Improving Outcomes in Paediatric Heart Transplantation

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For a dying person, a transplant is not a difficult decision. If a lion chases you to a river filled with crocodiles, you will leap into the water convinced you have a chance to swim to the other side. But you would never accept such odds if there were no lion…

Dr. Christiaan Barnard

DECLARATION OF ORIGINALITY

The contents of this thesis are original material, except where indicated in the text for illustrations and background material recreated from referenced sources.

This thesis accounts solely for my own work, except where indicated in the case of work done in collaboration with others, in particular:

- Figure 2.1 "Structure and function of actin/myosin complex" (Chapter 2) was produced by the Medical Illustration Department at Great Ormond Street Hospital
- Graphs concerning worldwide heart transplant data from the International Society for Heart and Lung Transplantation are reproduced from the ISHLT website (Chapter 3)
- Data collection and statistical analysis concerning basiliximab use post-transplant (Chapter 5) was performed in conjunction with Dr. Nicholas Grundy, with whom I shared first authorship in the paper "Pre-implantation Basiliximab Reduces Incidence of Early Acute Rejection in Pediatric Heart Transplantation" J.Heart Lung Transplant. 2009; 28:1279-1284
- Statistical analysis of data concerning extra-corporeal life support post-transplantation (Chapter 6) was performed in conjunction with Dr. Troy Dominguez and Dr. Kate Brown, who are co-authors on the paper "Extra-corporeal Life Support as Rescue Therapy in Paediatric Heart Transplantation" (about to be submitted)
- Flow-mediated dilatation measurements (Chapters 8 and 9) were performed by Elizabeth Ellins
- Coronary angiography intravascular ultrasound examinations (Chapter 9) were performed by Dr. Michael Burch and Dr. Matthew Fenton. In addition, offline IVUS analysis was performed in conjunction with Dr. Fenton to ensure reproducibility and accuracy

ABSTRACT

For patients with end-stage heart failure, heart transplantation remains the only viable long-term option. During the last fifty years, the procedure has become more successful, and the majority of candidates can now expect to recover and lead relatively normal day-to-day lives. Unfortunately, however, it is not a perfect cure. Daily lifelong immunosuppression is required to protect against rejection, and current drug regimens have substantial side-effects including infection, renal failure and hypertension, all of which further shorten life expectancy. Current post-transplant graft survival is estimated at 15 to 20 years, after which re-transplantation is indicated; with donation rates decreasing, and potential recipient numbers increasing, this is by no means certain.

This thesis represents a body of work aiming to show improving outcomes for children at different stages of the transplant journey. Pre-transplant diagnosis has long been thought a predictor of outcome, with worse results for patients transplanted for congenital heart disease than cardiomyopathy. This work showed that with increasing specific surgical expertise, this bias has now largely disappeared. Restrictive cardiomyopathy, pre-transplant extra-corporeal membranous oxygenation and extreme donor:recipient weight ratio were all shown to increase the need for extra-corporeal life support as a rescue therapy in the immediate post-operative phase; this was associated with excellent medium-term survival in patients surviving to hospital discharge. Pre-implantation use of the induction immunosuppression basiliximab was evaluated, indicating a reduction in acute rejection and mortality in the first 6 months post-transplant. Maintenance immunotherapy was also investigated, suggesting an improved side-effect profile seen in children taking tacrolimus rather than ciclosporin. Finally, cytomegalovirus was linked to the most important cause of death for patients over five years post-transplant – namely coronary allograft vasculopathy – as well as morphological and functional impairment in the systemic vasculature of heart transplant

recipients. In summary, this thesis indicates an improving outcome for children at every stage of the post-transplant journey.

ACKNOWLEDGEMENTS

The work presented here was possible only with the help of many individuals. I must thank all of the transplant patients and their families who have consented to take part in this research, many of whom have been incredibly supportive. They continue to be inspirational to all of us in the Heart Transplant Team at Great Ormond Street.

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In addition, there have been vital contributions from outside of the transplant team. Nigel Klein provided me not only with his expert immunological knowledge, but encouraged me to examine my data in different lights, which altered my path on more than one occasion. Victor Tsang was has been integral to my understanding of the surgical aspects of the discipline, and Kate Brown and Troy Dominguez have helped with intensive care elements; all three became driving forces behind different components of the research. Julian Halcox and Libby Ellins were integral to the vascular work, and David Cubitt was always available for his virology expertise.

I owe Silvia Schievano a special vote of thanks for generously providing the electronic template on which this thesis is based, without which I would still be lost in a maze of Microsoft Office help windows. Cruical to ironing out all the final formatting problems was Claudio Capelli, with whom I have continued to not talk about football for eight years. And Stella Fusco, who helped me through a similar jungle of bureaucracy and red tape, in order to submit this thesis on time.

The British Heart Foundation and Hearts and Lungs for Kids both generously supported parts of this research. It is important to single out James Datnow and his family, whose tireless work with Hearts and Lungs for Kids continues to form the backbone to on-going transplant research at Great Ormond Street.

And finally I must thank my family. My mother, who first encouraged me into a career in medicine, and my father, who showed me that there were always further layers of understanding and enjoyment to be found everywhere. And above all, my wife Emma, who is the reason for, and the engine behind, everything. CEGBD.

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
ATG	Anti-thymocyte globulin
BMI	Body mass index
BNP	Brain natriuretic peptide
CAD	Coronary artery disease
CAV	Cardiac allograft vasculopathy
CHD	Congenital heart disease
CHF	Congestive heart failure
CI	Confidence interval
cIMT	Carotid artery intima-media thickness
CM	Cardiomyopathy
CMV	Cytomegalovirus
CMV D+/R-	CMV donor positive, recipient negative
CO	Cardiac output
CPB	Cardiopulmonary bypass
CVVH	Continuous veno-venous filtration
D:R	Donor:recipient
DCM	Dilated cardiomyopathy
DIV	Double inlet ventricle
DNA	Deoxyribonucleic acid
DOV	Double outlet ventricle
ECG	Electrocardiogram
ECLS	Extra-corporeal life support
ECMO	Extra-corporeal membranous oxygenation

EDV	End-diastolic volume
EEM	External elastic membrane
eGFR	Estimated glomerular filtration rate
ESV	End-systolic volume
FMD	Flow-mediated dilatation
GFR	Glomerular filtration rate
GOS	Great Ormond Street Hospital for Children, London, UK
GTN	Glyceryl trinitrate
HCM	Hypertrophic cardiomyopathy
HDL	High density lipoprotein
HLHS	Hypoplastic left heart syndrome
HR	Heart rate
HRV	Hypoplastic right ventricle
hsCRP	High sensitivity C-reactive protein
HTx	Heart transplant
Ig	Immunoglobulin
IL	Interleukin
IL-2 Ra	Interleukin-2 Receptor antagonist
ISHLT	International Society for Heart and Lung Transplantation
IVC	Inferior vena cava
IVUS	Intravascular ultrasound
LAD	Left anterior descending coronary artery
LDL	Low density lipoprotein
MMF	Mycophenolate mofetil
nt-BNP	N-terminal brain natriuretic peptide
OR	Odds ratio
PA	Pulmonary atresia
PCR	Polymerase chain reaction
PRA	Panel reactive antibody
PTLD	Post-transplant lymphoproliferative disease
PVR (PVRI)	Pulmonary vascular resistance (Indexed to body surface area)
RCM	Restrictive cardiomyopathy

Re-Tx	Re-transplant
SD	Standard deviation
SR	Sarcoplasmic reticulum
SV	Stroke volume
SVC	Superior vena cava
TA	Tricuspid atresia
TAH	Total artificial heart
TGA	Transposition of the great arteries
TNF-α	Tumour necrosis factor-alpha
TPG	Trans-pulmonary gradient
TxCHD	(Heart) Transplant for congenital heart disease
TxCM	(Heart) Transplant for cardiomyopathy
VAD	Ventricular assist device

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Heart transplantation is a potentially life-saving procedure reserved for a very limited number of patients who suffer with end-stage, intractable heart failure. It is a rare treatment, performed in relatively few, super-specialised hospitals throughout the world. Paediatric heart transplantation is rarer still, which currently occurs in only two centres in the UK: the Freeman Hospital in Newcastle, and Great Ormond Street Hospital for Children in London.

The operation was first performed in a human subject by Christiaan Barnard in 1967. His early work, along with many other of the pioneers of heart transplantation, remains the basis for today's transplant units, although every area of the discipline has undergone continuous evolution – and occasional revolution – throughout the last fifty years.

An effective transplantation is dependent on much more than the operation alone. Rather, it relies on a combination of processes, all of which must be optimised to ensure the best chance of graft survival. Pre-transplant factors, both in the recipient (e.g. pulmonary hypertension) and the donor (e.g. ischaemia around the time of death), may preclude a successful outcome, regardless of the best attempts of surgeons, intensivists and physicians to maintain or revive graft function. And each of these areas – surgical technique, intensive care strategies, and post-transplant care – must also be delivered at the highest standard, if a transplant programme is to prosper.

To ensure constant improvement in our services, we must not forget the courage and drive for innovation that those early pioneers possessed, which allowed what was considered science fiction in the first part of the 20th Century to become the reality that transplant is today. Research must be at the very core of every unit's philosophy: reflecting on one's own practices, learning from other programmes, and developing novel strategies to prevent, reverse or reduce the potential mortality and morbidity that remains at every step of the transplant process. Great Ormond Street Hospital has always been at the forefront of such innovation, and I hope that – through this thesis – I have been able to advance our knowledge and understanding of paediatric heart transplantation.

1.2 AIMS AND OBJECTIVES

This work aims to demonstrate improving overall outcomes for children undergoing heart transplantation by careful analysis of several of the components of the transplant journey, namely: the impact of the original diagnosis of the patient on the success of transplantation; immediate post-operative intensive care management; immunosuppression (both induction and maintenance therapy) and the side-effects of treatment; and infections and their role in vascular health (peripheral and coronary). My thesis therefore combines the following objectives:

1. To compare outcomes of paediatric heart transplantation depending on indication (cardiomyopathy versus congenital heart disease)
2. To quantify the success and understand the risk factors associated with the need for extra-corporeal life support in the immediate post-operative setting
3. To understand the impact of recent changes in post-transplant medications with regards to their common side-effect profile
4. To examine the benefits of a specific induction therapy (basiliximab) in paediatric heart transplantation
5. To study the effect of cytomegalovirus on the peripheral and coronary vasculature in the post-transplant setting

1.3 THESIS OUTLINE

The thesis consists of an overview of paediatric heart failure and heart transplantation, followed by original research designed to answer the questions posed by the aims and objectives cited above. Specifically:

Chapter 2 outlines the physiology of paediatric heart failure, and describes current modalities of treatment (both pharmacological and mechanical). It then goes on to describe an overview of paediatric heart transplantation, outlining a brief history, clinical indications and timing of transplant, mechanical support as a bridge to transplantation, surgical procedure, immunosuppression, and common post-transplant morbidities (such as renal disease, lymphoma and cardiac allograft vasculopathy).

Chapter 3 compares the overall outcome in children transplanted for cardiomyopathies against those transplanted for congenital heart disease, and further investigates post-transplant survival for different congenital heart disease diagnoses.

Chapter 4 reviews the use of extra-corporeal life support as an emergency life-saving measure in the immediate post-transplant phase, indicating its relatively high incidence, highlighting certain risk factors for its requirement (e.g. restrictive cardiomyopathy), and – despite its high early morbidity – an excellent outlook for those who survive to hospital discharge.

Chapter 5 examines the results of the evolution of induction immunosuppression, from the early use of the broad spectrum anti-thymocyte globulin, to the more targeted CD25 monoclonal antibody basiliximab, demonstrating a reduction in both acute rejection and post-transplant lymphoproliferative disease.

Chapter 6 explores the impact of post-transplant drug therapy with regards to associated morbidities (such as renal impairment, diabetes, hyperlipidaemia and hypertension).

Chapters 7 and 8 investigate the vascular effects of cytomegalovirus (CMV) in the post-transplant setting. Chapter 7 reveals a reduction in peripheral vascular health associated with post-transplant CMV replication, and Chapter 8 indicates the accelerated morphological changes within the coronary arteries of transplant patients with CMV.

Chapter 9 summarises the results of this thesis, describing the effect of the improvements seen in surgical, medical and intensive care of paediatric heart transplant patients, and highlights on-going challenges and potential future directions in the field.

CHAPTER 2

PAEDIATRIC HEART FAILURE AND HEART TRANSPLANTATION

Paediatric heart failure is a relatively uncommon presentation, but one which has potentially devastating results. The most severe cases will require intensive care, with ventilatory, circulatory and perhaps mechanical support; it is these children who may go on to require and receive a heart transplant. It is of vital importance, therefore, to understand the pathological basis of heart failure, in order to implement and develop treatment strategies that improve outcomes in these patients, both in terms of quality of life, and overall (transplant-free) survival. And in order to understand heart failure, an in-depth understanding of normal cardiac physiology is required.

Most of what we know of heart function and failure is derived from adult studies. Although this information is fundamental to how we think about paediatric cardiology, it is essential to bear in mind that "children are not small adults", and extrapolations from adult practice must be translated into the paediatric sphere only with a thorough grounding in the differences between the study population and the patient.

The first part of this chapter presents an overview of each of these matters. It begins with the basic physiology of myocardial contraction, and considers recent advances in our knowledge of cardiac function. This then serves as a basis on which to discuss the pathophysiology of heart failure, including intrinsic cardiac aspects, and extrinsic non-cardiac features, including neurohormonal and immunological adaptations that are originally compensatory, but end up compounding the severity of the myocardial dysfunction. I discuss the subtle differences between children and adults, and then review current treatment for heart failure in children, and possible future advances in the field.

The only long-term option for children with end-stage heart failure is heart transplantation. The second part of this chapter reviews the current state of play in the discipline. Features of those patients requiring transplantation are discussed, including indications for and timing of transplantation, and the subtle but important differences in paediatric compared to adult transplant. Specific issues concerning pre-transplant status are also mentioned, with particular reference to transplantation for congenital heart disease and the use of extra-corporeal life support post-transplant – two of the important areas that are investigated later in this thesis. Post-transplant problems – including immunosuppression and cardiac allograft vasculopathy – are then examined, before reviewing the overall survival figures published by The International Society for Heart and Lung Transplantation.

2.1 INTRODUCTION – PAEDIATRIC HEART FAILURE

Worldwide, heart failure places a substantial burden on healthcare systems. The majority of adult cases are secondary to ischaemic heart disease and myocardial infarction. Whilst ischaemic heart disease is uncommon in children, heart failure is a significant problem. Most cases in the paediatric age group are due to congenital heart disease and cardiomyopathies, but other causes, (e.g. rhythm disturbances) or non-cardiac causes (e.g. sepsis) are recognised. Over recent years, knowledge of the pathophysiology of heart failure has increased, which has led to improvements in the treatment of this disease. Most importantly, it is now possible to target therapies not only at improving myocardial function directly, but which aim to counteract other physiological changes that prove deleterious in the long term. The majority of this work has – inevitably – been derived from adult studies; nonetheless, with careful consideration in certain circumstances, this knowledge can be transferred to paediatric patients. However, in order to understand the pathophysiology of the failing heart, it is first essential to understand the physiology of the working heart.

2.2 PHYSIOLOGY OF MYOCARDIAL CONTRACTION

Cardiac muscle is composed of a latticework of muscle fibres, each composed of myofibrils made up of actin and myosin filaments. The actin and myosin filaments interdigitate, and are linked by small projections from the sides of the myosin filaments known as ‘cross-bridges’; together, these filaments compose the functional unit of cardiac muscle – the sarcomere. The interaction of these cross-bridges generates mechanical forces that cause muscle contraction by the sliding filament mechanism.

The myosin filament is comprised of 200 or more myosin molecules which are arranged into three portions.[1] The body of the myosin molecules are collected together to form the central portion of the filament. The arm protrudes from the central body and, together with the head, forms the cross-bridge which interacts with the actin filament.

The actin filament also has three components. The backbone of the filament is two helical strands of F-actin, on which are situated many active sites (actually each a molecule of ADP) that interact with the myosin cross-bridges to cause contraction. In the resting state, however, these active sites are hidden by the second element of the actin filament, tropomyosin, which is a protein that wraps around the F-actin helix. The final portion of the actin filament is the protein troponin, itself comprised of three subunits (T, I and C), each of which plays a specific role in controlling muscle contraction. Troponin T binds troponin to tropomyosin, anchoring the protein to the filament. Troponin I has a strong affinity for actin and inhibits the interaction of actin and myosin, thus preventing contraction. When troponin C binds to calcium, a conformational change occurs in the troponin complex, uncovering the active sites of the actin, allowing them to interact with the myosin cross-bridges, and contraction follows (Figure 2.1).

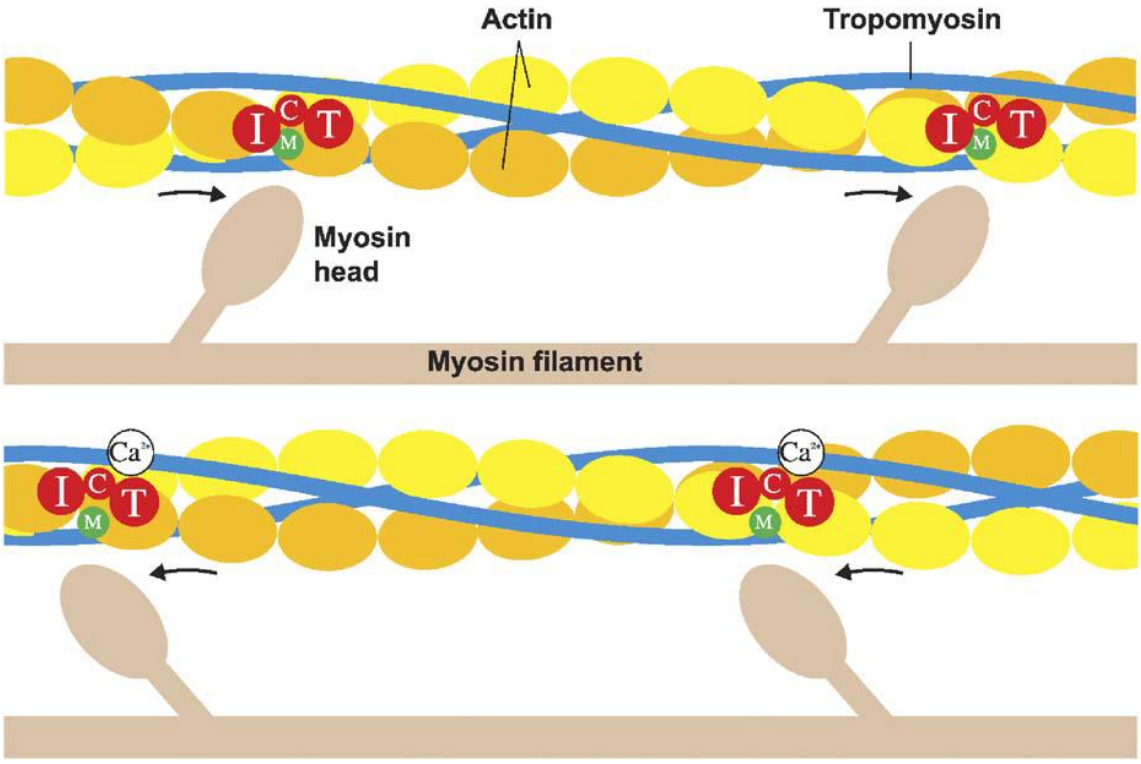

Figure 2.1 Structure and function of myosin/actin complex. Without calcium (top), the I-subunit of troponin covers the myosin binding site, inhibiting contraction. As calcium binds to the C-subunit of troponin (bottom), the I-subunit is pulled away, permitting binding of the myosin head to the actin filament. This is followed by a conformational change in myosin, which pulls the actin filament from right to left. I, I-subunit of troponin; C, C-subunit of troponin; T, T-subunit of troponin; M, myosin-binding site. Figure produced by Great Ormond Street Hospital Medical Illustration Department.

