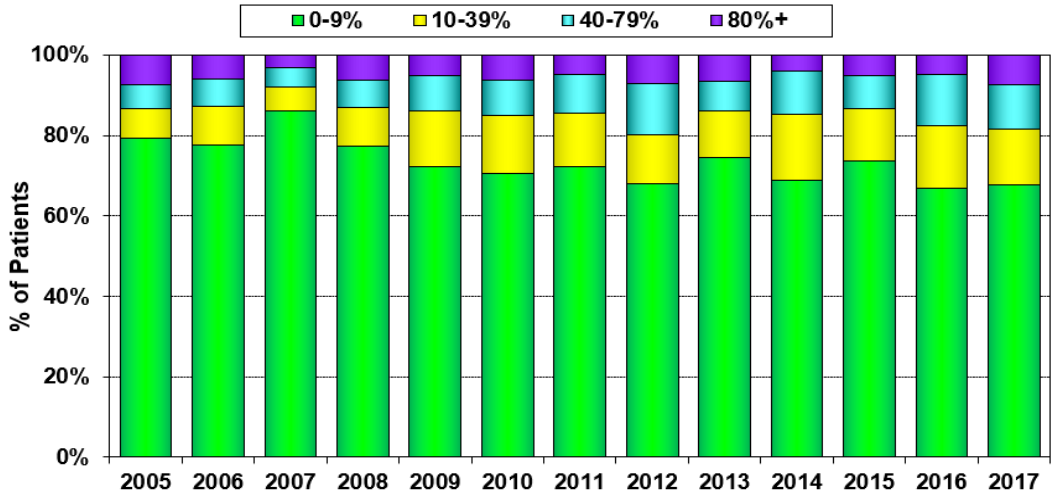


Fig. 15 PRA distribution by year (Transplants: January 2005-December 2017) [2]

Allosensitization is more common in older children than infants and among patients with CHD and retransplant than DCM or other types of heart disease (Fig. 16). This is believed to be due to blood products from previous surgeries, VADs and exposure to homograft material.

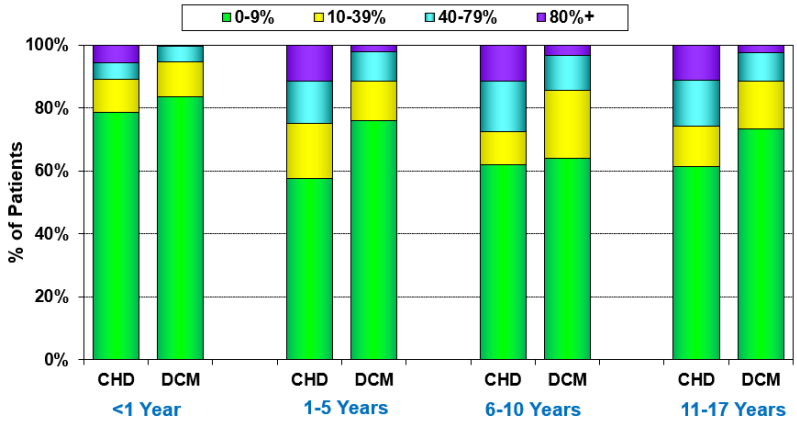


Fig.16 PRA distribution by age group and diagnosis (Transplants: January 2010-June 2018)[2]

Given the long duration of follow-up available in the ISHLT Registry, survival rates up to 25 years post-transplant can be determined. The overall median survival is more than 18 years, with the longest survival among children undergoing their transplant in infancy

(median survival 24.5 years) and the shortest survival among children undergoing transplant at 11 to 17 years of age (median survival 14.3 years) (Fig.17).

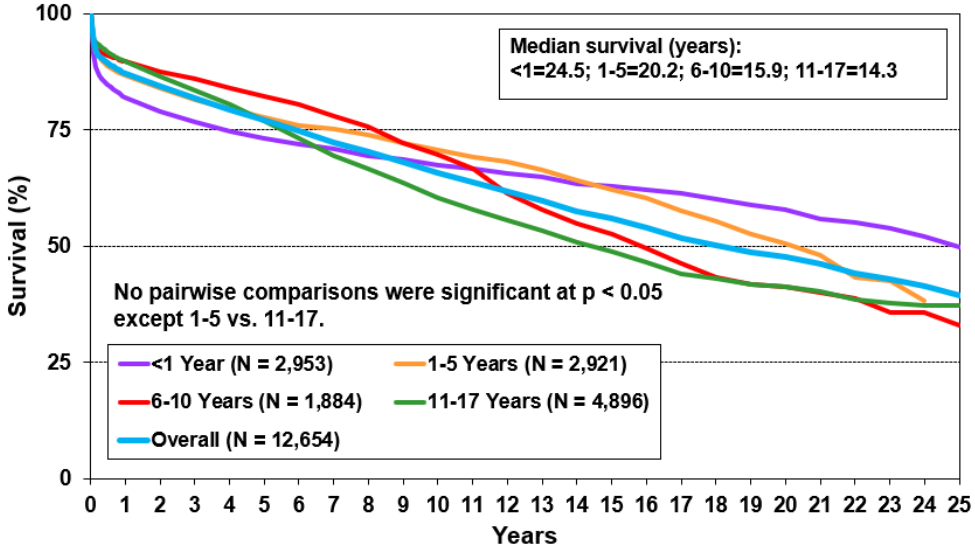


Fig.17 Kaplan-Meier survival conditional to survival to 1 year after transplant, by recipient age at transplant (Transplants: January 1992-June 2017) [2]

Outcome at 1 year and 5 years have improved when comparing 1982-1991 (1 year = 72,1%; 5 years 60.6%) to 2010-2017 (1 year = 91.5%, 5 years = 83%). Based on data from 2002 to 2009 the current 10-year survival is 68% with 15-year survival at 58.9% (Fig. 18).

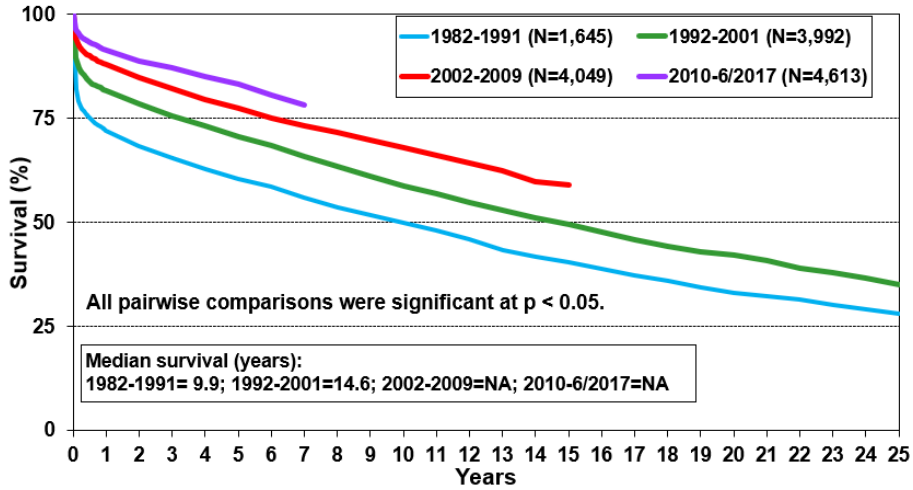


Fig. 18 Kaplan-Meier survival by era (Transplants: January 1992-June 2017) [2]

Looking at 10-year survival by age categories, patients transplanted between the ages of < 1-10 years old had no statistical difference in survival, with an average between 83% and 85%. There is however, a statistical difference between all of these age groups and those patients 11-17 years old with a 10-year survival of this group of 70.2%. When broken down by age group survival over era, all age groups survival statistics have

improved. Comparing outcomes across ages based on etiology of the transplant, those patients with DCM had higher survival, with DCM 1-year survival of 91-93% versus CHD at 82-88%. Outcomes are also greatly affected by the need and type of mechanical support while waiting for an organ. The 5-year survival for patients who needed no mechanical support or those who needed only a VAD or total artificial heart (TAH) had improved outcomes (85%) than those who needed ECMO (77%)[2].

HEART REJECTION

Despite advances in the care of heart transplant recipients, acute rejection remains a barrier to long-term success. Allograft rejection is one of the leading causes of death up to 10 years post-transplant in children [2,14,15], and treated rejection in the first year is associated with decreased graft and patient survival [15].

Additionally, rejection has been associated with the development of cardiac allograft vasculopathy [16-18], potentially further impacting graft longevity. The incidence of rejection has declined over time [14, 19, 20].

Despite this, between 10% and 30% of paediatric heart transplant recipients are diagnosed with rejection during the first years post-transplant in the most recent era depending on patient risk and induction strategy [20,21].

The continued risk of rejection combined with efforts to optimize long-term patient outcomes has led to the development of various strategies for rejection surveillance. This includes both invasive and non-invasive approaches. However, there is significant variability in the protocols for rejection surveillance among paediatric heart transplant centers [22,23].

1 | Definition and pathology

In histological terms, acute rejection is observed as an inflammatory response of the host to the transplanted organ.

An allogenic heart transplant is non-self to the recipient and prone to allograft rejection by the recipient's immune system. Heart transplant rejection occurs when the recipient immune system reacts to the foreign antigens in the donor organ by mounting an immune response.

Acute rejection follows allorecognition and involves different mechanisms including cellular (ACR) and antibody mediated (AMR) rejection.

Both types of rejection have distinctive histological and immunohistochemical findings [24, 25].

1.1 | Acute Cellular Rejection

Major and minor histocompatibility antigens are not expressed equally among all individuals; this increases the potential of such proteins to act as alloantigens and activate alloimmunity by stimulating cytotoxic T cells. T cells respond to these donor antigens either directly or indirectly based on the method of antigen presentation. T cells can either directly recognize donor MHC molecules on allograft or target when presented indirectly by recipient antigen-presenting cells (APC) (Fig. 19) [25]. Interleukin-2 (IL-2), tumor necrosis factor-beta (TNF-beta), and interferon-gamma (IFN-gamma), all act as significant mediators during rejection. ACR presents as a mononuclear inflammatory response infiltrating myocardial tissue with predominant lymphocytic cells. Immunohistologic assessment can confirm the presence of CD-4 and CD-8 positive T lymphocytes with high affinity to interleukin-2 receptors. Presence of increased intercellular adhesion molecules with high MHC-II expression on cardiac myocytes is present. These findings should be distinguished from Quilty effect, which carries no clinical significance. Quilty lesions extend to the endocardial surface and include significant B-lymphocytes distinguishing from acute cellular rejection [26].

1.2 | Antibody-Mediated Rejection

Antibody-mediated humoral rejection is poorly understood. The antibody reacts to donor MHC antigens (HLA-I and II) leading to capillary endothelial changes (Fig. 19).

The deposition of immunoglobulin and complements within myocardial capillary bed are detectable by immunofluorescence. AMR leads to intravascular macrophage accumulation with interstitial oedema, hemorrhage and neutrophilic infiltration in and around capillaries [27].