Initiation of contraction begins with the cardiac action potential.[2] As the action potential passes over the cardiac muscle membrane, it spreads to the interior of the muscle fibre along the membranes of the transverse tubules. These cause instantaneous release of calcium ions from the sarcoplasmic reticulum (SR) into the muscle sarcoplasm; these ions then diffuse into the myofibrils, causing the conformational change in the troponin complex leading to muscle contraction. During repolarization of the cardiac muscle, the calcium in the sarcoplasm is rapidly pumped back into the SR, causing relaxation of the muscle fibre and completing the cardiac cycle.

2.3 REGULATION OF CARDIAC OUTPUT

Cardiac output (CO) can be defined as the product of stroke volume (SV) and heart rate (HR):

$$CO = SV \times HR$$

Stroke volume is the amount of blood ejected from the ventricle in each cardiac cycle, or, in other terms, the difference between the end-diastolic volume (EDV) and the end-systolic volume (ESV). EDV is dictated by preload, myocardial compliance and active relaxation, and ESV is a function of contractility and afterload. As stated by Frank, when the preload increases, the left ventricle distends, the left ventricular pressure development becomes more rapid and rises to a higher peak pressure, and the stroke volume augments.[3] This increase in stroke volume, with a concomitant increase in heart rate, results in an increase in cardiac output (originally described by Starling).[4] This relationship between venous return and cardiac output (the Frank–Starling relationship) is demonstrated by the increase in cardiac output during exercise or following the administration of intravenous fluid.

The physiological basis on which the Frank–Starling law operates has two components.[5] Firstly, as in skeletal muscle, an increase in sarcomere length due to ventricular stretch optimizes the overlap of actin and myosin, forming more cross-bridges and increasing the force of contraction (although in contrast to skeletal muscle, cardiac muscle operates much closer to its optimal sarcomere length at all times; hence this effect is not as marked as in skeletal muscle). Secondly, an increased sarcomere length sensitizes troponin C to the prevailing calcium transient, leading to a steep increase in force as sarcomere length increases, i.e. length-dependent activation.[6]

An increase in cardiac output is also effected by an increase in afterload. This is described by the Anrep effect, i.e. an abrupt increase in aortic pressure is followed by a positive inotropic effect within 1–2 minutes.[7] One postulated explanation of the Anrep effect is that increased left ventricular wall tension acts on myocardial stretch receptors to increase cytosolic calcium, independently of sarcomere length; it is also believed that stretch results in an increase in myofilament calcium responsiveness.[8] Clinically, this increase in cardiac output may be seen in acute rises in blood pressure.

An increase in heart rate will obviously lead directly to an increase in cardiac output (since CO = SV x HR). However, an increase in heart rate also leads to an increase in stroke volume by a process known as the treppe phenomenon (or Bowditch effect);[9] during rapid stimulation, more sodium and calcium ions enter the myocardial cell than can be removed, leading to a stepwise increase in available calcium, increasing the force of contraction. This is also known as the force–frequency relationship.

2.4 PATHOPHYSIOLOGY OF HEART FAILURE

Cardiac failure is the inability of the heart to deliver oxygen to the tissues at a rate commensurate with the metabolic demands. Broadly, it can be divided into systolic and diastolic failure, although the two are closely interlinked and often occur together in the same patient, particularly in advanced failure once adaptive mechanisms start to fail. The most common causes in the paediatric age group are congenital heart disease, cardiomyopathies and myocardial dysfunction following cardiac surgery,[10] as well as rhythm disturbances and non-cardiac causes (e.g. bacterial sepsis).

2.4.1 Haemodynamic aspects of heart failure

Cardiac failure can be seen as an inadequacy of the aforementioned homeostatic mechanisms to fully respond to the demand for cardiac output. Each homeostatic mechanism has an upper threshold, beyond which cardiac performance falls. For instance, as heart rate increases (increasing cardiac output), diastolic time (i.e. filling time) decreases. Decreasing filling time will, eventually, decrease the amount of blood in the ventricles at the end of diastole (i.e. EDV), and stroke volume will be reduced. When the decrease in stroke volume is proportionally greater than the increase in heart rate, the product of the two will decrease, i.e. cardiac output will start to fall. This situation of a pathologically high heart rate can be seen in many rhythmic disturbances, e.g. supraventricular tachycardia.

Similarly, in the case of the Frank–Starling law of the heart, there is a point at which further increases in EDV are not met with an increased force of contraction, but rather stroke volume starts to tail off (Figure 2.2). This situation is thought of simply as volume overload. This condition arises in congenital left-to-right shunting lesions, such as a large patent ductus arteriosus or ventricular septal defect. As stroke volume falls, the volume overload itself gets worse, generating a vicious circle. In the case of afterload, there will come a point where further increases in arterial pressure cannot be matched with further increases in inotropy, and therefore the contractility of the heart will be insufficient to overcome the afterload. Again, stroke volume will decrease.

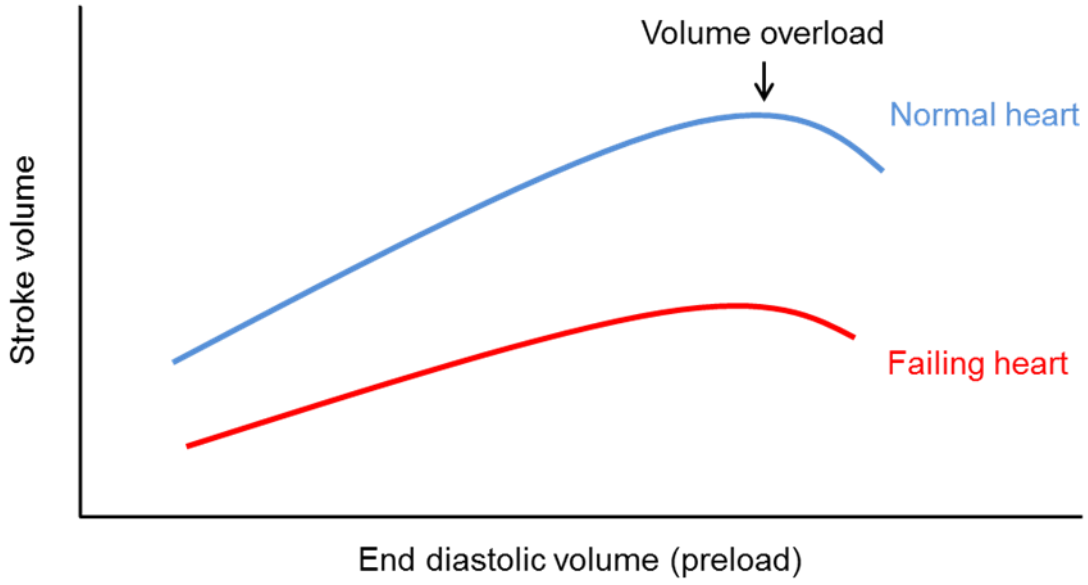

Figure 2.2 The Frank-Starling Curve. As preload increases, stroke volume increases in accordance with the Frank–Starling law. However, there is a point at which further increases in end-diastolic volume are met with a decrease in stroke volume, labelled 'volume overload'. Note also the depressed response of the failing heart.

In each of the situations above, we arrive at a position of impaired cardiac performance. When this occurs, the heart relies on a series of adaptive mechanisms for the preservation of its contractile state. Initially, each of these systems proves beneficial to the functioning circulation; however, in the case of chronic heart failure, over time, they become maladaptive and detrimental to cardiac function. These mechanisms can be divided into two broad groups: intracardiac and extracardiac mechanisms.

Intracardiac mechanisms involve changes to the myocardium on a molecular and cellular level. The most obvious of these is ventricular hypertrophy, which develops in response to an excessive pressure or volume load.[11] Haemodynamic overload is thought to re-activate growth factors present in the embryonic heart but dormant in the normal adult heart,[12] leading to increased protein synthesis and increased myofibril mass.[13] This is accompanied by an increase in energy demand, met by increased synthesis of mitochondria[14] and increased collagen.[15]

It is easy to see how these adaptive mechanisms prove beneficial to the circulatory system, at least in the short-term. Ventricular hypertrophy will increase contractile force, with support from more mitochondria meeting the increased energy demands, and it is also thought that an increase in collagen may reduce ventricular dilatation. Over time, however, there is a shift from compensatory hypertrophy to heart failure, as the myocytes deteriorate and die, and the mitochondria are exhausted. Failure of the compensatory mechanisms obviously results in impaired cardiac function once again, and a downward spiral is set in motion. In addition, the excess collagen starts to impair relaxation and ventricular filling, further exacerbating myocardial dysfunction.

So far, this chapter has dealt mainly with the intrinsic features of cardiac physiology and pathology, which constitute the haemodynamic model of heart failure. As knowledge of heart function and failure has increased, however, it has become evident that extracardiac mechanisms are at play, both in the physiological and pathological state. Broadly, these mechanisms form the neurohormonal and immunological models of cardiac failure. Again, these adjustments that are initially advantageous in maintaining adequate cardiac output, ultimately prove to be detrimental.

2.4.2 Neurohormonal aspects of heart failure

2.4.2.1 Sympathetic drive

The most immediate of these mechanisms is increased sympathetic activity. Primarily in response to decreased blood pressure detected by carotid artery baroreceptors, the sympathetic nervous system causes rapid vasoconstriction of the arteriolar system (causing an increase in arterial blood pressure), vasoconstriction of the venous system (increasing venous return), and increased heart rate. However, this control of blood pressure is very short lived, since the 'set-point' (the blood pressure at which the baroreceptors cause these effects) is reset within 1–2 days.[16] In fact, like other compensatory mechanisms, this also becomes counterproductive; adults with congestive heart failure (CHF) have increased levels of circulating catecholamines, but rather than producing the normal inotropic and chronotropic responses, the failing heart is relatively insensitive to this stimulation. Again, there is a situation of afterload mismatch, where stroke volume does not increase proportionally to the increased demands on the myocardium. There is, however, an increase in energy expenditure seen, as well as an increase in the likelihood of ventricular arrhythmia, with a concomitant negative effect on survival.[17, 18]

2.4.2.2 Renin-angiotensin system

The other main arm of the neurohormonal response is the renin–angiotensin system. Renin release in the kidney is augmented by β1-adrenoceptors in the juxtaglomerular apparatus (under sympathetic stimulation), and is further enhanced by activation of baroreceptors in the renal vascular bed (in response to decreased blood pressure). Renin converts angiotensinogen to angiotensin I, which is subsequently converted by angiotensin-converting enzyme (ACE) to angiotensin II, promoting re-absorption of salt and water by the tubules. Angiotensin II is also a powerful peripheral vasoconstrictor. Decreased arterial pressure and constriction of the afferent arterioles of the kidney (under sympathetic drive) also serve to decrease the glomerular filtration rate, which decreases urine output. This initial response by the kidneys to falling cardiac output causes salt and water retention (thereby augmenting preload). In chronic heart failure, angiotensin II stimulates the adrenal glands to produce aldosterone, which further increases sodium re-absorption by the tubules, and thereby fluid retention. In addition, the increase in sodium ions (and chloride ions) increases the extracellular osmolarity, which initiates the secretion of antidiuretic hormone by the hypothalamic-pituitary axis, exacerbating fluid retention further. While this may boost stroke volume in the short-term, long-term fluid overload leads to pulmonary and peripheral congestion.

The role of the renin–angiotensin system is not limited to its effect on the kidney. The myocardium itself is capable of synthesizing ACE and angiotensin receptors.[19] Under conditions of heart failure, the myocardium exhibits greater ACE activity. In addition, angiotensin II is known to have direct hypertrophic effects on the myocardium,[20] as well as increased collagen production.[19] This local renin–angiotensin system is now a crucial target in the medical therapies of heart failure.

2.4.2.3 Natriuretic peptides

The three natriuretic peptides found in the body (atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide) also have a crucial part to play in paediatric heart failure. ANP and BNP are both released from the heart in response to myocardial stretch, and act as physiological antagonists to vasoconstriction and sodium re-absorption mediated by the renin–angiotensin and sympathetic nervous systems.[21] Plasma levels of N-terminal pro-brain natriuretic peptide (nt-BNP) have been shown to reflect the severity of symptoms in heart failure and the impairment in cardiac function in children with CHF, and can be used as a marker of the effects of treatment.[22] Moreover, natriuretic peptides themselves have been considered as treatments for CHF in children.

2.4.3 Immunological aspects of heart failure

The immunological aspects of heart failure are perhaps the least studied and least well understood of the three arms in the pathophysiology of heart failure. However, over recent years, the importance of the immune system in the progression of CHF has become increasingly well recognised. Peptide growth factors have been shown to be expressed by cardiac cells,[23, 24] and have been shown to cause hypertrophy,[25] illustrating that they may well have a role in heart failure and may therefore be a target for future therapies.

In addition, inflammatory cytokines are also implicated in the development of heart failure. Tumour necrosis factor-α (TNF-α) and interleukin have been shown to depress myocardial activity both in vivo and in vitro; they have also been shown to induce myocardial apoptosis and promote cardiac hypertrophy and fibrosis.[26] It is also known that levels of TNF-α are increased in patients with heart failure.[27] TNF-α inhibition has been shown to inhibit myocardial remodelling in rats, promoting a possible concept of therapy.[28] Two proposed agents were etanercept (a recombinant human TNF receptor) and infliximab (a chimeric monoclonal antibody to TNF-α); neither, however, has shown benefit in clinical trials.[29, 30]

2.5 DIFFERENCES BETWEEN NEONATAL, PAEDIATRIC AND ADULT CARDIAC PHYSIOLOGY

The vast majority of clinical trials (as well as many physiological and pathological studies) in heart failure have taken place in adult populations; the paediatric cardiologist is therefore forced to base much of his knowledge on extrapolation from these. It is important, however, to bear in mind the axiom ‘children are not small adults’, and an understanding of some of the ways in which paediatric and adult cardiac physiology differ is essential if these extrapolations are to be justified.

One major difference is the underdeveloped sarcoplasmic reticulum (SR) of the neonatal myocyte.[31] Calcium handling is therefore dependent on greater activity of the L-type calcium channel[32] and the Na+/Ca2+ exchanger,[33] and it is vital to maintain normal ionised calcium levels in children for inotropic therapies to be effective. Similarly, calcium channel blockers are capable of producing a profound depression of myocardial contractility in the younger heart.

Recently, with greater application of Doppler tissue imaging, there has been interest in the biomechanics of myocardial contraction. Of particular significance is the 'twisting' of the myocardium during systole, caused by anticlockwise rotation of the apex and simultaneous clockwise rotation of the base (when viewed from the apex).[34] This torsion of the left ventricle stores significant energy that is released during the recoil of the isovolaemic relaxation stage of diastole, creating suction that is essential for rapid early filling of the ventricle. In a recent paper, age-related differences have been highlighted in this ventricular torsion: the neonatal heart exhibits relatively little torsion compared with adolescents and adults.[35] Furthermore, although torsion continues to increase throughout life, there is a slowing in recoil that may contribute to diastolic dysfunction in the elderly. It is important to consider these differences when thinking about diastolic dysfunction in children.

2.6 MEDICAL MANAGEMENT OF PAEDIATRIC HEART FAILURE

Aside from limited surgical interventions (either in congenital heart disease, or in the case of heart transplantation), the main approach to the treatment of paediatric heart failure is the use of drugs. However, it is important that other modalities of treatment are not forgotten. The most crucial of these is nutrition; it is essential that adequate nutrition be supplied to the child in heart failure, but excess sodium should be avoided. In the case of infants, reduced-volume, high-energy feeds may be required in order to supply the body with the increased energy demands of heart failure, without overburdening the circulation with excess fluid.

Previously, with limited understanding of the pathophysiology of heart failure, therapies were aimed at improving myocardial function directly. Although this must still be the approach in the acute setting, there is evidence that this strategy worsens both long-term morbidity and mortality.[36, 37] As knowledge has increased, greater importance has been placed on preventing the deleterious effects of long-term compensatory mechanisms. With a better understanding of the underlying pathophysiology, the physician is now in a better position to select the appropriate drug class for an individual patient, not only to improve cardiac function in the acute setting, but also to prolong the long-term viability of the myocardium.

2.6.1 Diuretics

Diuretics have long been used in the treatment of heart failure in both adults and children, for obvious reasons. They are certainly indicated in patients exhibiting symptoms or signs of congestive heart failure (CHF), such as dyspnoea, and produce symptomatic benefits more rapidly than any other drug for heart failure.[38] The reduction in preload not only decreases pulmonary congestion, but also leads to a reduction in myocardial wall stress, which is a potent stimulus for remodelling. Loop diuretics also cause pulmonary vasodilatation, with further symptomatic relief. However, they possess the detrimental side-effect of neurohormonal activation;[39] for this reason, they may be relatively contra-indicated in patients without symptoms of congestion. A further issue is resistance to diuretics with long-term use in heart failure, which is associated with an increase in morbidity and mortality.[40] In this case, the European Society of Cardiology, the American College of Cardiology and the American Heart Association recommend adding metolazone (a thiazide diuretic) to a loop diuretic in order to promote sodium loss at different portions of the nephron,[38, 41] although it is only infrequently used in children.

2.6.2 Spironolactone

Spironolactone is an aldosterone receptor antagonist. It has only a mild diuretic therapy (as its site of action is the distal nephron, by which point much of the sodium of the filtrate has been re-absorbed), and was initially given concurrently with other diuretics to minimize electrolyte disturbance. However, the Randomized Aldactone Evaluation Study (RALES) found a 31% reduction in death from cardiac causes, and a reduction in hospitalization and symptoms in adults with heart failure treated concomitantly with an ACE inhibitor.[42] One of the possible explanations for this effect is that spironolactone may limit excessive extracellular matrix turnover, thereby reducing fibrosis and remodelling.[43]

2.6.3 Angiotensin converting enzyme (ACE) inhibitors

ACE inhibitors were a significant addition to the treatment of paediatric heart failure in the 1990s. They lower aortic pressure and systemic vascular resistance, and lower left and right atrial pressures in children with heart failure, without affecting pulmonary vascular resistance significantly. ACE inhibitors have also been shown to lower circulating norepinephrine levels, thereby decreasing the effects of the neurohormonal model of heart failure, and stimulate prostaglandin synthesis. Several studies have shown a decrease in mortality and morbidity in adult patients.[44, 45]

2.6.4 β-blockers

The rationale behind the use of β-blockers in heart failure is to prevent and reverse adrenergically mediated intrinsic myocardial dysfunction and remodelling,[46] one of the initially compensatory but ultimately deleterious factors in the progression of heart failure. Large multi-centre trials in adults have shown their benefit in morbidity and mortality in heart failure.[47-49] Disappointingly, the paediatric carvedilol study showed no benefit of carvedilol versus placebo on clinical outcomes in children with symptomatic systemic ventricular systolic dysfunction and heart failure.[50] The authors of the study have implicated a higher than expected placebo response rate, and a possible interaction between systemic ventricular anatomy and primary outcome as possible reasons for the disappointing result. In contrast, however, in a study on nine paediatric patients with dilated cardiomyopathy, carvedilol was found to significantly reduce neurohormonal activity (reduced renin–angiotensin system activation, reduced norepinephrine, aldosterone and dopamine) and improve left ventricular ejection fraction, with significant reductions in ventricular diameters.[51] The differences in the conclusions from these two articles further illustrate the difficulties associated with randomised controlled trials in paediatric cardiology. Despite this, β-blockers remain an important treatment in paediatric heart failure.

2.6.5 Digoxin

The use of digoxin in heart failure dates back over 200 years. It is a cardiac glycoside with a positive inotropic action, as well as a negative chronotropic action (by slowing conduction at the atrioventricular node). By inhibiting the Na+/K+-ATPase pump, digoxin leads to an increase in intracellular sodium, followed by inhibition of the Na+/Ca2+ exchanger, and therefore an increase in intracellular calcium, thereby increasing contractility. In normal subjects, this increase in contractility is offset by an increase in systemic vascular resistance, and thus digoxin leads to no change in cardiac output. However, in patients with systolic impairment (in sinus rhythm), digoxin does produce an increase in left ventricular ejection fraction.[52] Digoxin has also been shown to possess neurohormonal effects, including lowering norepinephrine levels[53] and sympathetic tone.[54] Recently, however, its use has become controversial[55] and its prescription has been limited in favour of β-blockers and ACE inhibitors, both of which have been proven to decrease mortality in randomised clinical trials. Digoxin has not been proven to reduce mortality in any age group, and shows little positive benefit in children with normal systolic function (for instance, children in heart failure secondary to shunt lesions).[56] However, a recently published Cochrane review of digoxin use in adults concluded that digoxin did have beneficial effects in treating symptomatic patients with heart failure, especially as an add-on in patients for whom a regimen of diuretics, ACE inhibitors and β-blockers has failed.[57]

2.6.6 Nesiritide

Nesiritide is a recombinant b-type natriuretic peptide that was shown in multiple clinical trials to be of benefit in the treatment of decompensated heart failure in adults.[58] Early studies in children showed improved diuresis and natriuresis in those already on inotropic support.[59, 60] However, concerns over increased mortality, increased renal failure and cost have severely tempered this early enthusiasm, and it cannot currently be recommended for use in paediatric heart failure.[61]

2.6.7 Statins

The use of statins (3-hydroxy-3methylglutaryl-CoA reductase inhibitors) in cardiovascular disease to lower cholesterol has been universal practice for many years. Statins are not currently used regularly in the treatment of paediatric heart failure. New evidence of the benefits of their use in heart failure patients with normal cholesterol levels indicate a potential broadening of the indications of their use. They have been shown to improve endothelial function and neurohormonal imbalance in patients with non-ischaemic heart failure and normal cholesterol levels.[62] Simvastatin has been shown to improve ejection fraction and improve symptoms compared with placebo in Japanese adults with dilated cardiomyopathy,[63] as well as improvements in endothelial function (measured by flow-mediated dilatation of the brachial artery). In the same study, levels of TNF-α, BNP and interleukin-6 were lower in those patients receiving simvastatin; a connection which the authors have suggested may be the mechanism for the clinical and echocardiographic improvements. An interesting preliminary report into the use of statins in diastolic heart failure has also strengthened the cause for their extended use in adults. In a trial comparing statins, β-blockers, ACE inhibitors, angiotensin-receptor blockers and calcium blockers in patients with diastolic heart failure (with an ejection fraction of ≥50%), only statin therapy was linked to a substantial improvement in survival.[64] In this publication, the authors postulated various possible mechanisms to explain their results, including anti-inflammatory effects, anti-oxidant effects, protection against left ventricular remodelling, antihypertensive effects and improvements in arterial distensibility.

2.7 CONCLUSION

As knowledge of the pathophysiology of heart failure has grown, theoretical advances in treatment have come to light. Although not all of these have been shown to have clinical benefit, others have become invaluable means of combating heart failure in children. However, the lack of clinical trials in the paediatric population requires that treatment protocols are largely based on extrapolation from adult studies, which may not always be wholly justified. There remains a pressing need for these challenging studies to be organised.

2.8 INTRODUCTION – PAEDIATRIC HEART TRANSPLANTATION

Heart transplantation remains the only realistic therapeutic option for children with end-stage heart disease. Though the first paediatric heart transplant was performed by Kantrowitz in New York in 1967,[65] only three days after Barnard's celebrated first adult transplant, it was soon understood that the discipline was not a mere extension of transplantation in adults, but rather a distinct clinical entity with its own set of principles, techniques and goals. Of course, there is much overlap with adult transplantation, and much of what we know and practice is a direct extrapolation of that much larger experience. However, although surgical techniques, post-operative intensive care and on-going pharmacological management share much in common between the age groups, there are vital differences that must be taken into account if a centre is going to produce a successful paediatric transplant programme.

2.8.1 Recipient population

It is important to state that paediatric heart transplantation is itself a very heterogeneous sub-specialty. Whereas approximately 90% of adult transplants are performed for cardiomyopathy (either ischemic or non-ischemic),[66] up to one in three paediatric recipients has a diagnosis of congenital heart disease;[67] that figure rises to almost 60% in infant recipients (Figure 2.3). The use of the word "diagnosis" in the singular form is somewhat of a misnomer, however, since this sub-group of patients is further sub-divided into a wide variation of congenital heart defects, with the possibility of multiple previous surgical interventions and wide diversity in clinical status. This variability in anatomic substrate must be thought about carefully and on an individual by individual basis by the surgical team prior to heart transplant listing; it is also accompanied by variability in physiological status that requires skilful management by the anaesthetic and intensive care specialists. Further complexity arises from the significant differences between neonates, young children and adolescents, again with regards to anatomy and physiology, but also in regard to psychology and social background. In addition, patients requiring heart transplantation in childhood often have co-existing conditions or syndromes that must be considered throughout the transplant process.

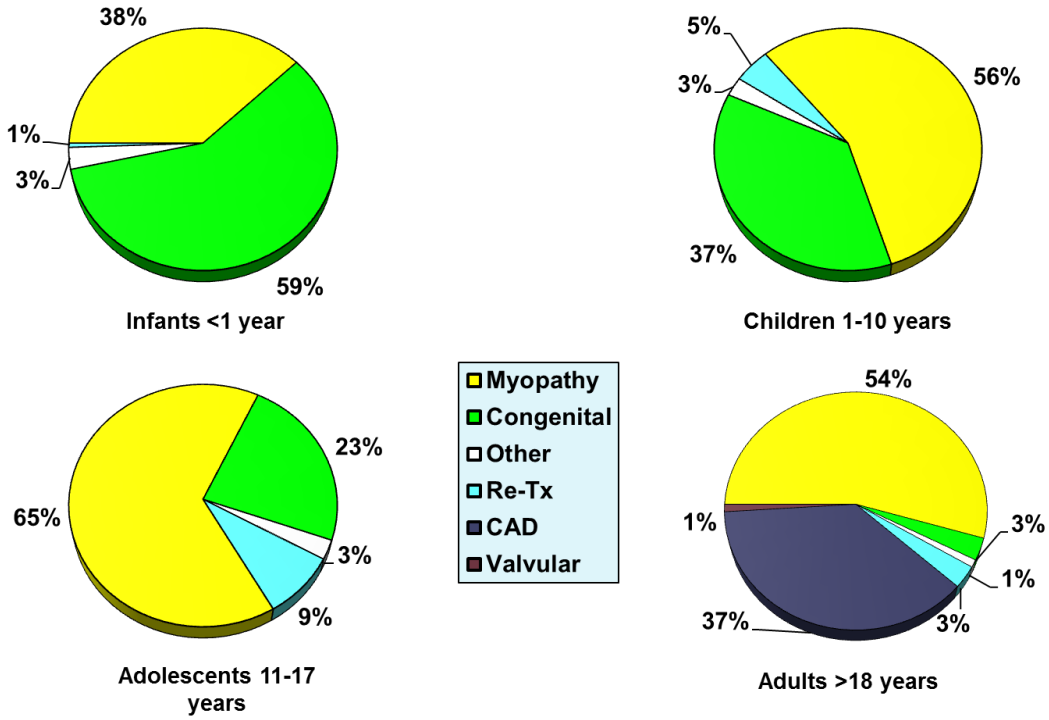

Figure 2.3 ISHLT figures displaying diagnosis of patients coming to transplant from 2000-2011, grouped by age. CAD – coronary artery disease.

2.8.2 Worldwide figures

With this heterogeneity in mind, paediatric transplant teams have been set up in many centres worldwide as distinct entities, with obvious benefits from the sub-specialization. However, the experience of these teams is generally much less than that of their adult counterparts. The latest figures from the International Society of Heart and Lung Transplantation reveal that only a quarter of European paediatric transplant centres performed more than 10 per year since 2000,[67] compared to 60% of adult centres.[68] Low volume of cases is known to be a risk factor for mortality post-transplantation, and it is easy to understand how increased experience and familiarity of every aspect of transplantation would lead to a more successful programme. The small numbers of procedures per centre also greatly limit both quantity and quality of research possibilities, yet this is counterbalanced by effective multi-centre clinical audit and research such as with the Paediatric Heart Transplant Study Group. However, even with these caveats, complications and complexities, paediatric heart transplantation has significantly better survival rates than adults (Table 2.1).

Age group	Median survival worldwide (years)
Infants	19.2
Children	15.6
Adolescents	11.9
18-29 years	12.2
60-69 years	8.9

Table 2.1 Latest ISHLT figures of overall survival by age at transplantation.[67]

One further important difference between paediatric and adult heart transplantation is how to define success for the paediatric transplant programme and for the individual patient. If a 55-year-old man with coronary artery disease survived 15 years after transplantation, it would be considered by many patients and health care providers alike as a successful and worthwhile intervention with obvious benefit to the patient and society as a whole. Although the success and worth of 15-year survival following infant transplantation could never be questioned, the prospect of re-transplantation or death in adolescence or early adulthood invites further philosophical, ethical and economic questions. Although difficult, these issues must be discussed with any prospective patient and family, to allow careful consideration before agreeing to be transplant listed.

2.8.3 Indications

Improvements to every stage of transplantation have greatly increased the success of the procedure, both in terms of longevity and quality of life of the childhood recipients. Simultaneously, there have been great strides made in the treatment of heart failure and the surgical treatment of congenital heart disease, allowing transplantation to be postponed or completely avoided in some patients. With this in mind, in 2007 the American Heart Association Council on Cardiovascular Disease in the Young developed a document describing the current indications for paediatric heart transplantation (Table 2.2).[69]

Predictably, the guidelines are largely based on clinical expert opinion, rather than large, multi-centre randomized controlled trials, due to the paucity of these in such a small field. They divided their indications into three classes:

- Class I where evidence or general opinion suggests that transplantation would be useful and effective
- Class II where there is conflicting evidence or a divergence of opinion, further subdivided into Class IIA (weight of evidence/opinion is in favour) and Class IIB (usefulness is less well established)
- Class III where evidence or general opinion is that transplantation is not useful.

Class I	Class IIA	Class IIB	Class III
•Stage D heart failure (CM or CHD) with systemic ventricular dysfunction •Stage C heart failure with: i. severe limitation of exercise and activity (peak max. oxygen consumption <50% predicated), or ii. significant growth failure, or iii. untreatable life threatening arrhythmias •Stage C heart failure in RCM with reactive pulmonary hypertension	•Stage C heart failure with reactive pulmonary hypertension and potential of developing fixed PVR •Anatomical or physiological conditions likely to worsen prognosis of univentricular CHD which can lead to HTx as primary therapy •Anatomical or physiological conditions likely to worsen prognosis of operated CHD without impaired ventricular function	Efficacy of HTx not established for •Patients with history of: i. Hepatitis B or C or HIV ii. Use of tobacco or illicit drugs, or alcohol abuse iii. psychological, behavioural disorders, poor family support structures, documented noncompliance with previous therapies	HTx not efficacious: •Severe irreversible disease in other organs •Severe irreversible, fixed elevation of PVR •Severe hypoplasia of central branch pulmonary arteries

Table 2.2 Indications for paediatric heart transplantation. CM – cardiomyopathy, CHD – congenital heart disease, RCM – restrictive cardiomyopathy, PVR – pulmonary vascular resistance, HTx – heart transplantation.

It is important to stress that heart transplantation should not be used as the primary therapy for any infant with congenital heart disease without the presence of concomitant coronary, valvular or ventricular impairment. It is, however, reasonable to consider transplantation in the setting of previously repaired congenital heart disease without ventricular dysfunction if there is risk of developing fixed raised PVR that could preclude future transplantation, severe aortic or systemic valve insufficiency, severe cyanosis, persistent arrhythmia or persistent protein losing enteropathy.

2.8.4 Timing of listing for transplant of ambulatory patients

While increasing proportions of heart transplants are performed in very ill patients on high dose inotropes or mechanical support, some patients are transplanted while still ambulatory and at home. The timing of listing for ambulatory patients is difficult and controversial, even in adult cardiology. Many paediatric cardiologists base timing on evidence of left ventricular systolic dysfunction and symptoms of heart failure. Severity of ventricular dysfunction has been found to be predictive of outcome in some studies but not in others. Symptoms appear to provide poor prognostic capability too, because even asymptomatic children with incidental discovery of cardiomyopathy can have a poor prognosis. Finding ways to identify those patients with the highest risk of clinical deterioration and/or death would greatly assist in clinical decision-making, including the indication and prioritization for transplant.

2.8.4.1 Exercise testing

Extensive evidence supports the use of cardiopulmonary exercise testing as a tool to select patients with increased short-term mortality that should be offered transplantation. Besides peak oxygen uptake, several additional variables, for instance ventilatory efficiency, have been shown to have high prognostic value in adults with heart failure. However, despite widespread use in the adult population, information regarding the practical clinical value of exercise testing as a prognostic tool in paediatrics is very limited. At Great Ormond Street, exercise testing is possible in children over 10 years of age and sometimes younger. Data acquired here is broadly supportive of a peak oxygen consumption of less than 50% of predicted for age, although peak blood pressure response to exercise has also been found to be an important prognostic test.[70]

Exercise testing is complicated in congenital heart disease. Peak heart rate appears to be a useful prognostic marker. Oxygen consumption and ventilatory efficiency are also useful. However, the values at which transplantation should be considered vary with the type of congenital heart disease. Patients with a Fontan circulation have much lower baseline levels of oxygen consumption and ventilatory efficiency. A simple 6-minute flat walking test is still valuable in congenital heart disease.

For younger children, pre-school age and infants, exercise testing is impossible. It is in this age group that biomarkers of heart failure are particularly useful. In patients with chronic heart failure, B-type natriuretic peptide level of over 300 pg/ml may be predictive of poor outcome in paediatric cardiomyopathy.[71] Other biomarkers are currently being assessed.[72]

2.9 SPECIFIC PRE-TRANSPLANT ISSUES

2.9.1 Transplantation for congenital heart disease

Transplantation for congenital heart disease illustrates best many of the peculiarities of heart transplant in the paediatric age-group. As experience with paediatric transplant has grown, the boundaries of what anatomy is considered transplantable have extended to the point now that the only contraindications from a purely anatomical point of view are severe pulmonary artery hypoplasia and pulmonary vein stenosis. However, these may be correctable by heart-lung transplantation or using extended donor pulmonary arteries for pulmonary hypoplasia.

Concurrently, improved conventional surgical techniques have led to the treatment of more and more congenital heart disease without the need for early transplantation. However, these methods, along with the success of non-curative surgical procedures such as the Fontan pathway, have only postponed the need for transplant in many patients, reducing strain on the youngest, most scarce donors, but in many cases adding to the waiting list in later childhood. In reality, these improvements may have even increased the overall numbers of potential recipients as many babies would previously have died during neonatal operations, or on neonatal transplant waiting lists. Many of these children are now surviving infancy, but with circulatory systems that are destined to fail at some point, and by the time a heart transplant is required, some families are finding transplantation a more attractive option, and palliative care strategies less so.