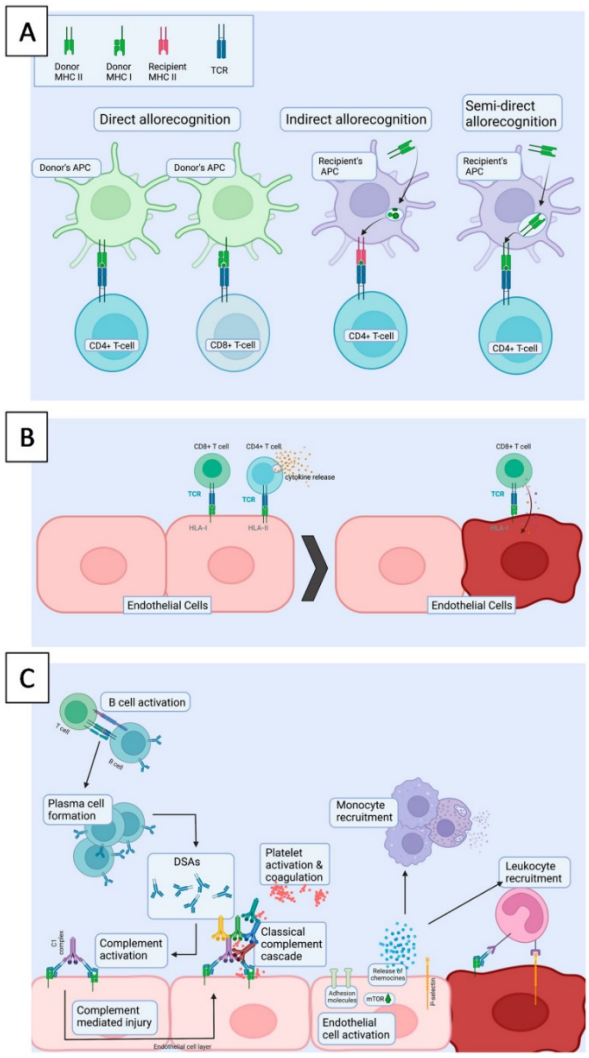


Fig. 19. (A) Different T cell allorecognition pathways. In direct allorecognition, donor derived APCs present donor allopeptides on a donor MHC to the recipient's T-cells, which leads to donor allorecognition. In indirect allorecognition, recipient derived APCs present a donor allopeptide on MHC molecule to the recipient's T-cell. In semi-direct allorecognition, recipient APC catches a donor MHC molecule, which is transported to the cell surface and presented to T-cells. (B). In cellular rejection, alloreactive cytotoxic CD8+ T cells have been activated in secondary lymphoid organs by activated antigen presenting cells either via direct or indirect allorecognition. Once they encounter cells presenting target antigens on HLA I molecule, the target cells, which are typically ECs, will be killed. (C). AMR is characterized by injury of the allograft endothelium and presents as microvascular inflammation. First, donor derived antigen is presented by APCs to CDA+ T-cells in the secondary lymphoid organ. Hence, CD4+ T cells activate B cells and the formation of plasma cells, producing donor specific antibodies (DSAa). Upon DSA (IgG) binding to target cells, which are typically ECs, the activation of complement cascade is triggered, leading to the activation of membrane attack complex. HLA binding activates intracellular signaling in ECs, e.g., via mTOR, which induces upregulation of adhesion molecules and further leukocyte recruitment. APC = Antigen presenting cell, TCR = T cell receptor, MHC = major histocompatibility complex, mTOR = mammalian target of rapamycin [25]

2 | Classification

In 1990, an international grading system for cardiac allograft biopsies was adopted by the International Society for Heart Transplantation [27]. This system has served the heart transplant community well, facilitating communication between transplant centers, especially with regard to patient management and research. In 2004, under the direction

of the International Society for Heart and Lung Transplantation (ISHLT), a multidisciplinary review of the cardiac biopsy grading system was undertaken to address challenges and inconsistencies in its use and to address recent advances in the knowledge of antibody-mediated rejection [29].

2.1 | Acute Cellular Rejection

The new classification system includes 0R (no rejection, no change from 1990), 1R (mild rejection, 1990 grades 1A, 1B and 2), 2R (moderate rejection, 1990 grade 3A), and 3R (severe rejection, 1990 grades 3B and 4). The new classification scheme is outlined in Table 1 [29].

2004		1990	
Grade 0 R[a]	No rejection	Grade 0	No rejection
Grade 1 R, mild	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage	Grade 1, mild A—Focal	Focal perivascular and/or interstitial infiltrate without myocyte damage
		B—Diffuse	Diffuse infiltrate without myocyte damage
		Grade 2 moderate (focal)	One focus of infiltrate with associated myocyte damage
Grade 2 R, moderate	Two or more foci of infiltrate with associated myocyte damage	Grade 3, moderate A—Focal	Multifocal infiltrate with myocyte damage
Grade 3 R, severe	Diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis	B—Diffuse	Diffuse infiltrate with myocyte damage
		Grade 4, severe	Diffuse, polymorphous infiltrate with extensive myocyte damage ± edema, ± hemorrhage + vasculitis

Table 1 ISHLT Standardized Cardiac Biopsy Grading: Acute Cellular Rejection
[a]Where "R" denotes revised grade to avoid confusion with 1990 scheme [29]

2.1.1 | 0R Rejection

In Grade 0 R there is no evidence of mononuclear (lymphocytes/macrophages) inflammation or myocyte damage (Fig. 20) [29].

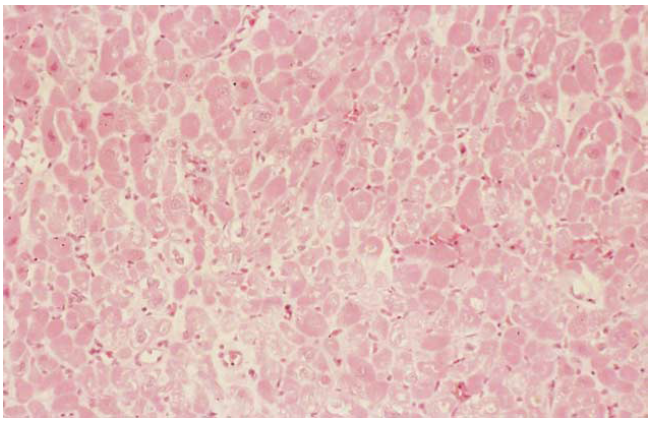


Fig. 20. Endocardial biopsy showing 0R rejection [29]

2.1.2 | 1R Rejection

On histology this appears as infiltration of perivascular and/or interstitial mononuclear cells without distortion of the normal architecture or as a single focus of mononuclear cells with associated myocyte damage (Fig. 21) [29].

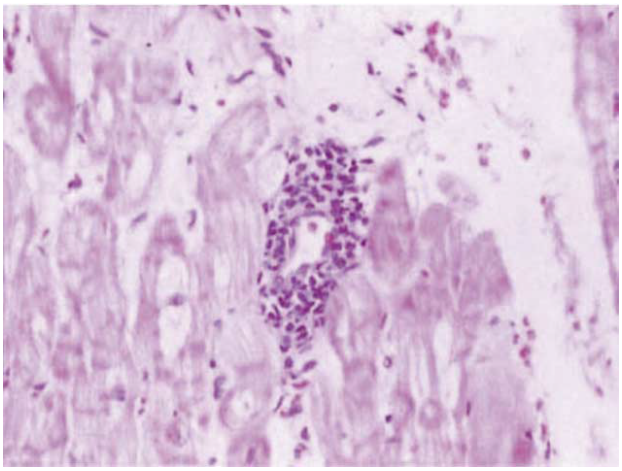


Fig. 21. High power view of the endocardial biopsy showing 1R rejection [29]

2.1.3 | 2R Rejection

In Grade 2 R two or more foci of mononuclear cells (lymphocytes/macrophages) with associated myocyte damage are present. Eosinophils may be present. The foci may be distributed in one or more than one biopsy fragment. Intervening areas of uninvolved myocardium are present between the foci of rejection (Fig. 22) [29].

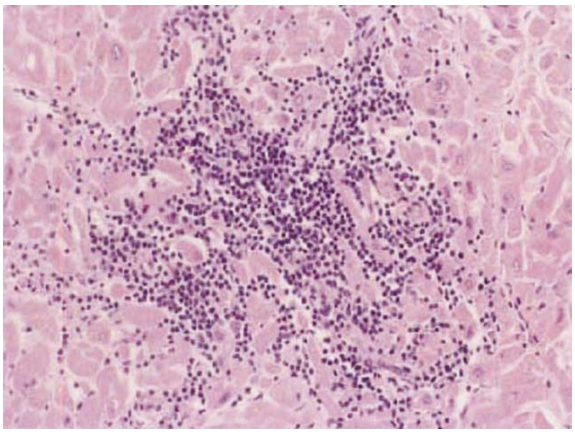


Fig. 22. High power view of the endocardial biopsy showing 2R rejection [29]

2.1.4 | 3R Rejection

Severe cellular rejection, also known as Grade 3R rejection (equivalent to 1990 Grade 3B and 4) is defined as a cardiac biopsy with diffuse cellular infiltrates and associated multifocal myocyte damage, and may include oedema, hemorrhage and/or vasculitis (Fig. 23) [29]. It is mostly seen with hemodynamic compromise, which can be defined as cardiac dysfunction necessitating new inotropic support, decreased cardiac index, elevated filling pressures or arrhythmias.

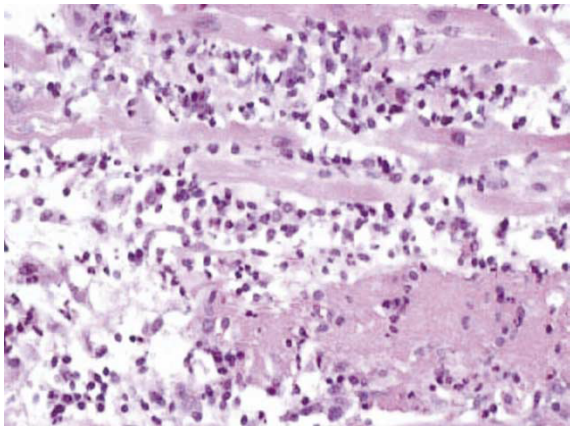


Fig. 23. High power view of the endocardial biopsy showing 3R rejection [29]

2.2 | Acute antibody-mediated rejection

Over the last two decades, acute antibody-mediated rejection (AMR) has been increasingly recognized as a separate clinical entity from acute cellular rejection (ACR), and a major contributor to graft loss after heart transplantation. Much work has been done on the pathological definitions of AMR and treatment methods are evolving [30].

Clinical outcomes after AMR in children have only recently been studied, and much is still unknown. A recent study from the Pediatric Heart Transplant Study (PHTS) group showed 16% mortality after developing AMR over a 5-year study period [31].

A study by Everitt et al [32] showed that paediatric recipients with severe AMR have worse cardiovascular outcomes (increased cardiovascular mortality and cardiac allograft vasculopathy - CAV) compared to those without AMR.