In most registries, congenital heart disease remains a risk factor for transplantation.[67] Attrition is early and related to the complexity of the reconstruction. Yet it would be wrong to be uniformly negative about transplantation for congenital heart disease. Registry data has shown that transplantation following previous Senning or Mustard procedures can now be achieved with a low mortality. Previously palliated infants and young children with a Glenn circulation also appear to have relatively low risk. However, it is the Fontan patients who represent the highest risk with a recent paper showing a relative risk of death of 8.6 compared to other congenital operations.[73] The Fontan patients often have liver or renal problems and may suffer more infections by virtue of protein losing enteropathy and lymphopenia. Protein losing enteropathy increases the risk of transplant, perhaps because it may be a marker of increased pulmonary vascular resistance. Resistance is hard to calculate in the Fontan patients with multiple sources of pulmonary blood supply, pulmonary arteriovenous malformations, collaterals and fenestrations.

For all congenital patients it is obvious that risk can be reduced by having an experienced congenital heart surgeon perform the operation with appropriate anaesthetic and intensive care teams. This is particularly important in adult congenital heart disease, where few adult transplant teams have extensive congenital experience.

Since the lifespan of a transplanted organ is limited, no potential recipient should be transplanted too early. The aim of paediatric programmes is often to delay transplantation for as long as possible by optimizing medical management. However, this delay must not be at the expense of other organ damage (most notably renal failure or cirrhosis in Fontan patients), increasing pulmonary resistance or inducing HLA sensitisation by virtue of blood transfusions. Any of these complications of delaying transplant may reduce the success of future transplantation, and have the effect of an overall decrease in life expectancy.

Prior to listing for transplantation, detailed scanning of the heart and pulmonary vasculature with an appropriate combination of echocardiography, angiography, CT or MR must be undertaken to ensure that the anatomy is favourable. These scans must be available to the surgeon before accepting an offer of a potential organ, in order that he can request extended portions of the great vessels which may be required to facilitate non-standard anastomoses. Since it is likely that other organs may be being harvested from the same donor, it is important for the cardiac surgeon to speak to other transplant teams to enable the most judicious use of donor vessels, or perhaps arrange for alternative tissue to be collected. For instance, if the heart transplant surgeon feels he is unable to harvest enough of the pulmonary arteries for a particular recipient, he may be able to use donor pericardium or descending aorta.

2.9.2 Surgical technique

Heart transplantation is performed via a median sternotomy. In patients with congenital heart disease, previous sternotomies may have resulted in dense mediastinal adhesions which can cause heavy intra-operative haemorrhage. Significant bleeding can also occur from substernal great vessels or additional collateral vessels – the results of years of cyanosis. Under such circumstances, some surgeons may opt to expose the femoral vessels prior to sternotomy to facilitate emergency femoro-femoral bypass should the need arise.

Venous cannulae are placed in the SVC and IVC, and an arterial cannula in the ascending aorta at the level of the innominate artery, facilitating cardiopulmonary bypass. Body cooling is carried out to 32°C, unless deeper hypothermia is required in the case of longer predicted operating times, for instance when additional correction of complex heart defects is necessary (e.g. the abnormal venous anatomy seen in patients with isomerism of the atrial appendages); in such instances, the body is cooled to a nasopharyngeal temperature of 18°C. It is essential to anticipate long recipient dissection times to closely coordinate with the arrival of the donor organ, thereby minimising ischaemic times.

The aorta is subsequently cross-clamped, and the right and left atrial walls are then divided close to the atrioventricular groove and the atrial septum, being careful to leave a sufficient rim of atrial tissue for later suturing of the donor graft. A similar cuff of right atrial tissue is left following transection of the caval veins, and the rest of the right atrium removed. The great arteries are transected just superior to their valves; further surgical modification of the remaining tissue is often required to compensate for any discrepancy in size between the donor and recipient vessels. The recipient heart is now completely free, and is removed.

The donor heart is placed over the left side of the divided sternum, parallel to the remnant of the excised recipient heart. The graft is positioned in such a way that the left atrial surfaces of the donor and recipient hearts are in contact, with leftwards rotation of the donor heart resulting in its posterior surface facing anteromedially. The two left atria are then sewn together with continuous polypropylene, starting at the base of the left atrial appendage of the donor heart close to the caudal end of the recipient left upper pulmonary vein. The anastomosis continues around the superior and inferior walls of the left atrium onto the atrial septum. The Great Ormond Street surgical team favour a bicaval anastomosis for the systemic venous pathways, with both caval veins being sutured separately. However, due to the risk of kinking or purse-stringing of the SVC, right atrial anastomosis is sometimes preferred in small infants.

Connection of both great arteries is then performed. Following meticulous de-airing of the left heart, the aortic cross-clamp is removed and rewarming of the heart can begin. Similarly, the caval snares are removed and the right heart is de-aired. In order to guide intensive care decisions – particularly concerning the use of post-operative ventilation and inotropic drugs – monitoring lines are placed in the left atrium and the pulmonary artery, and right atrial and right ventricular pacing wires are inserted as a precaution against the effects of post-operative arrhythmias, such as heart block. As the heart rewarms, spontaneous re-activation occurs; direct application of a DC current is needed in the case of ventricular fibrillation. As graft function improves, the patient can be weaned from cardiopulmonary bypass, and transferred back to the intensive care unit.

2.9.3 Pulmonary vascular resistance

The assessment of pulmonary vascular resistance is particularly crucial in order to reduce the rate of right heart failure post-transplant,[74] but it can be technically difficult, particularly in congenital heart disease. In general the guidelines document referred to above advises that transplantation is possible if PVR can be reduced using pulmonary vasodilators to a transpulmonary gradient of under 15mmHg and a PVR of ≤ 6 Woods units.m^2.[69] There is a tendency for paediatric centres to push the boundaries of transplantable PVR, and some units will take on children with resistance higher than this, but it is clear that risk is increased. A long period of inotrope or vasodilator therapy may reduce PVR, as may a period of mechanical support by reducing left ventricular end diastolic pressure.

2.9.4 Mechanical Support

Traditionally, paediatric units offered only ECMO support as a bridge to transplant, as most ventricular assist devices were not suitable for small children. This strategy allowed only a short time for support before serious complications ensued. The development of a pneumatic assist device suitable for children (the Berlin Heart) has allowed long-term bridge to transplantation in young children.[75] Successful support is possible for many months, although few children are discharged from hospital. Most experience has been obtained in children with dilated cardiomyopathy who are older than 1 month of age. Usually the device is implanted in children with progressive cardiac failure and inotrope dependency. Little data exists on support for very young children less than 4 kg. It seems likely that the neonatal group will be more difficult to manage with the small 10ml pumps and anticoagulation complexities increasing their risk of thrombo-embolic complications.[76] Typically the device is used as a bridge to transplant although there are reports of use as bridge to recovery, and it has been used in myocarditis. In general, fulminant myocarditis is still treated with ECMO in most centres although this may change as experience with the Berlin Heart increases. However, ECMO does avoid a left ventricular scar. Conversion from ECMO to Berlin Heart is often performed, although primary implantation of Berlin Heart is preferred in most units. The use in single ventricle circulations and particularly the Fontan circulation also appears more complex, but possible.[77, 78] The limited data available does suggest a higher mortality in the single ventricle group. However, the recent huge increase in Berlin Heart insertion in the USA illustrates the success of this device.

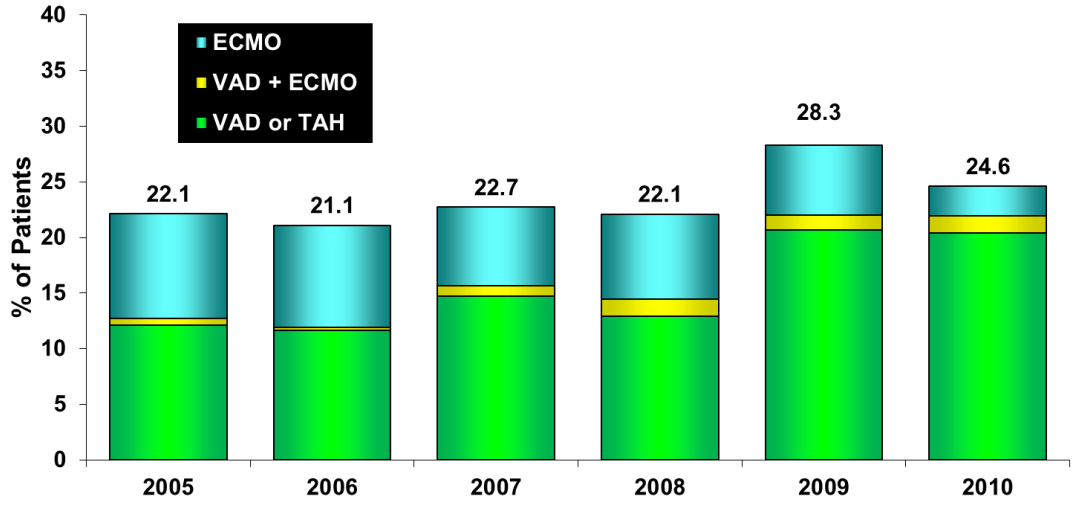

Figure 2.4 ISHLT figures showing use of mechanical support prior to paediatric heart transplantation from 2005-2010. ECMO – extra-corporeal membranous oxygenation; VAD – ventricular assist device; TAH – total artificial heart.

2.9.5 ABO incompatibility

Compared with adult transplantation, the prospective donor pool of hearts suitable for infant recipients is small. Infants with blood group O have a further reduced donor pool. This imbalance has prompted advances in transplanting across blood groups – the so-called ABO-incompatible transplant. In adults, heart transplants across ABO blood groups have occurred only by mistake, with high rates of both hyperacute rejection and mortality.

In contrast to mature adult immune systems, however, infants have very low levels of isohemagglutinins, allowing the possibility of transplanting an organ of blood type B, say, into a recipient of type A. In 1996, Prof. Lori West led the team that performed the first intentional heart transplant across an ABO incompatibility.[79] The recipient was a 25 day-old baby with hypoplastic left heart syndrome of blood group O that received an organ of blood group AB. During cardiopulmonary bypass, plasma exchange was performed until the levels of isohemagglutinins were non-detectable. In the paper reporting the first ten ABO-incompatible transplants, rates of mortality, rejection and morbidity were equivalent to ABO-compatible transplants, with two deaths and one other graft loss unrelated to ABO activation,[79] data which has recently been supported with excellent long-term outcome.[80] It is thought that donor-specific B-cell elimination results in immunological tolerance in recipients of ABO-incompatible hearts. In order to ensure the on-going success of the graft, future transfusion of blood products must occur according to strict criteria (Table 2.3). The exact age and level of isohemagglutinins that are acceptable for ABO mismatch transplant are unclear but an isohemagglutinin dilution of 1:16 is used at Great Ormond Street as a cut-off value, and few such transplants have been performed over the age of 2 years.

Donor Blood Group	Recipient Blood Group	Indicated plasma	Indicated red cells	Indicated platelets
AB	O	AB	O	AB
B	O	AB or B	O	AB or B
A	O	AB or A	O	AB or A
AB	B	AB	O or B	AB
A	B	AB	O or B	AB
AB	A	AB	O or A	AB
B	A	AB	O or A	AB

Table 2.3 Required blood groups for transfusion after ABO incompatible paediatric heart transplantation.

2.9.6 Lymphocytotoxic antibodies

Another problem in paediatric transplantation is the presence of pre-existing human leukocyte antigen (HLA) antibodies, which have been linked to increased hyperacute, cellular and humoral rejection, and increased mortality post-transplant.[81] There are several factors that cause the formation of such antibodies. However, most antibodies are formed in response to blood products (particularly white cells and platelets) or homograft tissue. Thus, prior HLA sensitization is much more common in children who have undergone surgery for congenital heart disease, or those who have required mechanical support. For children on longer term mechanical support the "prophylactic" use of supplements of iron, folic acid and erythropoietin may avoid blood transfusion.

When a patient is sensitized, a prospective cross match between donor and recipient serum prior to transplantation would be the perfect solution, but it is time-consuming – which limits the geographical area from which a sensitized recipient can receive an organ. Alternatively, it is possible to test antibodies against specific HLA antigens, thereby facilitating a "virtual" cross-match once a potential donor has been found. Although this will not be as specific as a prospective cross match it has been used safely in both adult and paediatric transplantation.[82]

It is also possible to reduce the level of HLA antibodies prior to transplantation. Strategies for de-sensitization include intravenous immunoglobulin, methotrexate, mycophenolate mofetil, cyclophosphamide and rituximab, and newer monoclonal antibodies to plasma cells, to lower the level of PRA prior to transplantation.[81, 83, 84] However, there has not been uniform success with these strategies and there is often a limited window of low antibody levels in which transplantation can be performed. This has led some North American centres to transplant across the HLA barrier using intra-operative plasma exchange, and post-operative plasmapheresis with other antibody reducing strategies. Results are still at a preliminary stage, but it appears that primary graft failure can often be avoided, and while antibody mediated rejection is common, it may resolve with possible accommodation to the antibody. The long-term consequences of this strategy are as yet unknown.

2.10 POST-TRANSPLANT LIFE

2.10.1 **Immunosuppression**

It does appear that acute cellular rejection, in particular that associated with hemodynamic compromise, is becoming less common. This may be the result of newer drugs and increased experience. Over the last decade or so, there has been a gradual shift towards using induction therapy in paediatric heart transplantation, with current levels at about 70%.[67] As opposed to adult practice (which has similar overall levels of induction use), antithymocyte globulin remains the preferred agent in children, although the proportion of centres using an interleukin-2 receptor antagonist such as basiliximab is increasing.[85]

Maintenance therapy is most commonly a combination of a calcineurin inhibitor and cell cycle inhibitor. Tacrolimus has become the calcineurin inhibitor of choice in two-thirds of paediatric patients,[67] taking over from ciclosporin at least in part due to the latter's unwanted side-effect profile of hirsuitism and gingival hypertrophy, which can lead to problems with compliance in paediatric patients. Cell cycle inhibitors are used by 88% in the first year, with mycophenolate mofetil (MMF) the most common, being used in 70% of those children; approximately one-third of all patients are prescribed it in combination with tacrolimus. This figure is slightly less than the equivalent value in adults, approximately half of whom are taking tacrolimus and MMF in combination.

Steroids still hold a place in paediatric practice, particularly in the first year post-transplant, and in the treatment of rejection. The proportion of patients using steroids is slightly less than in adults, with 55% of children taking prednisolone at one year; by five years post-transplant this figure has fallen to less than 40%.[67]

2.10.2 **Renal disease**

Chronic use of calcineurin inhibitors induces renal dysfunction. This is clearly crucial in paediatrics where patients are still young and hopeful of a second heart transplant when the first one eventually fails. Nephrotoxicity can be reduced by using lower levels both early post-transplant and later in the recipient's course. The main paediatric use of sirolimus and everolimus has been in children with impaired renal function post-transplant along with reduced doses of calcineurin inhibitors. Others have used calcineurin free regimes.[86] The latter may be associated with an increased risk of rejection and greater surveillance with biopsy and non-invasive testing is needed.

2.10.3 **Lymphoma**

Unlike adult transplant medicine, paediatric transplant teams do not have to deal with a wide range of malignancies post-transplant. Even skin malignancies are uncommon in children, but this does not preclude the need for sun protection and surveillance. The main malignancy in children post-transplant is lymphoma. Usually, this is a B cell lymphoma and is triggered by Ebstein Barr Virus. Surveillance using quantitative PCR for the virus may give early warning of incipient lymphoma and many teams will reduce immunosuppression when viral loads are high. The risk of lymphoma appears greater in children that seroconvert post-transplant. The site of the lymphoma varies but is often in the gut (typically associated with a low albumin), lungs (where chronic chest infections may be wrongly diagnosed) or tonsils. Tissue diagnosis is usually needed. Treatment can be simply with reduction of immunosuppression but rituximab is also increasingly used in the initial stages. Treatment may escalate to conventional chemotherapy and even specific T cells have been generated to destroy the EBV associated B cells.

2.10.4 Cardiac allograft vasculopathy

Like adults, the major obstacle to late patient survival in paediatric heart transplantation is cardiac allograft vasculopathy (CAV), an accelerated form of obliterative cardiovascular disease (Figure 2.5). CAV causes considerable morbidity and mortality, affecting approximately 50% of patients 5 years post-transplant. It is the leading cause of death in adult patients more than 5 years post-transplant,[87] and in children more than 3 years post-transplant.[88] As it is accompanied by remodelling and compensatory luminal dilatation in the early phases, it is best diagnosed not with conventional angiography, but with intravascular ultrasound. However, since this technique is not widely available in children, it is likely that the prevalence of the disease has been underestimated. Moreover, once a diagnosis of CAV has been made, graft survival is limited to 50% at 2 years.[89] It is thus the greatest limitation on long-term outcome, and remains the most pressing problem for transplant programmes worldwide. The cause remains unknown but conventional risk factors such as smoking, hypertension, diabetes, hypercholesterolemia, and obesity are less of a problem in paediatrics than in adults. In children chronic viral infection such as cytomegalovirus and recurrent acute or chronic cellular rejection appear important. It does seem that chronic inflammation is important in the development of CAV.

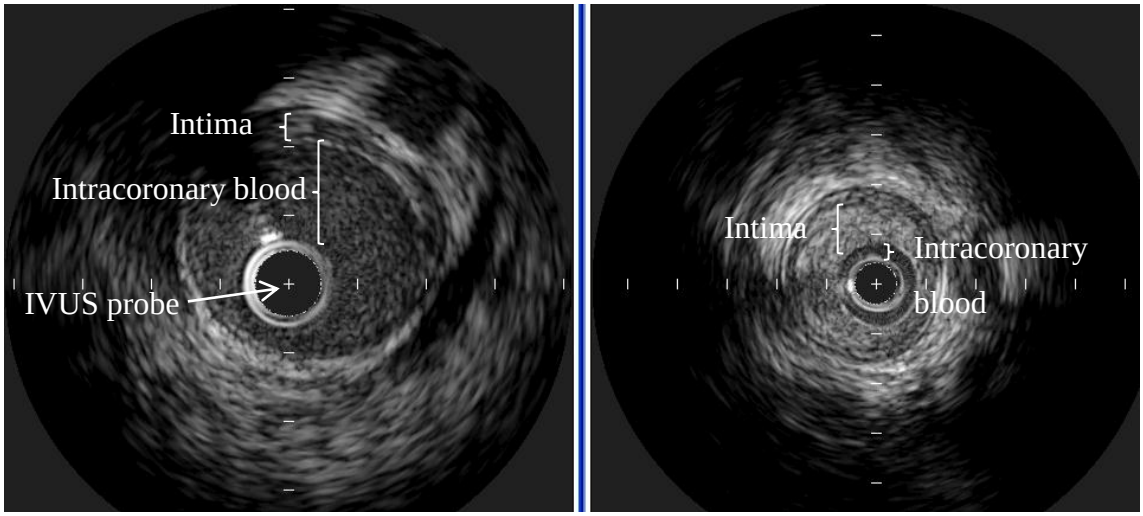

Figure 2.5 Intravascular ultrasound images of the left anterior descending coronary artery of the same patient taken 1 and 2 years post-transplant. The figure on the left shows a relatively healthy artery, with good lumen size, and only a small amount of intimal thickening in the upper left quadrant. The figure on the right shows the effective lumen now decreased to only slightly larger than the ultrasound probe, with the rest of the artery replaced by grossly thickened intima. The patient had clinically significant CMV disease during the period between the images.