Younger age, congenital heart disease, homograft material, positive donor specific crossmatch, positive panel reactive antibody (PRA) titers, sensitization to OKT3, cytomegalovirus seropositivity, previous transplantation, blood transfusions, use of ventricular assist devices, presence of positive B-cell flow cytometry cross and female gender have been identified as risk factors for AMR [29, 31, 33].

The International Society of Heart and Lung Transplantation in 1990 defined AMR as the presence of antibody by immunofluorescence, vasculitis or severe interstitial oedema in the absence of cellular infiltrate in a heart biopsy specimen especially occurring during the first 6 weeks after transplantation [28].

The 2004 revision of the ISHLT cardiac biopsy grading system concluded that routine screening for immunopathologic evidence of AMR is not advocated, but if histological features suggestive of AMR were present, heart biopsy specimens then should be submitted for immunohistochemistry and serum should be tested for donor specific antibodies to confirm the diagnosis [29] (Table 2).

	2004	1990
AMR 0	Negative for acute antibody-mediated rejection No histologic or immunopathologic features of AMR	
AMR 1	Positive for AMR Histologic features of AMR Positive immunofluorescence or immunoperoxidase staining for AMR (positive CD68, C4d)	Humoral rejection (positive immunoflourescence, vasculitis or severe edema in absence of cellular infiltrate) recorded as additional required information

Table 2 ISHLT Recommendations for Acute Antibody-Mediated Rejection (AMR) [29]

In a companion paper published by the Immunopathology Task Force, a combination of clinical evidence of allograft dysfunction, histologic features, evidence of antibody or complement deposition in the cardiac allograft and antibody in the serum was recommended to make a diagnosis of AMR [34].

The histologic lesion of AMR is demonstrated by evidence of capillary injury. This is generally noted as endothelial cell swelling with nuclear enlargement and the presence of macrophages within the capillaries (Fig. 24). As the severity of AMR progresses,

intracapillary neutrophils, interstitial oedema with fibrin deposition, and hemorrhage may be seen, all in the absence of a significant lymphoid infiltrate [35].

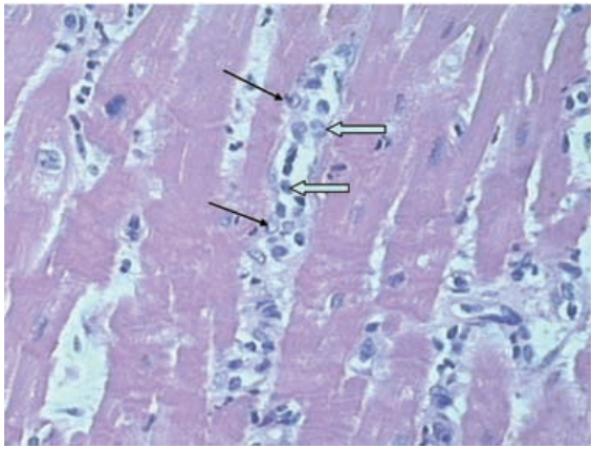


Fig. 24. Capillary endothelial swelling (black arrows) and leukocyte margination (green arrows) in antibody mediated rejection [35]

Immunohistological staining of frozen specimens demonstrating deposition of immunoglobulin (IgG or IgM), complement (C3/C4/C1q) and fibrin in an endovascular distribution has traditionally been used for confirmation (Fig. 25) [35].

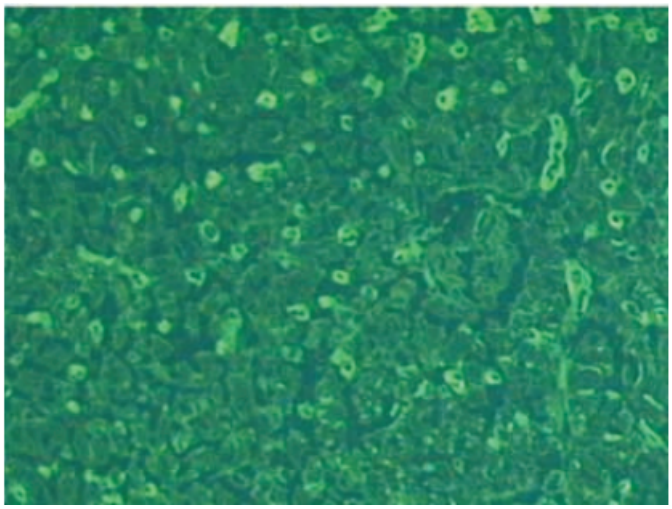


Fig. 25. Direct immunofluorescence using antibody directed against C4d complement fragment in antibody mediated rejection of a cardiac allograft. The immunolabeling delineates the capillary profiles [35]

3 | Multimodality detection of acute heart rejection

3.1 Invasive detection of acute heart rejection: endomyocardial biopsy

Endomyocardial biopsy (EMB) remains the gold standard for rejection surveillance in the heart transplant patient [36].

It has a high sensitivity and specificity for the diagnosis of acute cellular rejection [37,38]. Ideally, an initial biopsy of the donor heart should be obtained in the operating room at the time of transplantation. This biopsy can be valuable because it provides a means to assess the status of the donor myocardium for hypertrophy, ischemia, or the presence of any pathologic process such as myocarditis. The frequency of post-transplant surveillance biopsies varies highly between different institutions [39].

The early EMBs used the open approach with biopsy of the epicardial surface of the heart. These were followed by the use of the Vim-Silverman needle through a limited thoracotomy or transthoracic approach [40,41] (Fig. 26). They were associated with complications such as cardiac tamponade, and their use was somewhat restricted.

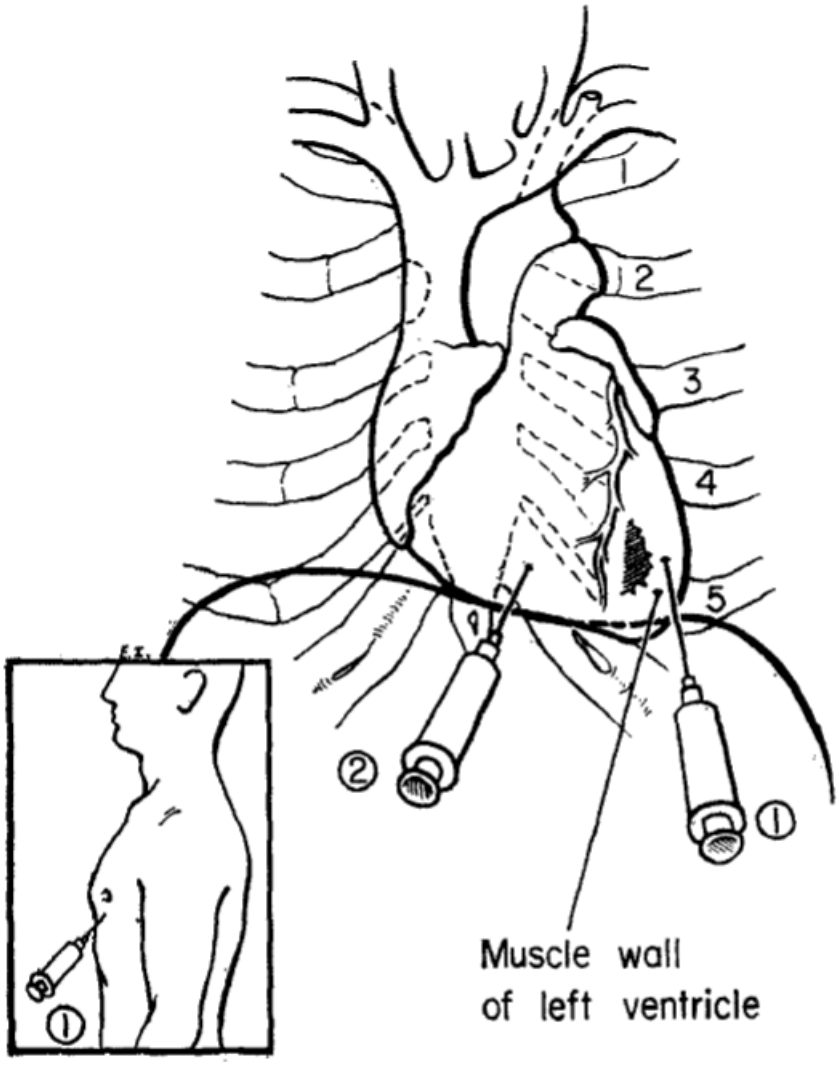


Fig. 26. 1. The needle was inserted into to the fourth or fifth left intercostal space, 10 to 15 mm, to the left of the palpable apex beat. 2. The needle was inserted at Larry's point and upward through the diaphragm [42]

In 1962, Sakakibara and Konno introduced a biopsy catheter or "bioptome" for the sampling of the endomyocardium through an endovascular approach rather than the previous trans-pericardial and transmural approach [40,43,44] (Fig. 27).

The benefit of this bioptome was the ability to obtain adequate EMB samples with fewer complications.

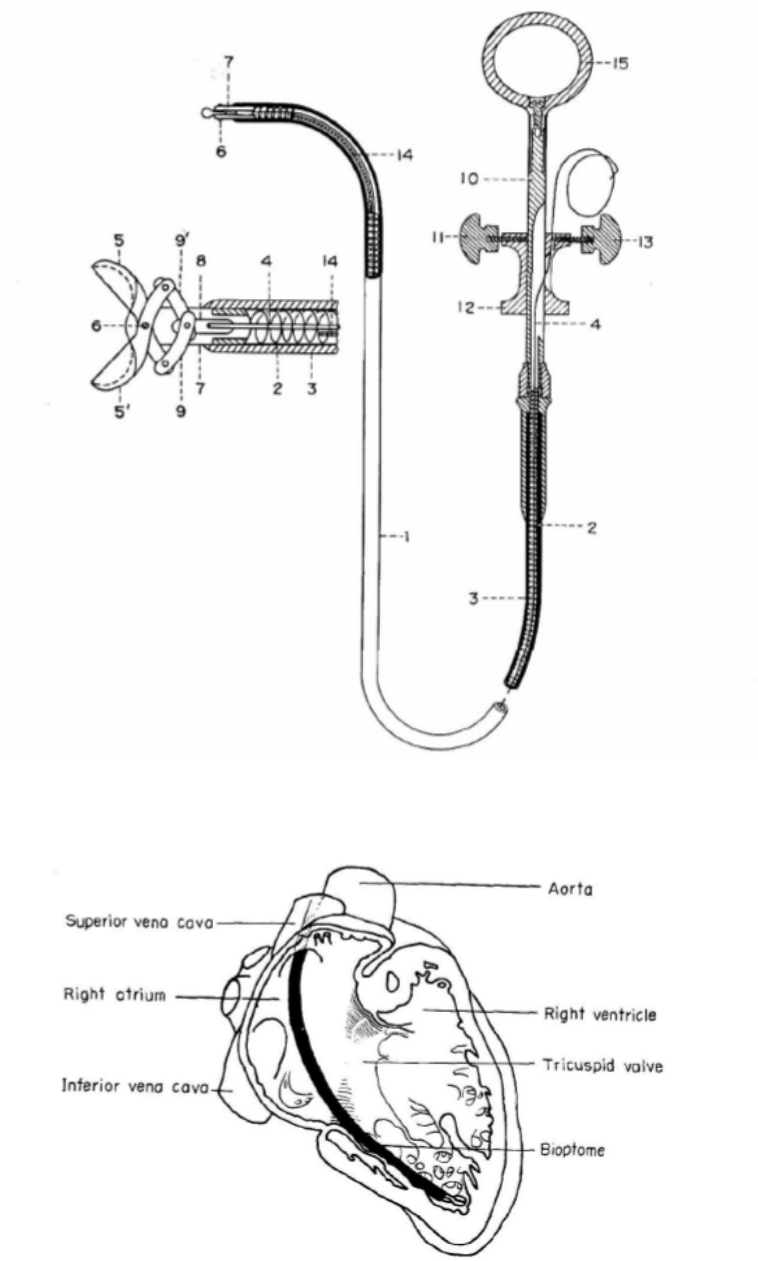


Fig. 27. Bioptome devised by Konno [43] and description of the way to nipp off the endocardium

The new transcatheter approach was followed by the introduction of the Stanford bioptome (Fig. 28) [44], both of which utilised the trans-vascular approach, under fluoroscopic control. While access initially was through arteries with biopsy of the left side of the interventricular septum, the technique soon evolved to a trans jugular approach through the neck veins, excluding the necessity for a "cut down". Today, virtually all biopsies are performed using this transvenous approach, via the neck; access through the jugular (or subclavian) vein gives good tissue samples. Today'catheters have been modified further so that the catheter lies inside a sheath, thereby allowing greater

flexibility and manoeuvrability in addition to the ability to perform repeated biopsies at the same sitting using the same venous puncture [45,46].

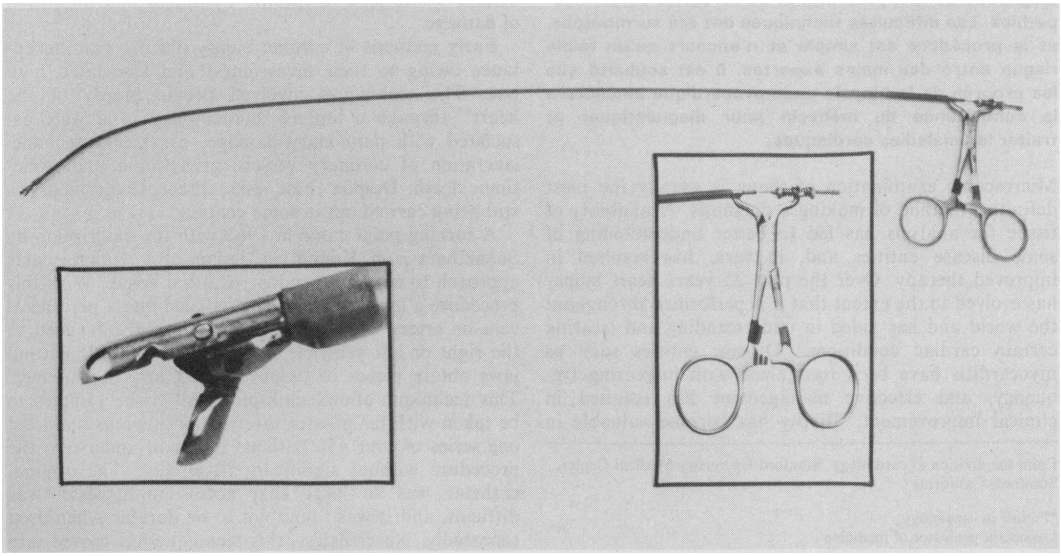


Fig. 28. Stanford right ventricle bioptome [44]

Serious adverse events have been described after EMB in children in both historical and modern publications, including pneumothorax, hemothorax, ventricular perforation, arrhythmia, tricuspid valve injury, and death [47-51].

Overall, however, cardiac catheterization with EMB is safe and well tolerated in paediatric HT recipients. A multicenter study from 2012 reported a 3% overall incidence of adverse events among paediatric HT recipients undergoing EMB and 1% incidence of high-severity adverse events [47].

Adverse events described in this study included tricuspid valve injury, transient complete heart block, arrhythmias, and right bundle branch block. There were no myocardial perforations or deaths reported in this study of 2665 EMB cases. The risks are slightly increased in infants but remain low. In their study of 43 EMBs performed in infant HT recipients, 5% (2/43) had complications: one arrhythmia requiring intervention and one pneumothorax requiring chest tube [48].

Loss of vascular access sites over time is another important consideration. Finally, there have been several described cases of paroxysmal complete atrioventricular block following routine cardiac catheterization in paediatric HT recipients in the absence of acute rejection, cardiac allograft vasculopathy (CAV) or underlying conduction system disease [52].

In addition to procedural complications, another important factor to consider is the risk of non-diagnostic EMB samples. Overall, 92-99% of paediatric EMBs yield adequate tissue for pathologic interpretation [47,53].

Longer time since transplant has been identified as an independent risk factor for non-diagnostic EMB, likely because of scar formation in the site of prior biopsies [47].

Additionally, there is also the potential for false negative results in the event of segmental inflammation, as even when multiple biopsies are taken only a very small portion of the total myocardium is sampled.

A study in the United States using the Pediatric Health Information System (PHIS) database demonstrated that the median cost of diagnostic catheterization after HT was over $ 8000 [54].

In the previously mentioned work by Duong et al [55], high-frequency centers had higher cumulative hospital-based costs in the first year post-HT ($390 315 vs $313 248) when compared with low-frequency RSB centers [23].

This suggests that increased surveillance EMB in the first year post-HT is associated with higher cost with no change in graft survival and CAV rates at medium-term follow-up.

Finally, additional negative consequences of frequent RSB include stress and time commitment for patients and parents. No study has directly evaluated the impact of high- versus low-frequency RSB schedules on quality of life (QOL). In a small 2007 study by Green et al. evaluating the key factors affecting the QOL of school-aged HT recipients, catheterizations and EMB were the second most common area of distress for patients [56].

EMB remains an imperfect gold standard, however, limited by its cost, procedural complication, interobserver variability, and sampling error. These limitations of EMB, combined with a persistently declining incidence of acute rejection, bring the current practice of an arbitrary frequency and duration of surveillance EMB into question [57].

3.2 Non-invasive detection of acute heart rejection

Since the beginning of the heart transplant era, an increasingly interest was devoted to finding a non-invasive test that can replace cardiac catheterization and EMB for routine rejection surveillance in paediatric heart transplant recipients.

a) Echocardiography

Most research has focused on standard echocardiographic approaches including two-dimensional (2D) imaging, M-mode imaging, Doppler and measures of systolic function.

Given its low cost, ease of access, and portable non-invasive nature, serial echocardiography undoubtedly continues to have a key role in post-transplant care. It is potentially a tool to assess for signs of rejection, including new cardiac systolic or diastolic dysfunction, increased echogenicity of the ventricular myocardium, increased wall thickness, new valvular insufficiency, and a new pericardial effusion. The reliability of echocardiography to detect asymptomatic rejection is debated; in fact, these changes may not be reliably present even in moderate cellular rejection [58,59].

However, because early changes in shortening fraction or ejection fraction can be correlated to cellular rejection, 2D transthoracic echocardiography remains routine in the early post-operative phase and at intervals during later follow-up.

Recently advanced echocardiographic techniques including Doppler tissue imaging (DTI), deformation imaging and three-dimensional (3D) echocardiography have been investigated.

Sehgal et al. [60] demonstrated the clinical utility of peak systolic strain for detecting acute allograft rejection in children. They reported significant decreases in peak systolic global longitudinal strain (GLS) (11.7% vs 14.6%), circumferential strain (14.4% vs 21.7%), and radial strain (18.3% vs 26.5%) during rejection. Mingo-Santos et al. [61] reported similar utility of deformation imaging in detecting acute rejection and suggested that systolic strain measurements may reduce the burden of repeated biopsy.

The European Association of Cardiovascular Imaging recommendations for the assessment and follow-up of patients after heart transplantation suggest GLS as a suitable parameter to diagnose subclinical allograft dysfunction and that GLS could be used in association with EMB to characterize an acute rejection or global dysfunction episode [62].

b) Electrocardiography

Although acute rejection can be accompanied by alterations of QRS complexes and QT intervals, there are limited data to support the role of electrocardiography (ECG) in screening for rejection in paediatric HT recipients. Due to its low sensitivity for detection of acute rejection, surface ECG does not allow for discrimination between patients with and without significant acute rejection [63-66].

One historical study found that a 10% decline in electrocardiographic voltage from baseline had low specificity (87%) and positive predictive value (51%) but high sensitivity (94%) and negative predictive value (99%) for rejection [67].

Signal-averaged electrocardiography (SAECG) can demonstrate slowly conducting myocardium with delayed depolarization, potentially identifying areas of diseases myocardium. In 2006. Horestein et al. [68] published their findings that SAECG was not able to differentiate between HT recipients with and without rejection.

c) Cardiac magnetic resonance

Cardiac magnetic resonance (CMR) has shown promise for characterization of oedema, fibrosis/scar, and myocardial perfusion reserve, as well as potential application for the detection of microvasculopathic changes in the transplanted heart. CMR has great appeal because of reliable whole-heart imaging throughout the cardiac cycle, excellent border definition allowing accurate measures of ventricular mass, volume, and left ventricle ejection fraction and tissue characterization superior to that obtained with echocardiography.

CMR offers a diagnostic advantage in its ability to characterise the entire myocardium for evidence of scar or oedema. This ability renders CMR an ideal candidate for non-invasive rejection surveillance [69]. Besides being a more comfortable process for patients, CMR is able to visualize the entire myocardium, unlike EMB, where samples may miss foci of rejection [70]. In addition, CMR may be repeated as often as desired without increasing the risk of malignancy because there is no radiation exposure [71].

CMR parametric mapping is widely regarded as the 4th era of myocardial CMR development, which include T1-, T2- and extracellular volume (ECV)-mapping.

Parametric magnetic resonance (MR) relaxometry mapping methods (such as T1- and T2-mapping) are quantitative magnetic resonance imaging (MRI) techniques that provide a pixel by-pixel representation of absolutely denominated numerical T1 or T2 properties, expressed in units of time (e.g. milliseconds). T1 and T2 relaxation times can be used to infer tissue type and composition in view of the surrounding environment. This is in contrast to conventional T1- or T2-weighted MRI methods, which rely on the relative image signal intensities to highlight and label areas deemed abnormal, compared to areas deemed normal. Traditional MRI is subject mainly to visual assessment, but allows semi-quantitative analysis in terms of signal intensity ratios or differences. In this regard, conventional MR images are not ideal to detect diffuse and homogenous disease presentations. The advantages of directly quantitative parametric mapping include that they can detect diffuse disease by comparing to previously established normal ranges.