Current treatment is severely limited. The use of statins has been shown to improve outcome after transplantation, perhaps in part due to their anti-inflammatory properties, as well as a lipid lowering effect. They are recommended as a preventative measure in all adult transplant recipients. We have used pravastatin in all children including infants with few problems. Pravastatin has the advantage of not interfering with cytochrome p450 and thus calcineurin inhibitors. Reversal of CAV is much harder. Sirolimus use has been used as an antiproliferative in both adults and children. Ultimately, however, many patients with CAV face the prospect of re-transplantation relatively soon after diagnosis. In the paediatric age group, it is the most common indication for re-transplantation, making up just over half of all re-transplants.

2.10.5 Psychological, developmental and behavioural issues

Ultimately, the success of individual transplants and the programme as a whole depends on well-informed, psychologically stable and motivated patients. In paediatric transplant these are even more vital as the advent of adolescence often magnifies any psychological stressors. The prevalence of behavioural problems in post-transplant children is over 25%, a frequency that exceeds that of children following conventional cardiac surgery.[90] Moreover, the effects of such problems, in particular non-compliance with immunosuppression, are potentially more serious. It is therefore of paramount importance to make psychological and social assessments of potential recipients prior to transplant, and try to address any issues that may arise.

Cognitive development of children following cardiac transplant is obviously an important outcome variable. Many studies have tried to evaluate accurately the impact of transplantation. In general, the transplant group has mean mental and psychomotor scores at the low end of the normal range.[90, 91] Many factors are postulated as causes for this discrepancy between the transplant and healthy population, including hospitalization, missed school, cardiopulmonary bypass and the side effects of post-transplant drugs. Encouragingly, the vast majority of paediatric heart transplant recipients should look forward to reintegrating fully into normal schooling and social activities.

Adherence to post-transplant medication regimens is not universally high amongst childhood recipients, and it is commonly cited as a cause of acute rejection and death, particularly amongst teenagers.[92] Early recognition of clues to non-compliance, for instance wildly variable immunosuppressant levels and erratic lifestyles (including school avoidance and irregular sleeping patterns) must be taken seriously, and causes to the root problems must be addressed.[93] Addressing concerns over the cosmetic side effects from medication and more convenient medicine regimens may help.

Ultimately, psychological and behavioural difficulties must be considered on an individual basis. A coordinated multidisciplinary approach to any problems, involving medical and nursing teams both at the transplant centre and locally must be undertaken where problems arise and include the family doctor, school teachers and nurses, family members and teams of specialists such as social workers and special educational providers where necessary.

2.11 OUTCOMES

There is no doubt that overall outcomes for all age groups following heart transplantation have steadily improved over the last thirty years, when large worldwide data sets from the International Society of Heart and Lung Transplantation have been available (Figure 2.6, Figure 2.7 and Figure 2.8). As useful as these data are, however, at producing an overall picture of heart transplant through the world, they are prone to the problems inherent in large registries; the patients, medical personnel and protocols obviously vary greatly between institutions and countries, and subtle improvements may well be masked, and lost in the fog of heterogeneity that is present.

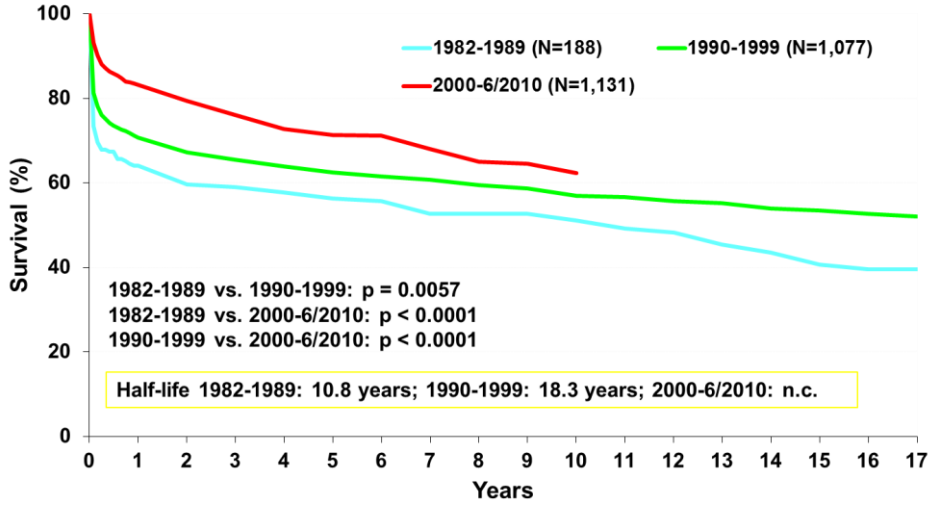

Figure 2.6 ISHLT registry data displaying post-transplant survival in recipients aged <1 year, over three eras.

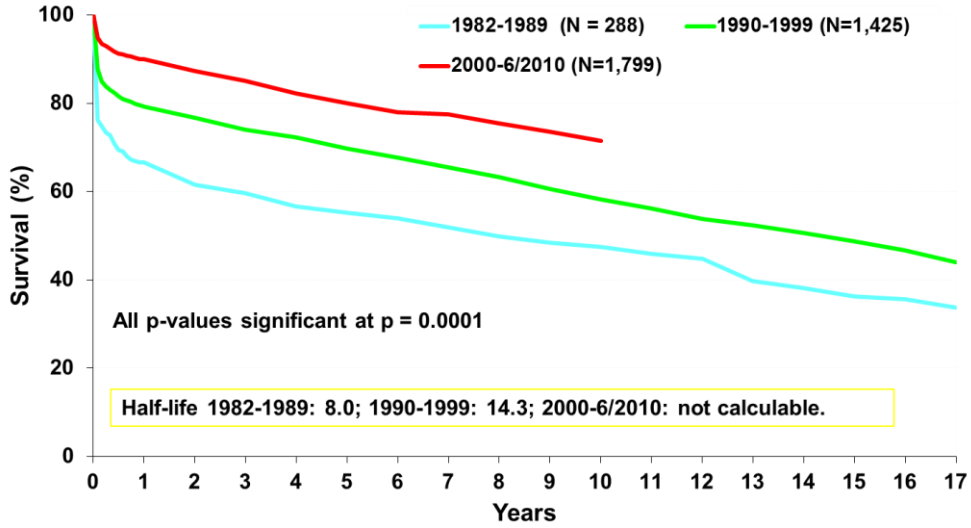

Figure 2.7 ISHLT registry data displaying post-transplant survival in recipients aged 1-10 years, over three eras.

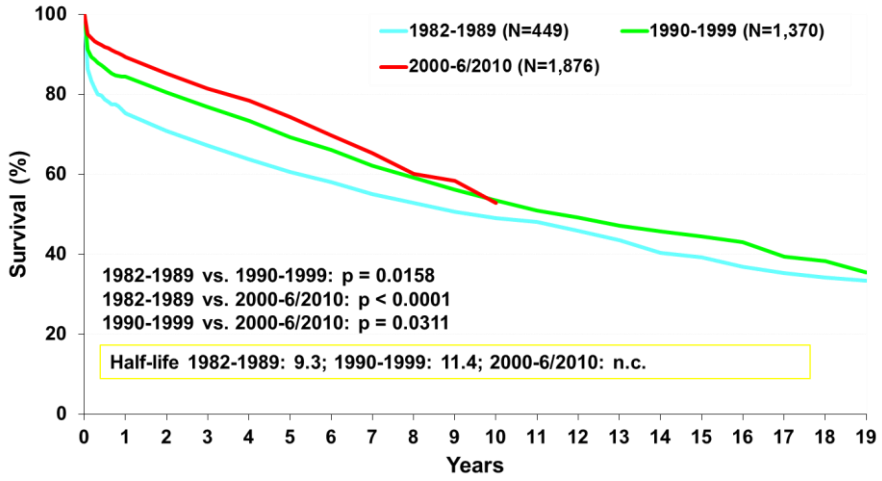

Figure 2.8 ISHLT registry data displaying post-transplant survival in recipients aged 11-17 years, over three eras.

Certainly, it was with that ethos in mind that this thesis was designed. The overall feeling at Great Ormond Street was that our heart transplant protocols were based on good theories, but we needed to back up those ideas with solid, local research. For instance, the accepted thinking on transplantation for congenital heart disease is that outcome is worse than those patients with an original diagnosis of cardiomyopathy (Figure 2.9), but with at least anecdotally good success with some children with difficult anatomy, I wondered whether with our increasing expertise, outlook for these patients was higher than had been previously reported (which became the basis for Chapter 3).

Similar questions arose with regards to the use of induction immunosuppression and extra-corporeal life support post-transplant, as well as the side-effects of transplant medications and effects of CMV post-transplant; those questions are revealed in the later chapters.

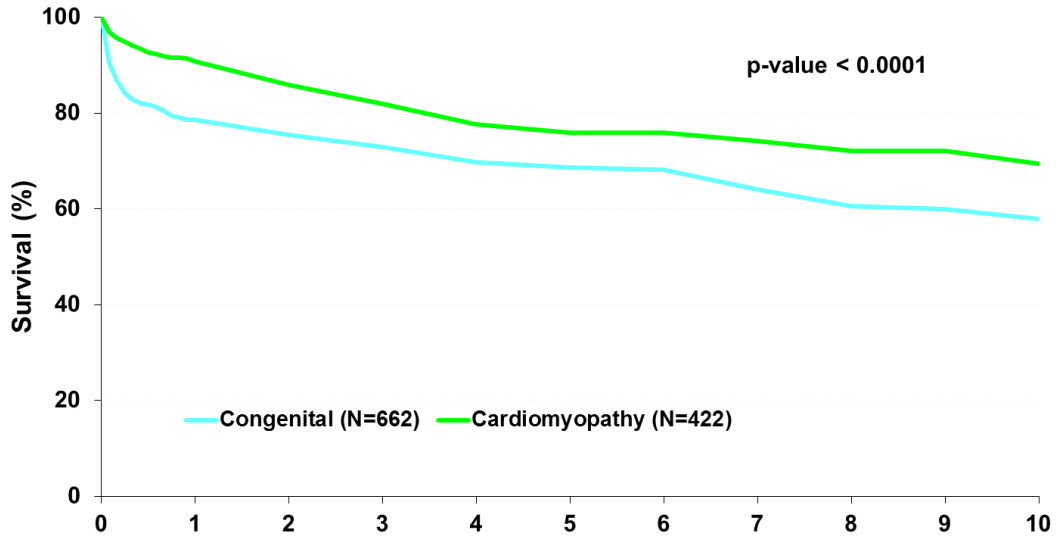

Figure 2.9 ISHLT registry data displaying post-transplant survival in recipients transplanted from 2000-2010 with an original diagnosis of cardiomyopathy vs. congenital heart disease.

CHAPTER 3

HEART TRANSPLANTATION FOR CONGENITAL HEART DISEASE

With growing numbers of children with complex congenital heart disease surviving initial surgical procedures, more patients are presenting in later childhood or early adulthood in cardiac failure. This presents an obvious increased burden on transplant centres, and a further strain on a limited donor pool. Historically, results for heart transplant following congenital heart disease (CHD) have been worse than those following cardiomyopathy (CM). With increased surgical experience and intensive care expertise, the gap between the two aetiologies in our practice is decreasing. This chapter reviews the current protocols for transplantation in this setting, presenting a large single-centre experience over 20 years, and speculates on possible future advancements in this very challenging field.

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3.1 INTRODUCTION

Historically, transplantation for congenital heart disease (CHD) has had a worse prognosis than that for heart failure of other aetiologies.[94] It has been estimated that between 10 and 20% of children with complex CHD will at some stage require transplantation; since it accounts for approximately 25% of all heart transplants in the paediatric age group[95] – and, with more and more children with CHD reaching adulthood, a growing number of early adult transplants – it is important to strive to redress this imbalance. Over the 40-year history of heart transplantation, various improvements in pre-operative, operative and post-operative care have dramatically improved the immediate and long-term outlook for all transplant recipients. Furthermore, a number of adaptations specific to congenital heart disease recipients have combined to shrink the gap between transplantation for CHD and other indications, and patients transplanted for CHD can now reasonably expect to live for a decade or more. However, CHD remains a highly significant risk factor for one-year and five-year mortality in transplant recipients,[95, 96] and the wide diversity of this group with respect to age, original diagnosis, previous operations and clinical status makes it very difficult to analyse accurately different practices or produce clear formal protocols. This chapter was designed to study the evolution and outcomes for transplantation for CHD in a single centre over almost two decades, and speculates on future advancements in this very challenging field.

3.2 METHODS

3.2.1 Study population and design

The notes of the first 259 patients undergoing heart transplant at Great Ormond Street Hospital from the beginning of the programme were reviewed. Only first transplantations were included, excluding 9 re-transplants. A total of 250 first transplants were studied, of which 73 were for CHD (29.4%). The initial diagnoses of the CHD group are displayed in Table 3.1. In addition, one patient – whose initial diagnosis was tricuspid atresia with VSD – was re-transplanted 3.6 years after her initial transplant for failing graft. She is currently well, 13.9 years after her second transplant. Only her first transplant is used for this analysis.

Biventricular (35)		Univentricular (38)	
TGA	12	HLHS	14
Aortic disease	7	DOV	8
Mitral disease	4	DIV	6
Septal defects	4	HRV/PA/TA	10
Tetralogy of Fallot	4		
ccTGA	2		
Ebstein's anomaly	2		

Table 3.1 Diagnoses of 73 patients transplanted for congenital heart disease. TGA – transposition of the great arteries; ccTGA – congenitally corrected transposition of the great arteries; HLHS – hypoplastic left heart syndrome; DOV – double outlet ventricle; DIV – double inlet ventricle; HRV hypoplastic right ventricle; PA – pulmonary atresia; TA – tricuspid atresia.

Transplant management has evolved over the years: for clarity, the initial analysis divided the entire cohort chronologically into five groups of fifty transplants. The relatively small numbers of patients with congenital heart disease required that for meaningful secondary analysis aimed to further probe the success of transplant for congenital heart disease (TxCHD), the entire cohort was re-divided into two eras - the Millennium was chosen as an arbitrary time point that created two almost equal groups of TxCHD patients: 38 transplants were performed prior to 2000, and 35 since.

3.2.2 Statistical analysis

The entire data set was used for all statistical analyses. Data were analysed with SPSS 14.1 for Windows (SPSS Inc.). “Survival” refers to graft and patient survival: censoring for the purpose of survival curves was taken at the date of death or re-transplantation, whichever came first. The Kaplan-Meier method was used to analyse survival, and Wilcoxon tests were used for comparison between groups.

3.3 RESULTS

3.3.1 Survival of entire cohort

Figure 3.1 displays a Kaplan-Meier curve of survival for the entire cohort of patients following first heart transplantation at Great Ormond Street. Survival over this twenty-year period was 90.8% at 30 days, 86.4% at one year, 73.9% at 5 years, and 59.5% at 10 years.

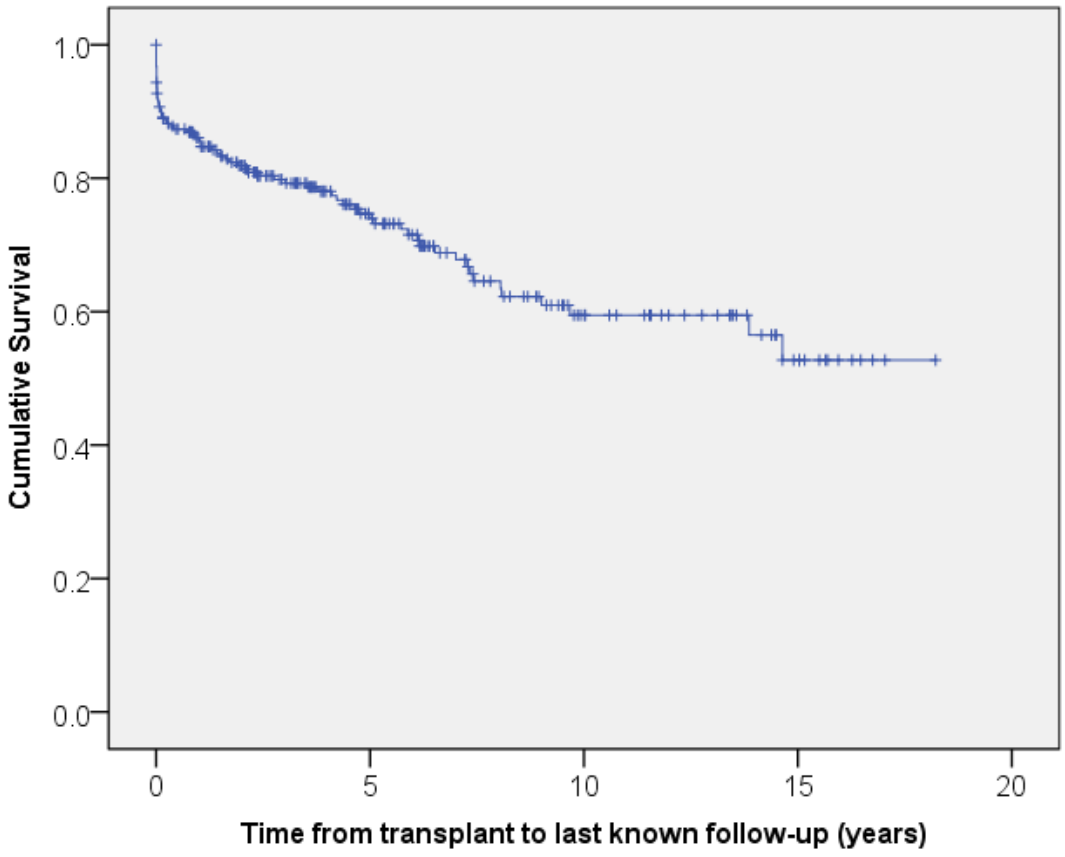

Figure 3.1 Kaplan-Meier curve depicting overall survival of entire cohort of 250 patients.

3.3.2 Survival by Era

By dividing the cohort into groups of fifty transplants, the Kaplan-Meier graph shown in Figure 3.2 illustrates that overall survival following transplantation has improved steadily from the beginning of the programme (Log Rank (Mantel-Cox) p = 0.004)). Importantly, the most obvious improvement occurs within the first year, with 30-day and one-year survival improving consistently through the groups (Table 3.2). After the first year, the slope of each of the curves is largely parallel, indicating little difference in long-term outcome conditional on survival to one year post-transplant.

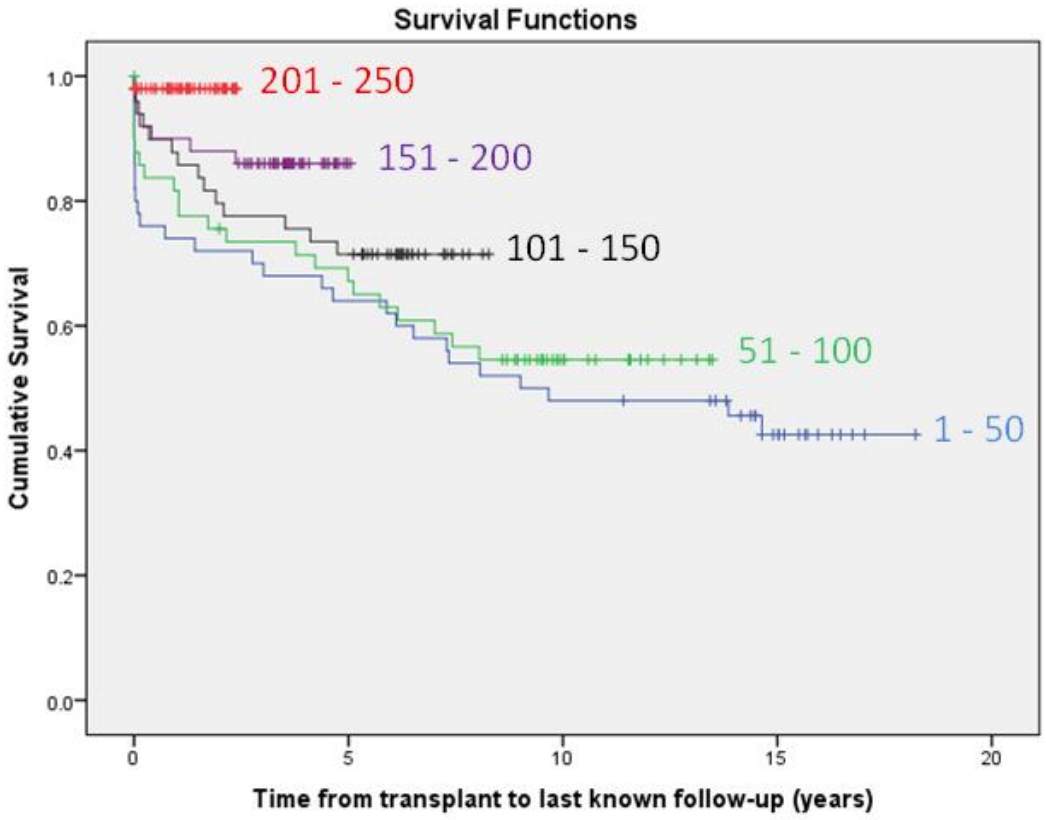

Figure 3.2 Survival of the entire cohort, divided into chronological groups of 50 transplants.

Transplant number	30-day survival (%)	1-year survival (%)
1-50	78.0	74.0
51-100	87.8	81.7
101-150	96.0	87.8
151-200	98.0	90.0
201-250	98.0	98.0

Table 3.2 30-day and 1-year survival grouped chronologically for the entire cohort.

3.3.3 Cardiomyopathy vs. Congenital Heart Disease

Figure 3.3, Figure 3.4 and Figure 3.5 display survival curves of transplantation for CM and CHD divided by the Millennium. 1-year survival for TxCHD improved from 66% pre-2000 to 90% post-2000 ($p = 0.005$). Prior to 2000, 1-year survival for TxCHD was significantly worse than that of transplant for cardiomyopathy (TxCM) (66% vs. 84%, $p = 0.036$). There was no such difference in 1-year survival for TxCHD compared to TxCM in the post-Millennium era (90% vs. 94%, $p = 0.76$).

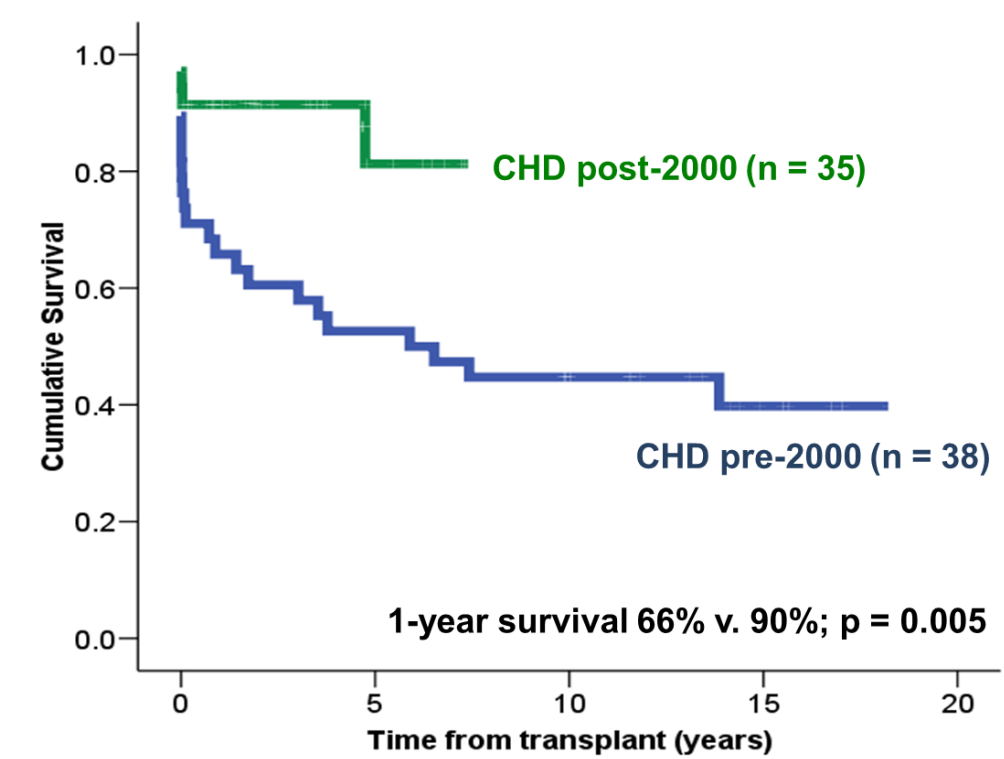

Figure 3.3 Survival following heart transplantation for congenital heart disease. Improving results post-Millennium.

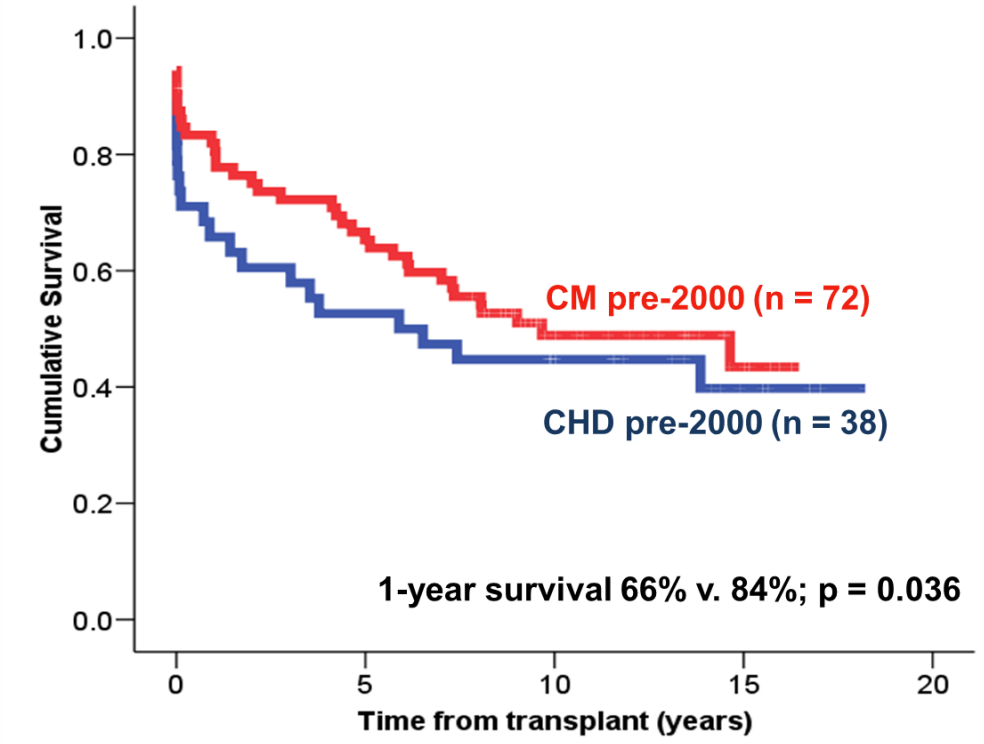

Figure 3.4 Survival following heart transplantation prior to the Millennium. There is a better outlook for patients transplanted for cardiomyopathy than those transplanted for congenital heart disease.

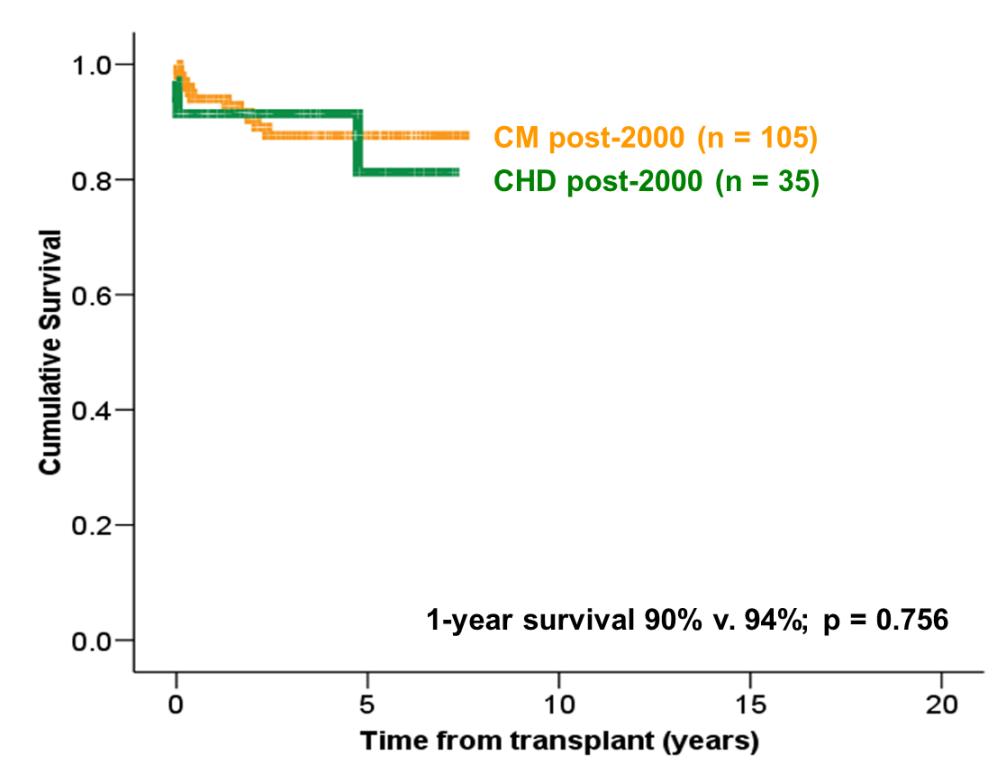

Figure 3.5 Survival following heart transplantation post-Millennium. There is no difference between cardiomyopathy and congenital heart disease patients.

3.3.4 Survival post-TxCHD based on original diagnosis

Figure 3.6 displays survival curves based on univentricular (n = 38) versus biventricular (n = 35) circulations. 1-year post-transplant survival for patients with originally univentricular circulations was similar to those with originally biventricular hearts (75% vs. 78%). 8 of 38 univentricular (21%) and 5 of 35 biventricular (14%) patients died within the first 30 days post-transplant (p = 0.45).

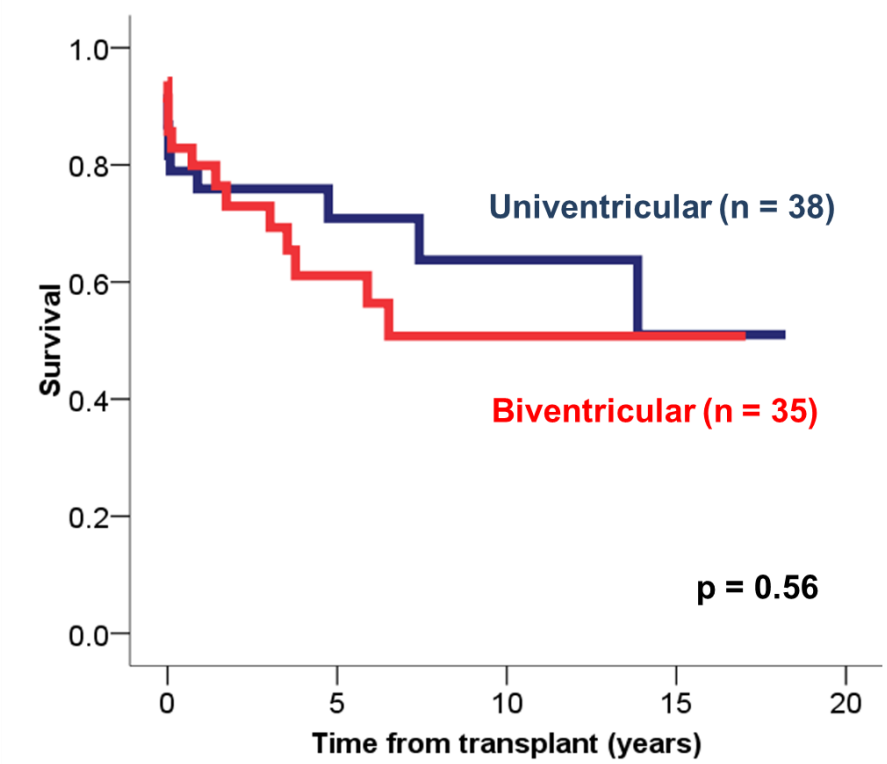

Figure 3.6 Survival following heart transplantation for congenital heart disease, univentricular vs. biventricular anatomies.

3.3.5 Survival post-TxCHD in patients with univentricular circulations based on Fontan repair stage

Table 3.3 illustrates the repair stage of those patients with univentricular circulations at the time of their transplant. Of the univentricular deaths there were 5/15 Fontans, 1/13 Glenns, 4/8 Norwoods (Figure 3.7; p = 0.052). Both patients transplanted as a primary therapy for a univentricular circulation (i.e. with no prior operation), died in the early post-operative period.

Repair Stage	n
No prior operation	2
Norwood Stage 1	8
Glenn	13
Fontan	15

Table 3.3 Repair stage at transplantation of patients with univentricular anatomies.

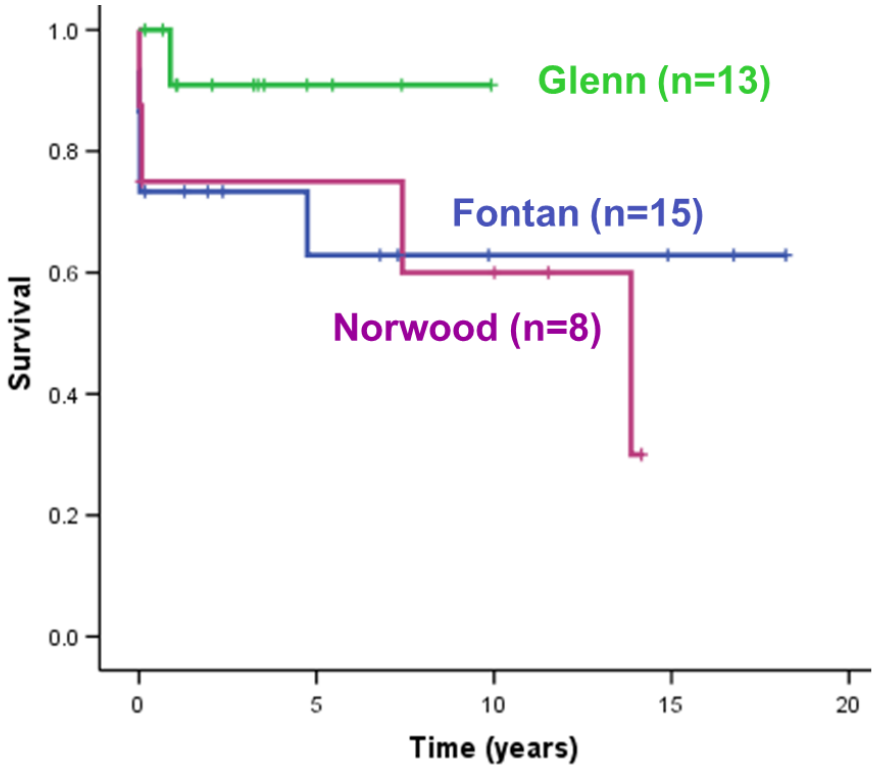

Figure 3.7 Survival of patients with univentricular circulations post-heart transplant.

Since 2001, Great Ormond Street has also been using mechanical support as a bridge to transplant in children, with either extra corporeal membranous oxygenation (ECMO) or Berlin Heart. Although we have successfully used these techniques in many patients with cardiomyopathy, our experience with mechanical support in TxCHD remains limited to two patients. One was a 6 year-old boy with a diagnosis of double inlet left ventricle who had had a total cavo-pulmonary connection performed; the other was a 13 year-old girl who had previously undergone mitral valve repair. Unfortunately, both patients died within 10 days of their operation.