Native T1-mapping refers to T1-mapping at rest and before the administration of any contrast or stress agents, including exercise stress. Native T1 represents a composite

signal from both the intracellular and extracellular compartments. Each tissue type has a specific normal range of T1 values, deviation from each may indicate disease or change in physiology. Native T1-mapping methods are characterized by a relatively narrow normal range of myocardial T1 with a small standard deviation [72,73] and have been demonstrated in multiple studies to be sensitive to changes in a wide range of common myocardial diseases [74,75].

Native T1 values increase with free water content in tissue, and T1-mapping is particularly useful for detecting acute myocardial inflammation/oedema [76-78] or in chronic pathologies in which the myocardium has an expanded interstitial space where free water can accumulate, such as in areas of chronic fibrosis [74,79].

Myocardial T1 is relatively long, and is potently shortened by administration of modest amounts of extravascular gadolinium-based contrast agent.

Late gadolinium enhancement (LGE) imaging uses intravenously administered gadolinium-based contrast agents to accentuate differences in tissue T1 relaxation characteristics. As a result of their molecular sizes, the gadolinium chelates used are interstitial agents that cannot penetrate healthy intact cell membranes. Therefore, they remain in the interstitial space and accumulate in areas of cell injury/necrosis and fibrosis where this is expanded, while in healthy regions, contrast more readily wash out [80]. Gadolinium is paramagnetic and shortens T1 in proportion to its concentration. Thus, if imaging is performed after an adequate interval to allow relative contrast wash-out from normal tissue (typically 10-15 min), areas of tissue injury with a higher gadolinium concentration will have a shorter T1 than surrounding healthy myocardium. This enhancement may reflect expansion of the extracellular space through myocyte necrosis (with loss of cell membrane integrity) and, in later phases, replacement fibrosis [81].

The post-contrast T1 measurement by itself (or isolated post-contrast T1) changes dynamically with time as gadolinium-based contrast agent is cleared from the body, and is affected by many other factors (such as renal function, age, hematocrit) to serve as a reliable biomarker.

This variability limits the clinical utility of post-contrast T1. From native and post contrast T1 data from planes that can be co-registered, using either the patient's hematocrit or a syntetic hematocrit, extracellular volume (ECV) can be calculated.

The myocardial ECV is estimated by measuring the pre- and post-contrast relaxivity changes (R1=1/T1) of myocardium and blood, adjusting the ratio by the known extracellular volume of blood (i.e. 1-Hematocrit) [82].

ECV of the myocardium is calculated as follows:

$$ECV = \frac{\Delta R1_{myo*(1-Hct)}}{\Delta R1_{blood}} = \frac{\left(\frac{1}{T1myo_{postGd}} - \frac{1}{T1myo_{native}}\right)}{\left(\frac{1}{T1blood_{postGd}} - \frac{1}{T1blood_{native}}\right)} x(1 - Hct)$$

As ECV is a ratio of native and post contrast T1 of blood and myocardium, it is independent of the many variables that affect post contrast T1, and thus can be used across institutions for multi-center study of myocardial disease. ECV is an approximation of extracellular matrix (ECM) expansion. The ECM may be expanded by infiltrative processes (e.g. amyloid, sarcoid) and by microfibrosis associated with ischemia and infarct. Increases in myocardial free water content, as occurs in acute myocardial oedema and inflammation, also prolong T1 and T2 relaxation times [74,77,83].

In addition, quantitative T2 mapping can uniquely assign a number to the degree of myocardial oedema. The T2 relaxation time is known to be prolonged in states of increased myocardial oedema [84,85] such as in acute allograft rejection [70,86]. This has been extensively studied in adult heart transplant patients [69,86,87,88] but to date there is limited data on its application in the paediatric population.

Thus, T1, T2 and ECV mapping may be useful for characterizing the transplanted myocardium, which may be subject to inflammation from rejection and from fibrosis from developing coronary vasculopathy (Fig. 29).

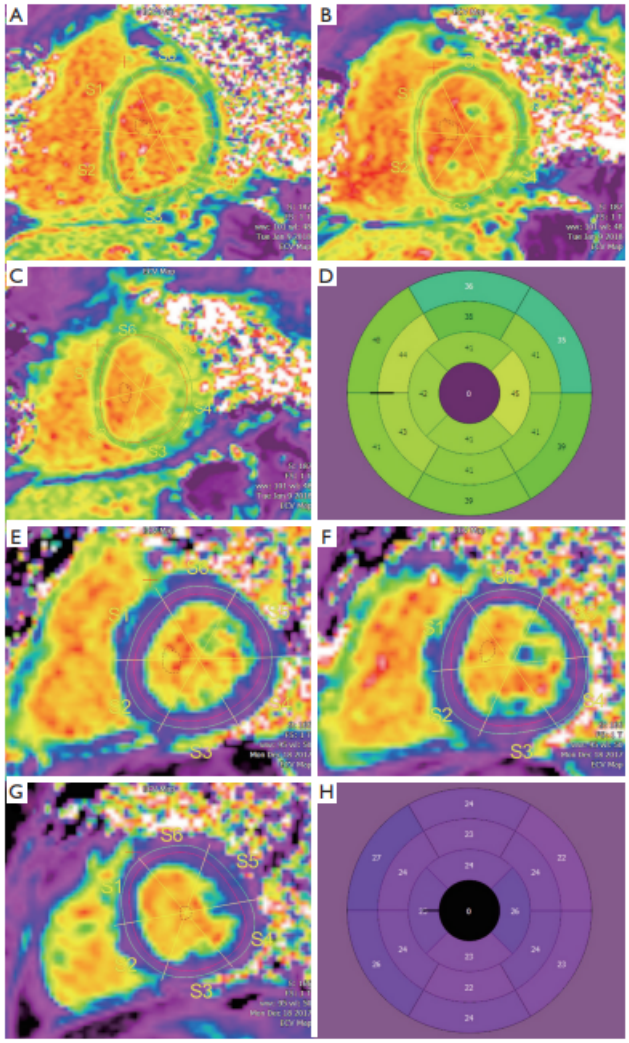


Fig. 29. Extracellular volume (ECV maps in the short axis at the base (A), mid left ventricle (B), and apex (C) in a paediatric patient with acute rejection. Bullseye (D) demonstrates significant ECV elevation. ECV maps at the base (E), mid left ventricle (F), and apex (G) in a paediatric transplant patient who presented for routine endomyocardial biopsy without evidence of rejection. Bullseye (H) demonstrates ECV values in the normal range [89]

Strain analysis by CMR has also been used in patients after heart transplant. Of note, the temporal resolution of echo strain analysis may be higher than that achieved by CMR, which could affect results [90]. Initial paediatric heart transplant CMR studies using grid tags demonstrated abnormalities in twist in heart transplant patients as compared to controls [91]. A recent analysis by Latus [92] using feature tracking demonstrated findings similar to echo, with decrements in GLS and maintained GCS and global radial strain. Miller et al. [93] evaluated adult heart transplant patients early post-transplant (within 6 months) and found a significant difference between circumferential strain in patients with and without acute cellular rejection but also noted significant overlap between the two patient populations. The poor discrimination may have been partly related to the early time period after transplant.

d) Donor-specific antibodies

Defining positive donor-specific antibodies (DSA) as a mean fluorescent intensity (MFI) of ≥ 2000 using single antigen bead testing, Ware et al. demonstrated that DSA had a sensitivity of 93% and NPV of 98,5% for antibody-mediated rejection (AMR) (though lower specificity and PPV at 62% and 24%, respectively) [94]. These findings support that DSA testing can help in the non-invasive prediction of AMR absence in paediatric heart transplantation.

There is strong evidence in the adult heart transplant literature that de novo DSA production is associated with poor patient survival, with worse survival particularly described for persistent DSA and for class II DQ-specific DSA [95-97]. The 15-year survival appears to be highest in patients who never develop DSA compared to those that develop de novo DSA post-transplant (70% vs 47%) [97].

There is also emerging paediatric evidence that de novo DSA has a strong impact on CAV, rejection, and graft survival. In a retrospective cohort of 105 paediatric patients with negative T-cell and B-cell crossmatches, 43% (45/105) patients developed de novo DSA. Compared with DSA-negative patients, DSA-positive patients had significantly higher rates of CAV (36% vs 13%), 2,5 times more rejection events per year, and significantly worse 5-year survival (21% vs 72%) [98].

e) B-type natriuretic peptide/NT-proBNP

A hormone released by myocardial cells in response to volume expansion and increased wall stress, BNP/NT-proBNP has been explored as a potential biomarker to predict rejection. It is a sensitive marker for rejection in small studies. Lindblade et al. [99] evaluated 211 consecutive BNP measurements in 59 paediatric HT recipients along with EMB samples. Patients with rejection had significantly higher BNP levels than those with a negative biopsy. A retrospective single-center study by Knecht et al. [100] found significant inter-subject variability in NT-proBNP levels and reported that increases in a patient's NT-proBNP level were predictive of rejection, with greater increases being associated with greater risk. This study supports the notion that serial measurements of BNP/NT-proBNP may be more useful than isolated measurements, and that changes from a patient's baseline may be more predictive of rejection than specific universal cutoffs. Conversely, Hall et al. found no significant difference in BNP based on grade of cellular rejection in 62 paediatric HT recipients [101].

Whether it is specific enough, however, for a certain threshold value to trigger the need for EMB, especially with significant interpatient variability, remains unclear. Trends over time are likely most beneficial, leading many paediatric heart transplant centers to incorporate it into their surveillance protocols [102]. Of additional importance, BNP needs to be interpreted with caution in the first year post-HT as it is typically elevated immediately post-transplant and decreases over several months before reaching a nadir [101,103,104].

f) Troponin

Cardiac troponin T and I are serologic markers of myocyte damage. Dyer et al. [105] performed a small study in which biomarker data (NT-proBNP and high sensitivity cardiac troponin T) at the time of EMB was reviewed. There were 7 episodes of rejection over 53 EMB; biopsies with acute rejection (grade $\geq$ 2R) were associated with higher troponin T and NT-proBNP. Conversely, Moran et al. [106] retrospectively compared histologic rejection grades with biochemical markers in 37 patients and found poor concordance between serum markers (troponin T, troponin I, and creatinine kinase-MB fraction) and rejection. The data are too conflicting at this time to recommend troponin as screening tool for rejection in paediatric HT recipients.

g) Gene expression profiling scores: AlloMap®

AlloMap® (CareDx, Inc.) is a test that performs gene expression profiling in 20 genes in peripheral blood mononuclear cells to detect rejection in transplant recipients. It has been approved for use as a non-invasive screening tool for HT recipients 15 years of age and older [107-108]. It has been shown to be non-inferior to RSB for rejection surveillance in low-risk adult HT recipients with respect to clinical outcomes in a large randomized controlled trial [109]. However, the data on AlloMap® in paediatric HT recipients are sparse [110-112].

h) Circulating donor-derived cell-free DNA

Donor organ injury, such as from rejection, can lead to an increased release of donor DNA in the recipient plasma; cell-free DNA can be extracted from the plasma and the fraction of donor-derived cell-free DNA (dd-cfDNA) to recipient-derived cell-free DNA can be determined. Evidence is emerging to support quantification of circulating dd-cfDNA as a contender in the market of non-invasive markers of rejection in paediatric HT recipients [113-115].

INTRODUCTION

Heart transplantation is the therapy of choice for paediatric end-stage heart failure to improve survival and quality of life. Survival after cardiac transplantation is linked to the occurrence of complications, especially acute rejection [116,117].

The diagnosis of acute rejection in cardiac transplant recipients requires invasive technique with endomyocardial biopsy (EMB) which has risks and limitations [118,119].

Cardiovascular magnetic resonance (CMR) is the gold standard imaging modality for assessing cardiac morphology, ventricular volumes, systolic function and myocardial mass [120]. In addition, CMR allows for assessing the activity of inflammatory changes using markers for myocardial oedema, hyperaemia, capillary leak and irreversible injury applying a combination of non-contrast T2 weighted imaging and more recently parametric mapping techniques (T1 and T2 mapping) and gadolinium enhanced technique [121-123].

Native T1 values are higher with increased extracellular compartment by fibrosis [123] and oedema [124]. From native and post-contrast T1, it can calculate extracellular volume fraction (ECV) which represents the interstitial volume [125]. Expansion of interstitial volume occurs with diffuse fibrosis, oedema and infiltrative diseases [126].

T1 and T2 mapping sequences accurately diagnoses interstitial oedema and extracellular space expansion and can potentially detect acute allograft rejection.

In paediatric heart transplantation, few studies have assessed these mapping techniques [127-130].

There is a clear need for a non-invasive and accurate method of detecting acute allograft rejection and late graft disfunction in paediatric heart transplant patients. The overall aim of this study was to determine the utility of multiparametric CMR in identifying acute graft rejection in paediatric heart transplant recipients. In secondary analysis, we aimed to compare textural features of parametric maps in cases of rejection versus those without rejection.

MATERIAL AND METHODS

Population and study design

This single center, prospective, cross-sectional study was approved by the Institutional Research Ethics Board (Study Code: 460/2023/Oss/AOUBo) and included children and young adults (transplanted when they were ≤ 18 years) who underwent an EMB for routine surveillance between February 2022 and May 2023. All consecutive and eligible patients without contraindications to contrast-enhanced CMR during the study period

were enrolled. Following written informed consent, CMR was performed at least two days before cardiac catheterization and EMB. We also did not recruit recipients who were < 3 months post-heart transplantation to reduce the possibility of confounding from ischemia-reperfusion injury that occurs with the heart transplant procedure.

Patient charts were reviewed for data demographics, transplant history (age of the organ donor, time from transplant, ischemia time of the donor heart, immunosuppressive medications) and rejection history (number and severity of all previous episodes of rejection since heart transplant). Hemodynamic measurements from echocardiography (left ventricular -LV- ejection fraction, mitral E/e'), catheterization (right atrial mean pressure, right ventricle -RV- systolic pressure, RV end diastolic pressure -RVEDP-, main pulmonary artery mean pressure, and pulmonary capillary wedge pressure) and CMR (LV and RV end diastolic volume and global longitudinal and circumferential strain) were also included. Hemodynamic measurements listed above were considered markers of graft dysfunction.

Endomyocardial biopsy and right catheterization

RV septal EMB and right catheterization were performed by experienced clinicians via jugular vein. At least four tissue samples were obtained, stained with hematoxylin and eosin and evaluated using light microscopy. Evidence of cellular rejection and antibody mediated rejection on EMB were graded based on the International Society of Heart and Lung Transplantation (ISHLT) guidelines [28] by a hospital pathologist following standard clinical practises. Clinically, rejection was defined based on treatment plan created by transplant team following EMB procedure. At our institution, those cases with Grade 2 o above acute cellular rejection on biopsy received new rejection treatment including modification to immunosuppressive therapy, intravenous steroids, thymoglobulin, etc. Those cases with Grade 0 or 1 acute cellular rejection on biopsy did not receive any additional treatment.

Cases were divided into two groups: Group A included cases with no rejection and no changes made to their treatment regimen (grade 0 and 1); Group B included cases with Grade 2 o above acute cellular rejection.

Tissue samples were also evaluated for the presence of antibody-mediated rejection (AMR).

CMR acquisition and image analysis

CMR was performed using 1,5 Tesla scanner (Philips Ingenia). Images were analysed by an expert radiologist blinded to patient's clinical data and histology results.

Ventricular volumetry and late gadolinium enhancement

A stack of multiphase short axis slices was acquired using the steady state free precession technique for left and right ventricular volumes. Ventricular volumes were extracted from the cine short axis stack in end-diastole and end-systole in the routine clinical fashion using commercially available software (CVI 42). Ventricular volumes were reported as indexed to recipient body surface area. Ejection fraction for both ventricles were calculated using end-diastolic and end-systolic volumes. The presence of late gadolinium enhancement (LGE) was determined qualitatively on standard long-axis (4-chamber, 2-chamber and 3-chamber) and short-axis slices using phase-sensitive inversion-recovery acquisition (PSIR) > 7 min after the administration of 0,1 ml/kg gadobutrol (Gadovist®, Bayer Spa).

T1 mapping and extracellular volumes

A modified Look-Locker inversion recovery sequence (MOLLI) was used to measure native and post-contrast longitudinal relaxation T1 times of myocardium and blood. Images were acquired in diastole at a basal, mid-ventricular and apical level short axis slices orientation before and > 15 min after administration of contrast. Breathholds were used in cooperative patients and all other patients were scanned during free breathing. Longitudinal relaxation times (T1 times) were measured using commercial available software (CVI42 and Portal Philips). Contours were drawn in the interventricular septum, the left ventricular (LV) free wall and in a region encompassing the entire LV myocardium. T1 times in the blood pool were measured in the LV cavity avoiding papillary muscle. The ECV was calculated using pre- and post-gadolinium T1 times of blood and myocardium as well as the patient's hematocrit, obtained immediately before the scan. Myocardial T1-values were determined according to the American Heart Association 17-segment model and drawning ROIs in the septum [74].

T2 mapping

A modified GraSE sequence allowing for myocardial T2 mapping in a single breath-hold per slice using ECG-triggered acquisition of a black blood multi-echo series were acquired in diastole at a basal, mid-ventricular and apical level short axis slices orientation.

Transverse relaxation times (T2 times) were measured using commercial available software (CVI42 and Portal Philips) using the same method of contouring and ROIs of T1 mapping.

Statistical analysis

Categorical variables are presented as count (percentage) and continuos variables as mean ± standard deviation.

CMR data were stratified according to the presence (grade ≥ 2) or absence (grade 0 or 1) of significant acute cellular rejection on EMB.

In order to identify potential differences between cases with and without acute allograft rejection, the two groups were compared using t-student .

Pearson correlation was performed on T1, T2 and ECV values against markers of graft dysfunction, which included hemodynamic data from echocardiography, catheterization and CMR.

A p-value < 0.05 was considered statistically significant.

All statistical analyses were performed using SPSS, 25 version (IBM SPSS Statistics 25).

RESULTS

- *Study population*

15 patients (16,9 ± 5,1 years, 73% male) underwent study procedures for surveillance (47%) and follow-up of prior rejection (53%). Ten cases (66%) were in Group A (no rejection, no therapy changes), and 5 cases (34%) in Group B (rejection, therapy changes). Cardiac allograft age ranged from 4,5 months to 16,8 years at the time of the study. Average total ischemic time during the transplant surgery was 3 hours and 49 minutes ± 52 minutes (Group A 3:42 ± 0:35 hh:min versus Group B 4:02 ± 1:20 hh:min, p = 0.43).

3 patients were transplanted for critical congenital heart disease (one with Tetralogy of Fallot and two with Hypoplastic left heart syndrome).

12 patients were transplanted for primary or secondary cardiomyopathies: dilated cardiomyopathy (n = 8), hypertrophic cardiomyopathy (n = 1), restrictive cardiomyopathy (n = 2), anthracycline-induced cardiomyopathy (n = 1).

All patients were on a stable immunosuppressive regimen: 33.3% patients on ciclosporine plus mofetil micofenolate, 6.7% on tacrolimus, 6.7% on tacrolimus plus everolimus and 53.3% on tacrolimus plus mofetil micofenolate.

8 patients had a prior history of biopsy-proven rejection: four cases with grade 3, one with grade 2 and three with grade 1. One of the grade 3 patients had a history of significant non-adherence to immunosuppressive medications: this patient had five previous episodes of rejection including three times of grade 3.

46.7% of the cohort have received mechanical circulatory support as bridge-to-transplant for an average time of 254 ± 236 days. Patients with acute cellular rejection received mechanical circulatory support for a longer period time (Group B 340 days versus Group A 196 days, p = 0.41). A higher percentage of cases in Group B, compared to Group A, had positive panel reactive antibody (20% vs 10%, p = 0.57).

Echocardiographic data were comparable in the two groups.

Table 3 summarizes the demographic features of the population.

Demographic	All cases	Group A	Group B	P value
All cases	n=15	n=10 (66%)	n=5 (34%)	
Male	73%	70%	80%	0.57
Mean age at HT (years)	11.8 ± 5.6	12.2± 4.9	11.1 ± 7.2	0.12
Mean age at CMR (years)	16.9 ± 5.1	17.49±4,88	15.91± 4.88	0.59
Mean graft age at CMR (years)	5 ± 4.5	5.2 ± 3.3	4.5 ± 6.9	0.08
Mean donor age (years)	11.8 ± 5.6	12.4 ± 5.2	10.5 ± 6.9	0.35
Graft ischemic time at HT (hh:min)	3:49 ± 0:52	3:42 ± 0:35	4:02 ± 1:20	0.43
Number of prior rejection episodes	12	9	3	0.48
Number of prior EMB procedures	n=28	n=20	n=8	0.55
Positive panel reactive antibody	13.3%	10%	20%	0.57
Mechanical circulatory support as bridge-to HT	46.7%	26%	20%	0.42
Mechanical circulatory support (days)	254 ± 236	196 ± 105	340 ± 356	0.41
Initial cardiac diagnosis • **CHD** • **CMP**	 20% 80%	 20% 80%	 20% 80%	0.75
Echo LV ejection fraction (%)	64.5 ± 3.3	64.6 ± 3.2	64.4 ± 3.9	0.91
Mitral E/e'	7.5 ± 1.4	7.6 ±1.2	7.2 ± 1.8	0.56

Table 3 Patient cohort: demographics

- *Endomyocardial biopsy and right catheterization*

All endomyocardial biopsy samples were considered adequate and underwent pathology review. 13 endomyocardial biopsy (86%) were performed on general anaesthesia, 3 of which with endotracheal intubation. All patients were exposed to X-rays during biopsy for an average time of 5 min and 31 seconds ± 5 min and 38 seconds (range 1-22 min and 27 seconds). About complications (20% of cases), one patient developed pericardial effusion, one mild tricuspidal regurgitation and one patient presented experienced respiratory distress upon waking with need for steroid therapy. There were 10 cases with grade 0-1, 3 cases with grade 2 and 2 cases with grade 3. There were no cases of antibody mediated rejection.