3.4 CONCLUSION

This chapter presents the results of a single-centre paediatric heart transplant programme over a period of 20 years. These data are reassuring for several reasons. Firstly, they show that results of heart transplantation at Great Ormond Street have improved steadily from the early operations performed at the end of the late 1980's. and indicate that the historically high early attrition for transplant for congenital heart disease is no longer present.[94] In addition, transplantation in patients with a failing univentricular circulation was not a survival risk factor in our cohort (Figure 3.6).

There can be little doubt that results for transplantation in the setting of CHD have improved significantly over the last forty years, and, with many of the difficulties involved in this situation now at least partially remediable, the outlook for these patients is encouraging, similar to that of patients transplanted for dilated cardiomyopathy.[97]

Heart transplantation is a complex procedure that requires delicate and insightful understanding of the processes involved in listing for transplantation, preservation of recipient cardiac (and other organ) function, effective use of an increasingly strained donor organ pool, skilful operations, dedicated post-operative intensive care, and careful long-term medical management to prolong graft function. Many of these problems are common to all heart transplantation scenarios, but some are made even more difficult in the CHD population.

3.4.1 Pre-transplant assessment and listing for transplantation

With demand for donor organs at a premium, and the life of a transplanted graft being limited to approximately 15 years on average, the timing of listing for transplantation must be carefully considered, and cannot be premature. However, prolongation of native cardiovascular function may come at the price of other cardiac operations, and potentially additional organ failure, either of which may be detrimental to the success of future transplantation. For instance, post-transplant survival for children with a native single ventricle morphology has previously been shown to be related to pre-transplant operative stage, and some authors have suggested that listing for transplant with a Glenn shunt in situ rather than proceeding to a sub-optimal Fontan circulation may decrease the formation of lymphocytotoxic antibodies, restrict worsening pulmonary vascular resistance, and limit post-transplant complications such as diastolic dysfunction and protein-losing enteropathy, resulting in an overall longer, better quality life.[98-101] With improving long-term outlook for transplant recipients, these arguments hold even more influence. Although the numbers are small, the data in this chapter reaffirm that transplantation following the Glenn operation, but before completion of a Fontan circulation seems to be compatible with the best outlook, with no early or medium term deaths.

Paradoxically, given the increased importance associated with accurate listing for transplant for CHD, patients are often harder to assess. Specifically, it is important to measure pulmonary vascular resistance (PVR) to avoid the potential of donor right heart failure.[101] At Great Ormond Street, a PVR of $\leq$ 6 Woods units.m^2 (or transpulmonary gradient (TPG) $\leq$ 10mmHg) is considered compatible with a good result post-transplant. A PVR of 7-16 Woods units.m^2 (TPG 10-20 mmHg) is potentially more difficult, and requires pre-operative vasodilator testing with prostacyclin or nitric oxide, with the following inferences:

- PVR ≤ 5 Woods units.m^2 (TPG ≤ 10 mmHg) – proceed to transplant
- PVR 5-9 Woods units.m^2 (TPG 10-15 mmHg) – transplantation may convey increased risk
- PVR ≥ 9 Woods units.m^2 (TPG ≥ 15 mmHg) – heart transplantation contraindicated

However, PVR can be hard to estimate in the Fontan circuit, particularly when there is a fenestration and extensive venous collaterals. If problems with pulmonary vascular resistance are probable, it is wise to avoid undersized hearts and long ischaemic times during the operation.[101] Despite these precautions, post-transplant pulmonary vasodilatation with phosphodiesterase inhibitors, nitric oxide or prostacyclin may be required, as well as – in extreme cases of right ventricular dysfunction – mechanical assistance.[102]

3.4.2 Operative differences

Over the evolution of heart transplantation, the donor and recipient cardiectomy and implantation procedures have become largely standardised. However, specific anatomical abnormalities in the recipient with CHD – such as vascular and cardiac size, position and situs – can necessitate modification of each component. Previously, the most complex anatomies would themselves have contraindicated transplantation, despite an individual clinical scenario that would otherwise have been compatible with a successful operation. Surgical ingenuity has largely overcome anatomical contraindications to heart transplant,[103-106] with the possible exceptions of severe pulmonary artery hypoplasia and pulmonary vein stenosis. Crucially, each procedure must be appropriately adapted when considering a recipient with CHD.

With respect to the donor cardiectomy, it is advisable to harvest extended portions of the systemic veins, pulmonary arteries and aorta (in order to facilitate potentially complex anastomoses), and graft trimming should be postponed until implantation. Judicious retrieval may limit the need for additional prosthetic material in heart transplantation, but can hamper transplantation of other organs, notably the lungs. The superior and inferior venae cavae can be opened so as to produce a larger right atrial opening in small recipients, and the donor PA can be split open to enable connection with the distorted or undersized recipient pulmonary vasculature.

Dense pericardial and mediastinal adhesions with marked cardiomegaly in those recipients having undergone previous sternotomy can increase the chance of substantial haemorrhage. In addition, the presence of substernal great vessels and conduits may warrant exposure of the femoral vessels prior to sternotomy as a precautionary measure, permitting immediate femoro-femoral cardiopulmonary bypass in emergency situations.[97] In addition, chronically cyanotic patients may have developed collateral vessels which can be troublesome, especially in the posterior mediastinum.[107] It is also prudent actively to achieve haemostasis prior to implantation of the donor organ.

Pre-implantation, it may be necessary to re-establish normal recipient anatomy with, for instance, a left SVC/innominate vein reconstruction, in the case of bilateral cavopulmonary anastomoses. A case of situs inversus requires a spatial rearrangement of the systemic venous drainage, by the formation of a bicaval connection using the donor innominate vein and left-sided SVC;[108] it is also useful to open the left pleural space to accommodate the normally sited donor organ. These extra elements to the operation must be anticipated and corrected for in order to synchronise the donor and recipient components of transplantation, and avoid prolonging ischaemic times.

3.4.3 Post-operative complications

Post-operatively, CHD transplant recipients face the complications common to all heart transplants of, for instance, multi-organ failure, rejection, infection, coronary allograft vasculopathy, and – in the paediatric age group especially – non-compliance.[92] However, specific issues are more problematic in CHD patients. Higher rates of post-operative bleeding, infection and wound dehiscence in those having undergone previous thoracic procedures have been predicted, and, although studies have reported differing relative risks for this population,[97, 98, 109] they must be foreseen in this group. Years of sub-optimal end-organ perfusion also increase the chances of significant post-operative renal failure; the limited experience at Great Ormond Street of extracorporeal support as a bridge to transplant has suggested that this may optimise end organ function, in the immediate post-transplant phase at least. The effect this has on later function is currently unknown.

3.4.4 Lymphocytotoxic antibodies

The formation of lymphocytotoxic antibodies in response to repeated blood transfusion[110] or homograft tissue[111] in previous procedures is of particular relevance in the CHD group, and has been linked to increased rejection and worse actuarial survival post-transplant.[112] If feasible, it is important to limit pre-sensitization by restricting blood transfusion and homograft use[97] in patients with CHD. Various pre- and post-transplant protective measures, such as immunoglobulins, cytolitics, plasmapheresis, cyclophosphamide and rituximab have been attempted in the event of pre-sensitised patients, with varying success.[81, 83, 84] Again, with more transplants being performed on patients with multiple previous operations, this is likely to be a problem that increases over the coming years, and one that needs to be addressed actively.

It is also vital to recognise that a significant proportion of children with CHD will go on to need a transplant after they have been discharged into the care of adult cardiology services. However, it is not uncommon for adults with congenital heart disease with heart failure or a failing Fontan circulation to not be listed for transplant. This may be for a variety of reasons including abnormal renal function, sensitization to HLA antibodies, multiple previous surgical procedures, and complex anatomy requiring extensive repair. Careful surveillance of other organ function during childhood years, avoidance of liberal use of blood products and homograft tissue (with the potential adverse effects of immune modulation) and the availability of surgical experience with complex anatomical repair may ameliorate some of the problems.

Transplantation saves lives and improves the quality of life for many recipients. The gap between the number of organs needed and the supply of donors needs to be addressed. However, the lack of adult cardiac transplant centres with congenital experience on site remains a practical issue.

3.5 SUMMARY

Despite the problems associated with taking more marginal donors, and operating on more chronically and acutely sick patients, this chapter illustrates that the immediate and long-term outcomes for transplantation for congenital heart disease continue to improve. Noticeably, the historical discrepancy between prognosis following transplantation for CHD and cardiomyopathy is diminishing convincingly. This success is the result of specific advances in the understanding and management of CHD and heart transplantation, and the implementation of these by dedicated surgical, medical and intensive care teams. However, the heterogeneity of this population – even within sub-groups defined by original diagnosis or operative stage – makes it very difficult to perform robust longitudinal studies capable of identifying predictive factors for survival and improving allocation of scarce resources.

CHAPTER 4

PRE-IMPLANTATION BASILIXIMAB AS INDUCTION IMMUNOSUPPRESSION IN PAEDIATRIC HEART TRANSPLANTATION

Basiliximab is an anti-CD25 monoclonal antibody used as induction therapy in solid-organ transplantation. This chapter aims to determine whether basiliximab is beneficial in preventing early heart allograft rejection, and also if administration prior to cardiopulmonary bypass has additional protective effects. The effect of pre-implantation basiliximab on CD25 count and on acute rejection in children undergoing cardiothoracic transplantation is assessed. The notes of all children undergoing cardiothoracic transplantation at Great Ormond Street Hospital between January 2000 and June 2007 were retrospectively reviewed. 121 heart transplant recipients were included: 29 patients did not receive basiliximab; 33 patients received basiliximab after coming off cardiopulmonary bypass (CPB); and 59 patients received basiliximab prior to organ implantation. All patients receiving basiliximab had an effectively suppressed CD25 count (< 0.2%) on Days 1 and 10 post-transplant. Freedom from Grade 3A or greater rejection in the first year was significantly greater in the pre-implantation basiliximab group than in the post-implantation and no-basiliximab groups (95%, 70% and 72%, respectively; p = 0.02). Induction regimen was the only significant explanatory variable after multivariate Cox regression. The results of this chapter

confirm that basiliximab is effective at suppressing CD25 count whether given pre- or post-CPB. Basiliximab before transplantation appeared to reduce acute rejection, whereas post-CPB administration did not suggest similar effects. These findings would benefit from independent validation in randomised trials and further studies should seek to mechanistically delineate these observations.

4.1 INTRODUCTION

Anti-lymphocyte induction has been used in cardiothoracic transplantation for more than 20 years, with demonstrated safety, efficacy and reduction in the incidence of acute rejection episodes in heart[113] and other solid-organ transplantation.[114, 115] At many centres, children undergoing cardiothoracic transplantation currently receive either anti-thymocyte globulin (ATG),[116] or an interleukin-2 receptor antagonist (IL-2Ra), such as basiliximab.[117] The more specific immunosuppressive action of basiliximab has resulted in reduced rates of infectious deaths and post-transplant lymphoma compared with ATG, but studies aimed at showing a reduction in mortality or rejection have been inconclusive.[113, 118-120] The majority of research in cardiothoracic transplantation has investigated a two-dose regimen starting after the recipient has come off cardiopulmonary bypass (CPB), due to concerns over wash-out of the drug.

Great Ormond Street Hospital began using basiliximab in 2001, initially utilising a two-dose regimen on Day 0 (coming off bypass) and Day 4 post-operatively. In 2002, the protocol was changed on the basis that it was unlikely that a monoclonal antibody would be washed out by CPB, and because it is given before implantation in other solid-organ transplantation. After this change, the first dose would be given after confirmation of the donor organ immediately before the patient went on CPB.

The purpose of this chapter was to ascertain whether timing of basiliximab administration determines efficacy of CD25 suppression, or influences the incidence of acute rejection.

4.2 METHODS

4.2.1 Patients and Treatment Protocol

The notes of all children undergoing cardiac transplantation (n = 142) at Great Ormond Street Hospital (GOS) between January 2000 and September 2007 were reviewed with regards to CD25 count, post-operative infection, allograft rejection and mortality. At GOS, CD25 counts were measured on the first day post-transplant and thereafter weekly until discharge.

Exclusion criteria included insufficient data on drug administration (n = 9), insufficient CD25 count data (n = 1), insufficient biopsy data (n = 4), previous solid-organ transplantation (n = 2), metabolic disease (n = 2) and death or graft failure within 24 hours of transplantation (n = 3). In total, 121 children undergoing cardiac transplantation were included, divided into three groups by transplantation era:

1. Children who did not receive basiliximab (n = 29). 14 of these patients receive anti-thymocyte globulin. Analysis of these patients showed no difference in outcomes compared to patients with no induction therapy; they were therefore considered as one group to aid statistical testing.
2. Children who received the first dose of basiliximab after coming off CPB (n = 33).
3. Children who received the first dose of basiliximab prior to organ implantation (n = 59).

Those patients given basiliximab received a second dose on Day 4 post-operatively. All patients received primary immunosuppression (ciclosporin, tacrolimus or sirolimus), an anti-proliferative agent (mycophenolate mofetil [MMF] or azathioprine) and prednisolone. These changes were made at the institutional level and saw tacrolimus replace ciclosporin and MMF replace azathioprine because of better side-effect profiles. Sirolimus was used as primary immunosuppression when there were concerns over severe renal impairment. All patients transplanted after 2002 underwent a standard

biopsy schedule of 3 biopsies in the first 6 months, regardless of clinical status, with subsequent biopsies only if clinically indicated or to follow up rejection.[121]

4.2.2 Study Outcome Measures

The primary end-points were CD25 counts on days 1 and 10 post-surgery and freedom from acute graft rejection, defined as Grade ≥ 3A. When biopsy was contraindicated due to clinical instability, a clinical diagnosis was also counted. Secondary end-points were culture-positive infection in the first month after surgery, defined as any positive blood, respiratory tract, or urine culture, incidence of post-transplant lymphoproliferative disease (PTLD) and all-cause mortality.

4.2.3 Statistical Analysis

The full analysis set was used for all statistical analyses. Demographic and baseline characteristics were compared using 1-way analyses of variance (ANOVAs) for continuous data, and chi-square tests for categorical data. The primary outcome measure was analysed using Kaplan–Meier curves and the log-rank (Mantel–Cox) test. Of the secondary outcome measures, PTLD and infections were analysed using chi-square tests, and all-cause mortality was investigated using Kaplan–Meier curves. The possible effects of confounding variables (recipient age at transplant, donor-recipient cytomegalovirus mismatch status, primary immunosuppression received, anti-proliferative agent received) on freedom from graft rejection and on mortality were analysed using multivariate Cox regression analyses. The data set was right-censored for all survival analyses. All analyses were performed using SPSS, version 14.0 for Windows (SPSS, Inc., Chicago, IL).

4.3 RESULTS

4.3.1 Demographics and Baseline Characteristics

Demographics and baseline characteristics are displayed in Table 4.1. Two independent variables differed significantly between the groups: mean recipient age at transplantation and recipient cytomegalovirus (CMV) status. In light of evidence that the effect of recipient age is non-linear,[67] this variable was also analysed categorically with children divided into infants < 12 months of age and others; this was not significantly different between the groups. Both primary immunosuppression and anti-proliferative drug usage differed significantly by induction regimen, with tacrolimus use comparable in those children receiving post-operative (85%) and pre-operative (91%) basiliximab, but lower in those not receiving the drug (31%), and MMF use much higher in those receiving pre-operative (84%) than post-operative (21%) or no (7%) basiliximab. Mean follow-up was 40.7 ± 27.6 months.

	No basiliximab (n = 29)	Post-operative basiliximab (n = 33)	Pre-implantation basiliximab (n = 59)	p
Recipient characteristics				
Mean age ± SD (years)	10.8 ± 5.5	8.8 ± 6.0	6.9 ± 5.5	0.01
Infants < 1 year (%)	4 (14%)	2 (6%)	9 (15%)	0.42
Male %	48	36	48	0.53
CHD:CM %	24:76	18:82	31:69	0.42
% CMV positive: negative: unknown	52:41:7	33:64:3	24:76:0	0.02
Donor characteristics				
Mean age±SD (years)	16.9 ± 10.8	18.3 ± 14.5	16.3 ± 14.2	0.78
Male %	52%	49%	39%	0.77
CMV D+/R- %	15%	14%	20%	0.50
ABO mismatch %	0%	12%	15%	0.09

Table 4.1 Demographics and Baseline Characteristics. CHD – congenital heart disease; CM – cardiomyopathy; CMV – cytomegalovirus; CMV D+/R- – CMV donor IgG positive/recipient IgG negative.

4.3.2 CD25 Counts

All patients receiving basiliximab had a CD25 count of < 0.2% within the first 24 hours and for at least 10 days post-transplant, indicating effective early T-lymphocyte suppression.

4.3.3 Acute Rejection

Overall freedom from acute rejection within 12 months of surgery was 76% in the no-basiliximab group (22 of 29 children), 76% in the post-implantation basiliximab group (25 of 33 children) and 95% in the pre-implantation basiliximab group (56 of 59 children), which indicated a significant difference between the groups (log-rank test, p = 0.016). Induction regimen was the only significant explanatory variable (p = 0.023, Exp(B) 0.367, 95% confidence interval 0.154-0.873) after Cox regression analysis, controlling for the effects of possible confounding variables (chi-square 16.4, p = 0.021 for model).

There were 4 episodes of early acute rejection between 5 and 10 weeks post-transplant in our sample: 1 of these patients received ATG and 3 received pre-operative basiliximab. The only child of the 4 who received MMF also received sirolimus.

Extending the analysis to the entire follow-up period gave rates of freedom from acute rejection of 72% in the no-basiliximab group (21 of 29 children, mean follow-up 68.9 ± 25.2 months), 70% in the post-implantation basiliximab group (23 of 33 children, 50.4 ± 18.8 months) and 95% in the pre-implantation basiliximab group (56 of 59 children, 21.4 ± 15.3 months). This indicated a significant difference between groups (log-rank test, p = 0.01), as shown by Kaplan–Meier estimates (Figure 4.1). Multivariate Cox regression was performed as before. The final model was significant (chi-square 17.6, p = 0.02) and induction regimen was the only significant explanatory variable (pre-implantation basiliximab vs. no basiliximab: hazard ratio 7.3 [95% confidence interval 1.2-45.5]; pre-implantation basiliximab vs. post-implantation basiliximab: hazard ratio 5.4 [95% confidence interval 1.0-26.1]).