Right atrium mean pressure and right ventricle end-diastolic pressure were higher in Group B than Group A (5 ±2.1 mmHg vs 3.6 ±1.3 mmHg, p=0.14 and 7.6 ± 4.3 mmHg vs 4.7 ±1.7 mmHg, p=0.08, respectively).

Table 4 summarizes endomyocardial biopsy and hemodynamic results.

	All cases	Group A	Group B	P value
Complications: ○ **Pericardial effusion** ○ **Tricuspidal regurgitation** ○ **Respiratory distress**	n = 3 n =1 n=1 n=1	 n=0 n=1 n=1	 n=1 n=0 n=0	
Cath RA mean pressure (mmHg)	4.1±1.7	3.6±1.3	5 ±2.1	0.14
Cath RV systolic (mmHg)	26.3±6.2	26.3±7.2	26.4±4.1	0.97
Cath RVEDP (mmHg)	5.6±3.0	4.7 ±1.7	7.6 ±4.3	0.08
Cath average PCWP (mmHg)	7.9±13.4	7.1±14.3	3.9±17.2	0.97
Cath Cardiac Output (l/min)	4.5± 5.7	4.2 ±6.0	4.1± 6.0	0.94

Table 4. Endomyocardial biopsy and hemodynamic results

- *Cardiac magnetic resonance results*

Ventricular volumetry and late gadolinium enhancement

Table 5 displays the averaged findings of ventricular systolic function on cardiac magnetic resonance. There were no patient limitations to image acquisition, and all cardiac magnetic resonance exams were analysed. In two cases (a 6-year-old and an 8-year-old children) the exam was performed under deep sedation. The cohort was generally healthy from a cardiovascular perspective with normal filling pressures, cardiac index, ejection fraction and other routine CMR parameters. Although there is no statistically significant difference in cardiac magnetic resonance parameters in two groups, biventricular end-diastolic volumes were higher in patients with rejection.

Late gadolinium enhancement (LGE) was not observed in patients.

There were no adverse events.

CMR ventricular function	All cases	Group A	Group B	P value
Heart frequency (bpm)	88 ± 12	86 ± 12	91± 13	0.44
LV end-diastolic volume, ml/m^2 (SD)	77.8 ±14.3	76.9 ±16.9	79.6 ±18.6	0.74
LV stroke volume, ml/m^2 (SD)	38.9 ± 8.7	37.5 ±8.3	41.8± 9.9	0.54
LV ejection fraction, % (SD)	59.4 ±4.7	59.1± 4.9	60 ±4.8	0.74
LV myocardial mass index, gm/m^2 (SD)	51.7 ±6.9	52.1 ±7.5	50.8± 6.3	0.74
GCS	-19.4 ±-18	-19.3± –17.5	-20.9 ± -17.8	0.17
GLS	-17.6 ±-16.1	-17.1± -15.6	-18.8 ±-16.1	0.08
RV end-diastolic volume, ml/m^2 (SD)	73.8 ±14.6	71 ±12.3	79.6 ±18.6	0.3
RV stroke volume, ml/m^2 (SD)	38.93 ±8.7	37.5 ±8.3	41.8 ±9.9	0.39
RV ejection fraction, % (SD)	50.1 ±56.1	49.1 ±57.4	46 ±59.5	0.87

Table 5. CMR results

T1 mapping and ECV results

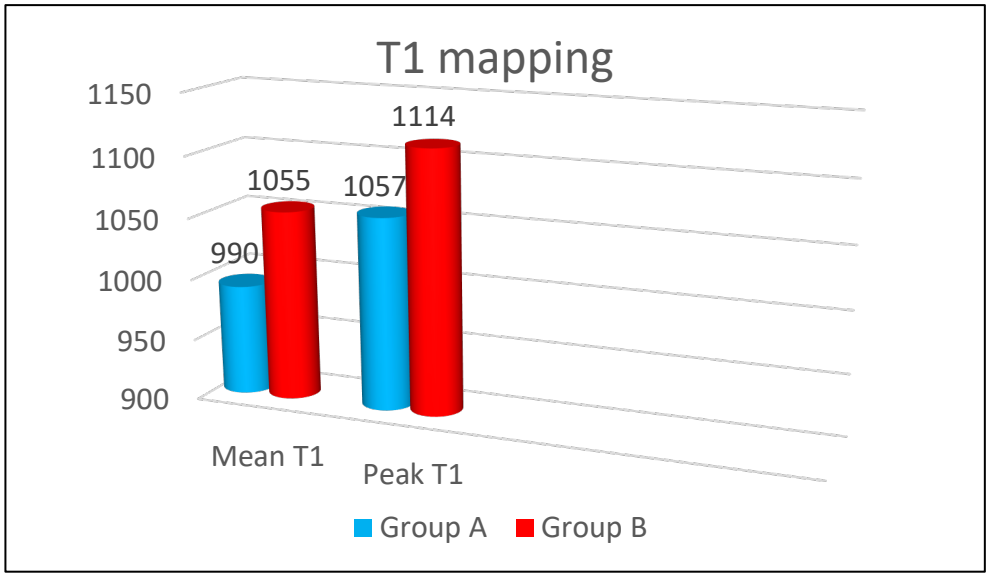
All T1 parametric maps were able to be analysed (Table 6).

There is significant difference in the mean T1 values in cases with and without rejection, 1055.6 ± 26.9 ms versus 990 ± 54.7 ms (p =0.02).

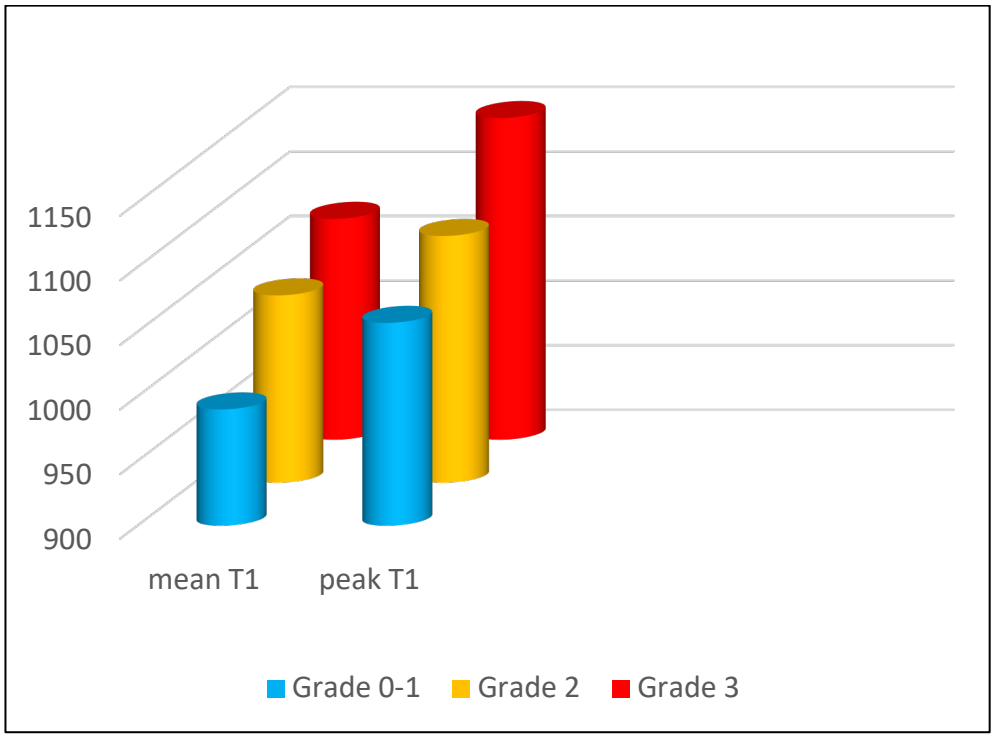
CMR parameter	All cases	Group A	Group B	P value
Peak T1 mapping (ms)	1076.2 ± 63.9	1057.1 ± 64.6	1114.4 ± 46.3	0.1
Mean T1 mapping (ms)	1012 ± 56.1	990.2 ± 54.7	1055.6 ± 26.9	0.02
Hematocrit (%)	37.1 ±7.1	37.3 ±7.1	36.6 ± 7.9	0.86
ECV (%)	29.6 ± 5.4	27 ±3.2	34.8 ± 5.5	0.04

Table 6. T1 mapping and ECV results

A monotonic, increasing trend was noted in both mean and peak T1 values, with increasing degree of rejection: mean T1 grade 0-1 990 ± 57.2 ms; grade 2 1045 ± 10 ms; grade 3 1071 ± 43.8 ms; peak T1 grade 0-1 1057 ± 64.6 ms; grade 2 1091 ± 27.4 ms; grade 3 1149 ± 54.4 ms (Fig. 30 a-b).



a)



b)

Fig. 30 T1 values for Group A and Group B (a) and for grade biopsy categories (b)

Studies in cases with a history of a prior rejection (n=8) had higher T1 values than cases with no prior rejection (n=7), peak T1 1085 ± 50.8 ms compared to 923 ± 390.4 ms (p=0.26) and mean T1 1031 ± 28.8 ms versus 998.2 ± 71.8 ms (p=0.25) (Fig. 31).

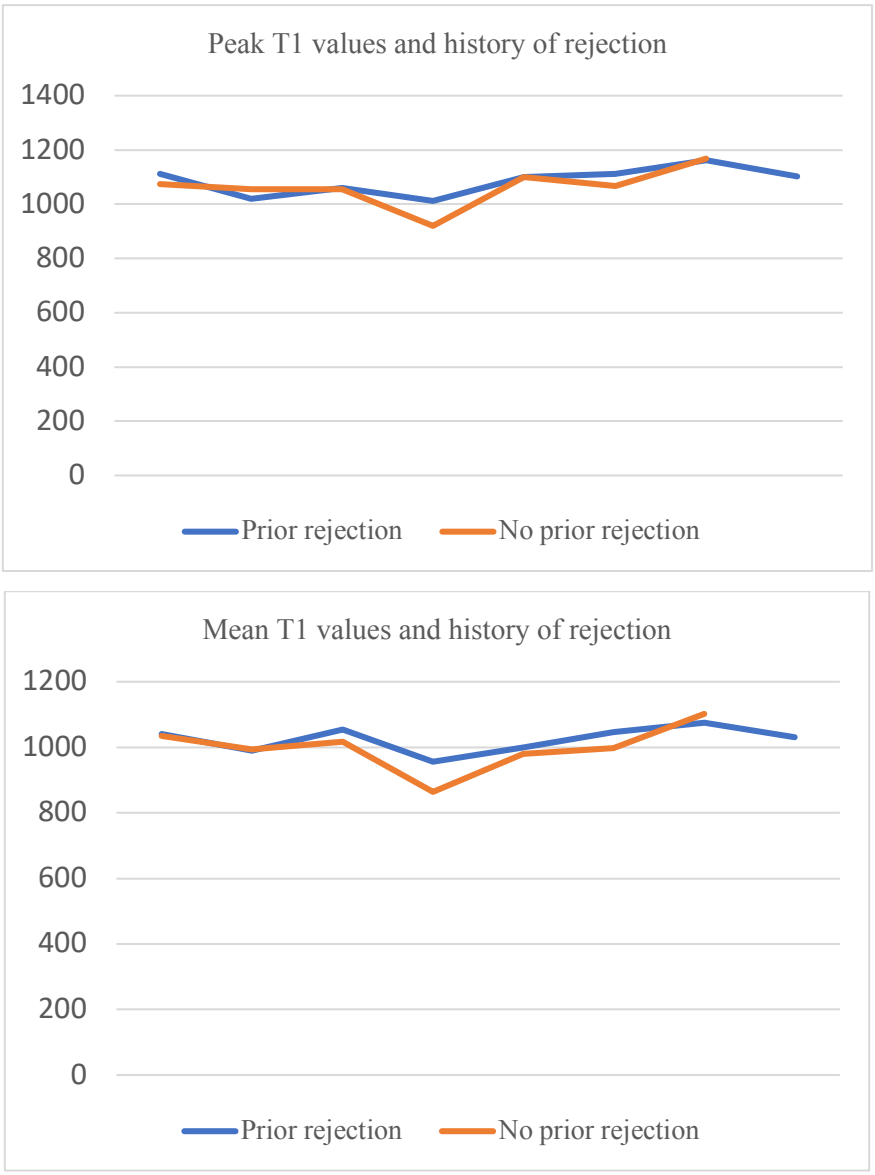
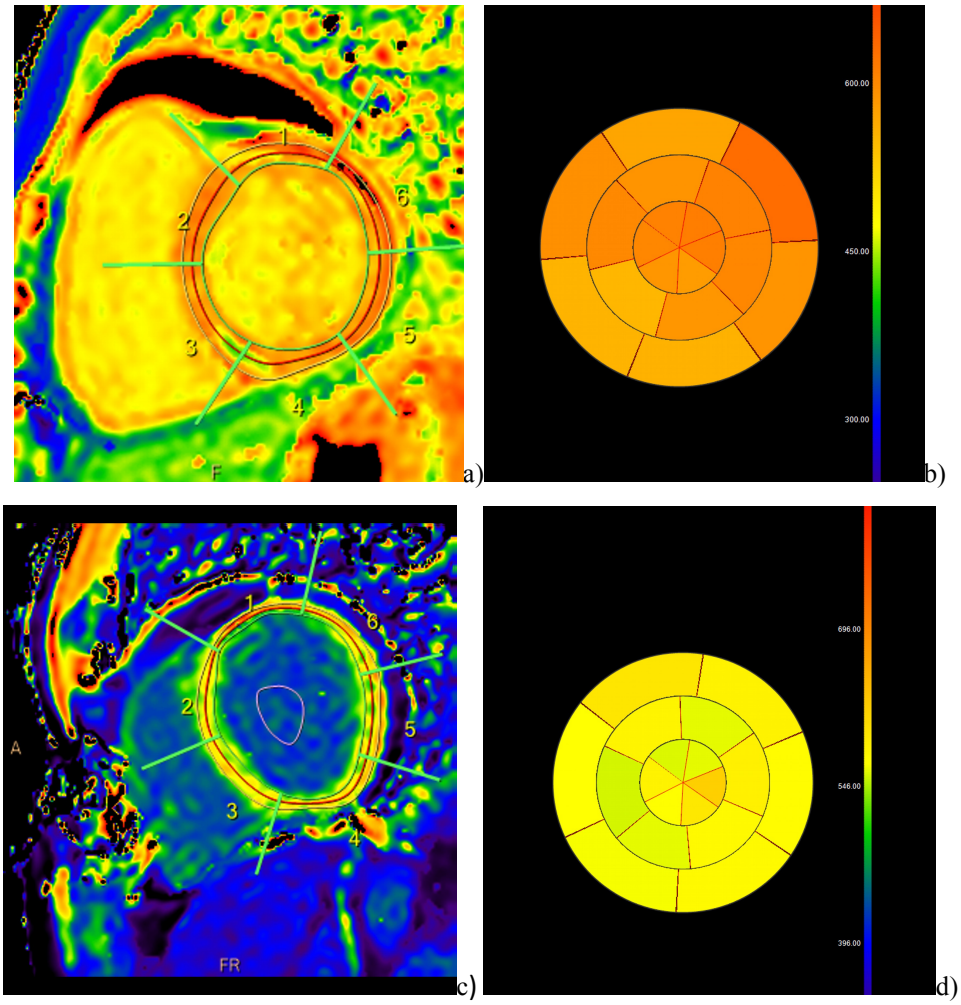


Figure.31 T1 values based on history of previous myocardial rejection

T1 values did not correlate with markers of graft disfunction.

ECV was significantly higher in patients with acute rejection (Group B 34.8 ±5.5% vs 27 ±3.2% Group A, p=0.04).

Fig.32 shows the difference in ECV values between patients with and without rejection.



a) b) c) d)

Fig. 32. Extracellular volume (ECV map in the short axis - a) in a patient with acute rejection.Bullseye (b) demonstrates significant ECV elevation. ECV map in a patient without rejection (c). Bullseye (d) shows normal ECV values

T2 mapping results

All T2 parametric maps were able to be analysed.

Although myocardial peak T2 value was higher in patients with a history of acute cellular rejection (55.6 ± 3.7 ms compared to 54.35 ± 3.5 ms, p=0.4), there was no difference in T2 signal between patients with and without active acute cellular rejection (mean T2 51.4 ±3.2 vs 51.1 ±2.8, p=0.85; peak T2 53.7 ±1.7 vs 55.5± 4.2, p=0.44).

Table 7 summarizes the results of T2 mapping.

CMR parameter	All cases	Group A	Group B	P value
Peak T2 mapping, (ms)	55 ±3.7	55.5± 4.2	53.75 ±1.7	0.44
Mean T2 mapping (ms)	51.2 ±2.8	51.1 ±2.8	51.4 ±3.2	0.85

Table 7. T2 mapping results

T2 values demonstrated moderate correlation only with RA mean pressure (r = 0.56, p=0,03).

DISCUSSION

Our study demonstrates the possible application of cardiac magnetic resonance-based quantitative T1, T2 mapping and ECV in acute cellular rejection detection in paediatric heart transplant recipients.

CMR imaging–based myocardial tissue characterization with T1 and T2 mapping has emerged as a non-invasive and highly sensitive method of detecting cardiac allograft rejection, with numerous studies demonstrating good correlation between CMR-based mapping and histopathology-determined rejection in adult patients [86, 88, 93, 131-136].

Surveillance EMB is important for cardiac rejection surveillance in paediatric populations because signs of allograft rejection may be more difficult to appreciate in this cohort [38].

Moreover, EMB is often performed under general anaesthesia in children, which adds to procedural risk and invasiveness and requires the use of X-rays.

Myocardial tissue characterization by CMR is feasible and informative in the paediatric setting [130,137].

However, there are few and conflicting paediatric studies in Literature.

Richmann et al. [128] demonstrated an increasing trend in both mean and peak T1 values with increasing degree of rejection and ROC analysis demonstrated 100% sensitivity at peak T1 values > 1050 ms.

In our cohort, there is a clear monotonic trend with prolongation of the T1 relaxation time with higher endomyocardial biopsy grades. Furthermore, the mean T1 values in cases of histologic rejection were statistically significantly higher than the mean T1 values in non-

rejection cases. Due to small sample size, multivariable logistic regression analyses or a receiver operator characteristics curve were not performed in our study.

However, in other studies in which rejection was defined solely by biopsy grade, no difference in T1 values was demonstrated.

In fact, Greenway et al. [129] affirmed that, rather than detecting acute rejection, CMR may have a greater role in identifying long-term changes in the myocardium perhaps associated with cardiac allograft vasculopathy.

In the cardiac transplant population, T1 values may be reflective of non-specific graft fibrosis. We identified, although not statistically significant, a difference in T1 values in cases with a history of prior rejection. It's probable that these prior episodes of rejection result in myocardial fibrosis which manifests as increased T1 values.

Sethi et al. [130] suggested that quantitative T2 myocardial imaging may add value to the endomyocardial biopsy in the detection of acute allograft rejection. In this paediatric study, T2 time appeared to rise similarly with acute rejection as in adult patients.

Usman et al. [86], based on a generated receiver operating characteristic curve, proposed a cut-off of 56 ms to maximise sensitivity and specificity in capturing true rejection cases that warrant treatment in an adult cohort.

In our cohort, we did not demonstrate a difference in the T2 in significant allograft rejection.

ECV which represents the interstitial volume can be calculated from native and post-contrast T1. Expansion of interstitial volume occurs with diffuse fibrosis, oedema and with acute rejection.

The combined use of T2 mapping and ECV quantification by T1 mapping may be useful in diagnosing myocardial rejection in adult transplant patients. Vermes et al. [138] claimed that EMB could have been avoided in more than one-half of the patients if CMR had been used for screening. However, this optimistic outlook was tempered by a study that used a multiparametric approach including T1 and T2 mapping and that was unable to distinguish between patients with and without significant rejection [93].

There are limitations to our work: this is a single center study including a relatively small cohort. We recognize that though this is a prospective study, the definition of rejection is retrospectively based on decision to treat. In addition, we were unable to evaluate CMR as a useful tool for response to therapy in patients with acute rejection ≥ 2.

Despite these limitations, we find it promising that multiparametric CMR serves as a non-invasive screening tool during surveillance encounters and may be used to identify those

patients that may be at higher risk of rejection and therefore require further evaluation. Transplant rejection surveillance remains a multi-faceted approach, including assessment of clinical presentation, echocardiography and catheterization hemodynamics.

CONCLUSIONS

EMB is commonly used as surveillance for acute rejection in paediatric heart transplant recipients. Given the potential morbidity associated with cardiac catheterization, a non-invasive tool to either diagnose or screen for AR in paediatric heart transplant recipients would be beneficial. Non-invasive imaging may also allow for more frequent screening at a lower cost. To date, no non-invasive test has been identified that consistently and accurately diagnoses or predicts rejection in paediatric heart transplant recipients with sufficient rigor to supplant EMB.

Multiparametric CMR is emerging as a useful tool for rejection screening and holds promise to substantially improve cardiac allograft rejection surveillance in the paediatric setting.

Native T1 mapping detects myocardial oedema and fibrosis and T2 mapping is sensitive for myocardial oedema. Extracellular volume (ECV) mapping, calculated from native and postcontrast T1 maps and hematocrit, is sensitive for extracellular matrix expansion, including oedema.

In view of our data, it could be considered to use multiparametric CMR as a supplementary examination but not yet a substitute for endomyocardial biopsy. Considering its non-invasive character, the absence of ionizing radiation and the non-strict need for sedation (especially in children > 8 years), resonance could be used as a screening examination limiting the biopsy only to suspected cases.

Future and multicenter studies are necessary to confirm these results and explore whether multiparametric CMR can decrease the number of surveillance EMBs in paediatric heart transplant recipients.

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