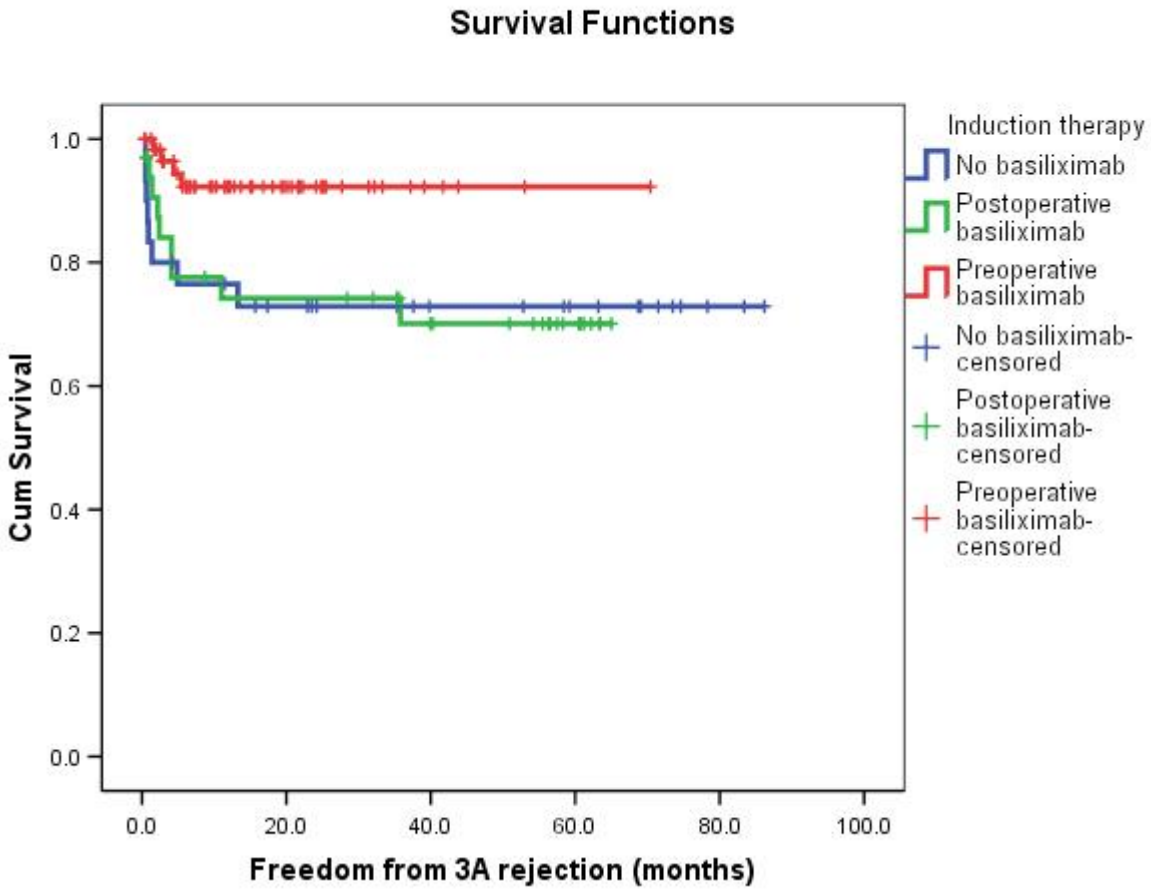

Figure 4.1 Kaplan–Meier survival curve showing freedom from acute rejection of Grade 3A or worse by induction regimen. Children who received pre-operative basiliximab were more likely to be free from acute rejection both within the 12 month pre-transplant and throughout the entire follow-up period when compared with children who received post-operative basiliximab or who did not receive basiliximab. These latter two groups had very similar rejection profiles at both 12 months and throughout the entire follow-up period.

4.3.4 Secondary End-points

There was no significant difference in culture-positive infections in the first 2 months between the groups (14%, 12% and 15%; log-rank, p = 0.92), nor in all-cause mortality rates (21%, 9% and 3%; log-rank, p = 0.24) (Figure 4.2). One child who received post-operative basiliximab developed PTLD 19 months post-transplant.

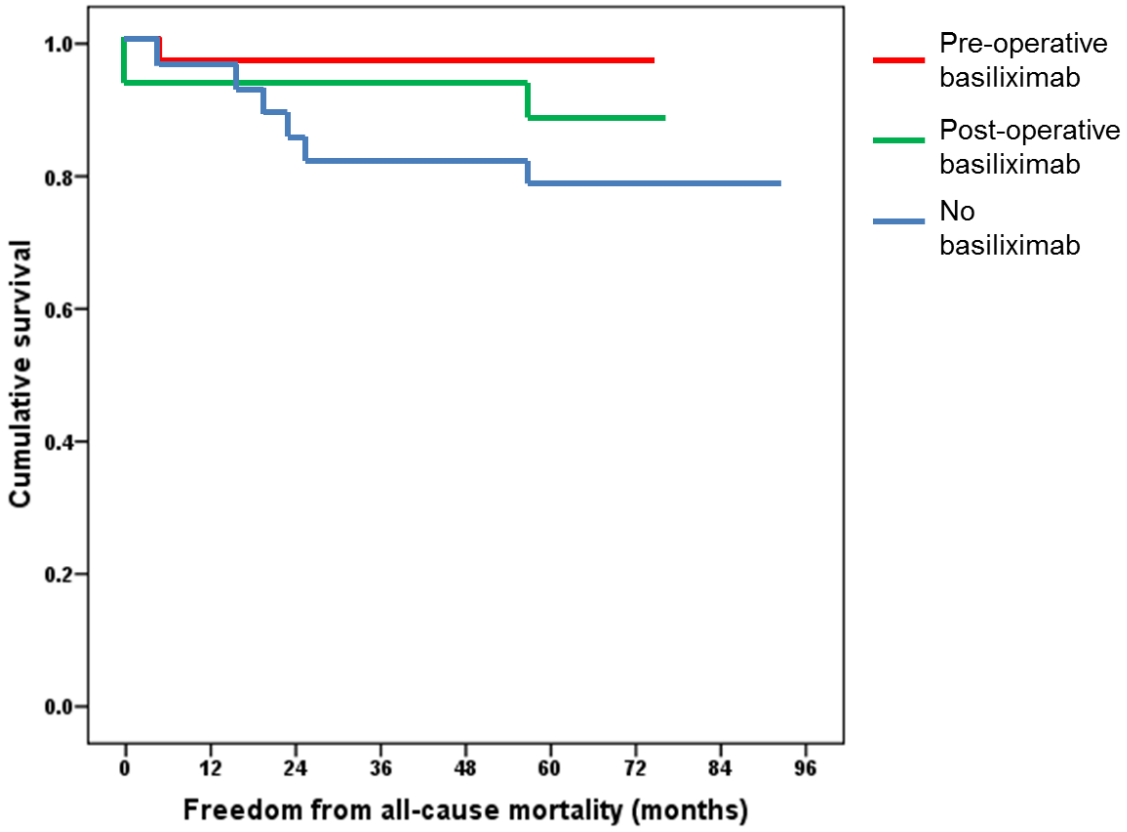

Figure 4.2 Kaplan-Meier curve showing all-cause mortality by induction regimen.

In light of the differences in other immunosuppressants received, as well as including primary immunosuppression and anti-proliferative type as variables in the main analysis, separate univariate analyses were constructed and showed no significant effect on rejection within the first year for primary immunosuppression (log-rank, $p = 0.12$) nor anti-proliferative agent (log-rank, $p = 0.19$). Neither had a significant effect over the whole follow-up period (primary immunosuppression: log-rank, $p = 0.31$; anti-proliferative agent: log-rank, $p = 0.12$). Analyses of binary groups comparing tacrolimus to any other agent (ciclosporin or sirolimus), and MMF to any other agent (azathioprine or nothing) were similarly not significant. Given the particular evidence for MMF, Kaplan–Meier curves illustrating rates of rejection among children who did and did not receive MMF were constructed for subgroups of children. The data suggest that children receiving postoperative basiliximab had slightly higher rates of acute rejection with MMF, and those receiving pre-operative basiliximab had slightly lower rates, but log-rank tests were non-significant in both cases. We also obtained data on calcineurin levels for the majority ($n = 106$, 87.6%) of children, but these are trough levels used to guide dosage rather than an indicator of level of immunosuppression. Calcineurin levels were nonetheless not significant on univariate analysis.

4.4 DISCUSSION

This chapter has demonstrated that basiliximab is effective at suppressing CD25 counts after paediatric heart transplantation, regardless of whether it is given before or after CPB. It further suggests that basiliximab is more effective at reducing rates of rejection if given before implantation.

In cardiothoracic transplantation, basiliximab is frequently given when coming off bypass due to concerns over "wash-out" of the drug during CPB, although the original research looking at basiliximab used a preoperative dose,[122] and the manufacturer, U.S. Food and Drug Administration (FDA) and British National Formulary (BNF) all advise giving the first dose pre-operatively. The CD25 results in our sample allay these concerns over wash-out, with all patients having a CD25 count of < 0.2% on the first day post-operatively, and none showing a rise in CD25 before day 10. It is of interest to note that there are data in renal transplantation suggesting that a single pre-operative dose is as effective as a two-dose protocol.[123]

The exact mechanism of basiliximab is unclear. It is a high-affinity chimeric monoclonal antibody that is a selective competitive antagonist to the alpha-chain (CD25) of the interleukin-2 receptor, which is present only on activated T-lymphocytes (IL-2Ra);[122] it may also reduce activation of T-lymphocytes. By preventing replication it reduces activation of B cells and thus inhibits antibody production. Basiliximab therefore avoids the broad cross-reactivity seen with older immunosuppressants such as ATG, and this selectivity appears to result in a reduced incidence of post-operative infections and infectious deaths,[119] and of post-transplant lymphoma;[124] it may also decrease rejection rates in critically ill children in whom calcineurin inhibitors cannot be used in normal doses.[125]

Previous studies investigating the use of basiliximab in cardiac transplantation have been inconclusive as to its efficacy compared with other agents at preventing clinically significant acute rejection,[113, 119, 120] although most of these studies gave the first dose of basiliximab post-operatively. Rates of acute rejection in this data set were markedly lower when basiliximab treatment was started before implantation, with the hazard ratios from our sample suggesting that children receiving no basiliximab were 7.3-fold more likely to undergo rejection during follow-up compared with those receiving the drug pre-implantation, and children who received basiliximab post-operatively were 5.4-fold more likely to undergo rejection. There are no other studies in cardiac transplantation in which basiliximab was administered pre-operatively, although there are studies in adult kidney[126] and liver[127] transplantation that indicated improvement compared with placebo when pre-operative basiliximab was given. Interestingly, there is evidence from renal transplantation that giving both basiliximab and low-dose ATG offers better rejection outcomes than one or the other.[128]

One possible explanation for our results is that pre-operative basiliximab prevents early immunologic insult to the donor organ, which would otherwise occur immediately after implantation, and there is evidence that activated IL-2 receptors are internalized in about 15 minutes.[129] Post-operative protocols vary, with the majority specifying that the first dose of basiliximab should be given on "Day 0" post-transplant, meaning that there is a window of opportunity for T-cell replication. It is possible that immunologic pathways have time to become activated peri-operatively, and that this process may be prevented by a pre-operative decrease in CD25 cells. Other investigators have demonstrated an advantage to starting ATG pre-operatively rather than post-operatively in renal transplantation,[130] suggesting any effect may not be specific to basiliximab.

There was a non-significant trend toward lower mortality in the pre-operative basiliximab group when compared with the no-basiliximab group, but not when compared with post-operative basiliximab, as illustrated in Figure 4.2. Although the follow-up in this study was limited, early acute rejection is known to be a risk factor for mortality in paediatric heart transplantation,[67] and longer follow-up of this cohort may demonstrate a mortality benefit in the pre-operative group.

There was no post-operative increase in infection in the pre-implantation basiliximab group, despite the decrease in acute rejection. Although this is a crude surrogate of immune function, it suggests that this group was not over-immunosuppressed in the first 2 months relative to the other drug regimens, which strengthens the argument that the explanation for the different rates of rejection lies in an early immunologic event prevented by pre-implantation induction. The fact that I did not show an increase in lymphoma or infection related death in the ATG group may be due to the relative small sample size and limited follow-up period.

4.4.1 Limitations

This study is limited by its retrospective nature, which prevented a comparison of pre-operative basiliximab to pre-operative ATG. The different groups also received a variety of other immunosuppressive medications, and other studies have demonstrated reduced rates of rejection with newer anti-proliferative agents and cell-cycle inhibitors. In this sample, neither primary immunosuppression nor anti-proliferative type was predictive of rejection within the three therapeutic groups on univariate analysis, and they were not significant in the main (multivariate) Cox regression analyses. However, there is evidence from the area of paediatric renal transplantation that co-administration of basiliximab and MMF extends the duration of CD25 saturation from 5 to 10 weeks, with the majority of acute rejection episodes occurring in this time window,[131] and only 1 of the 4 children in this sample who had early acute rejection episodes received MMF. There are also no data on pre-operative CD25 levels, and the study is therefore based on the assumption that patients had normal CD25 counts prior to transplantation.

Over the 6 years of the study, the average age at which patients were being transplanted decreased. However, the effect this had on graft survival was not statistically significant, and Cox regression analyses showed the hazard was in any event small (hazard ratio 1.08, 95% confidence interval 0.97 to 1.20).

There was one case of severe anaphylaxis in a 14-month-old child given basiliximab previously at an aborted transplant—an experience reported previously[132] and common to other findings in solid-organ transplantation.[133] This reinforces the importance of

delaying basiliximab until it is certain that the donor organ is suitable to avoid the risk of sensitization and anaphylaxis at subsequent transplant.

4.5 SUMMARY

In conclusion, these results demonstrate that pre-operative administration of basiliximab in paediatric cardiothoracic transplantation is feasible and safe, and it is effective at reducing CD25 counts despite subsequent CPB. Although the retrospective nature of this study necessitates caution in the assessment of rejection, there was a significantly lower incidence of acute rejection in cardiac recipients when compared with post-implantation administration, and this may prove beneficial to the long-term graft function of these patients. A randomized, controlled trial is needed to test the hypothesis that basiliximab is more effective at reducing episodes of acute rejection when given pre-implantation, and to establish whether reductions in acute rejection improve long-term graft function.

CHAPTER 5

EXTRA-CORPOREAL LIFE SUPPORT AS RESCUE THERAPY FOLLOWING PAEDIATRIC HEART TRANSPLANTATION

Extracorporeal life support (ECLS) is frequently employed following conventional cardiac surgery, with proven success. Its use following paediatric heart transplantation, however, is less well documented. This chapter reviews ECLS following paediatric heart transplantation, to understand better predisposing factors, morbidity and mortality connected to ECLS. The notes of all patients at Great Ormond Street Hospital undergoing orthotopic heart transplantation from 1999-2009 were reviewed (202 transplants; age 0.06-17.91 years). Patients were grouped by pre-transplant diagnosis: restrictive cardiomyopathy (n = 17), non-restrictive cardiomyopathy (n = 134) and anatomical heart disease (n = 51). 28 patients required ECLS post-transplantation (13.9%). Those requiring ECLS had a longer ischemic time (4.2 vs. 3.7 hours, p = 0.02). 35.3% of RCM patients required post-operative ECLS – a significantly higher proportion than for DCM (10.4%) or anatomical heart disease (15.7%) (Pearson's chi-square 7.99; p = 0.02). On multivariate logistic regression analysis, factors associated with post-transplant ECLS were RCM, longer ischemic time and ECMO pre-transplant. Graft survival was significantly higher in the non-ECLS group, with a one-year survival of 98.2% vs. 57.7% and five-year survival of 84.5% vs. 57.7%. The requirement for

ECLS was much higher in this cohort than would be expected for conventional cardiac surgery. Although just over one-half of patients requiring ECLS post-transplant survived to discharge, they had an excellent medium-term survival, with all still alive. ECLS is an expensive, invasive therapy, with significant morbidity and mortality. However, without ECLS, it is likely that those patients would have perished. Its judicious use, therefore, can be recommended in this setting.

5.1 INTRODUCTION

Modern improvements in cardiac surgery, intensive care and transplant medicine have improved the outcomes for paediatric heart transplant recipients over the last two decades.[134] In addition, conventional cardiac surgical techniques are able to palliate children born with congenital heart disease that previously would have not survived beyond infancy, a proportion of whom will come to the point where transplantation is their only therapeutic option.[135] Unfortunately, in contrast to this growing pool of potential recipients for paediatric heart transplantation, donation rates are relatively static.[136] This imbalance has led to the difficult situation of transplant programmes being burdened with a more heterogeneous – and therefore more technically demanding – group of transplant candidates waiting for longer on the transplant list (and becoming more unwell, with in particular potential for increasing pulmonary vascular resistance (PVR)), and being forced to accept donor organs from marginal donors.

The consequence of these shifts in recipient morbidity and donor quality is further strain on the critical immediate post-operative period, increasing the possibility of early graft failure. To combat this, transplant programmes have increasingly had to rely on additional rescue measures, such as nitric oxide and extra-corporeal life support (ECLS)[137] to attempt to support the circulation whilst waiting for a donated graft to recover. The indications, risk factors and outcomes of the use of ECLS in this setting are not well documented, particularly in the paediatric population. This chapter was designed to review the success of ECLS as a rescue therapy post-paediatric heart transplantation over the last decade at GOS, and to uncover any risk factors pre-disposing patients to the need for ECLS.

5.2 METHODS

5.2.1 Study Group

The study is a retrospective review of all paediatric orthotopic heart transplants at Great Ormond Street Hospital over an 11-year period from January 1999 to December 2009 inclusive, using institutional transplant and ECLS databases. The start of the study period reflected the date at which ECLS became a standard treatment option for graft failure immediately post-transplant at our hospital. During this period, 202 orthotopic transplants were performed (103 female recipients), including 8 re-transplants (age at transplantation 0.06-17.9 years (median 9.6 years)); in addition, 2 heterotopic heart transplants were conducted – these were not considered for analysis. The group was divided based on pre-transplant diagnosis (Figure 5.1): of 151 transplants for cardiomyopathy, 17 were for restrictive cardiomyopathy – the RCM group. Dilated (n = 129) and hypertrophic (n = 5) cardiomyopathies were considered as one – the non-restrictive cardiomyopathy group (n = 134). The third group was transplants for anatomical disease (n = 51, 48 of which were congenital heart disease, and 3 for acquired surgical heart disease: two patients with non-congenital valve pathology secondary to Marfan's disease and one with infective endocarditis).

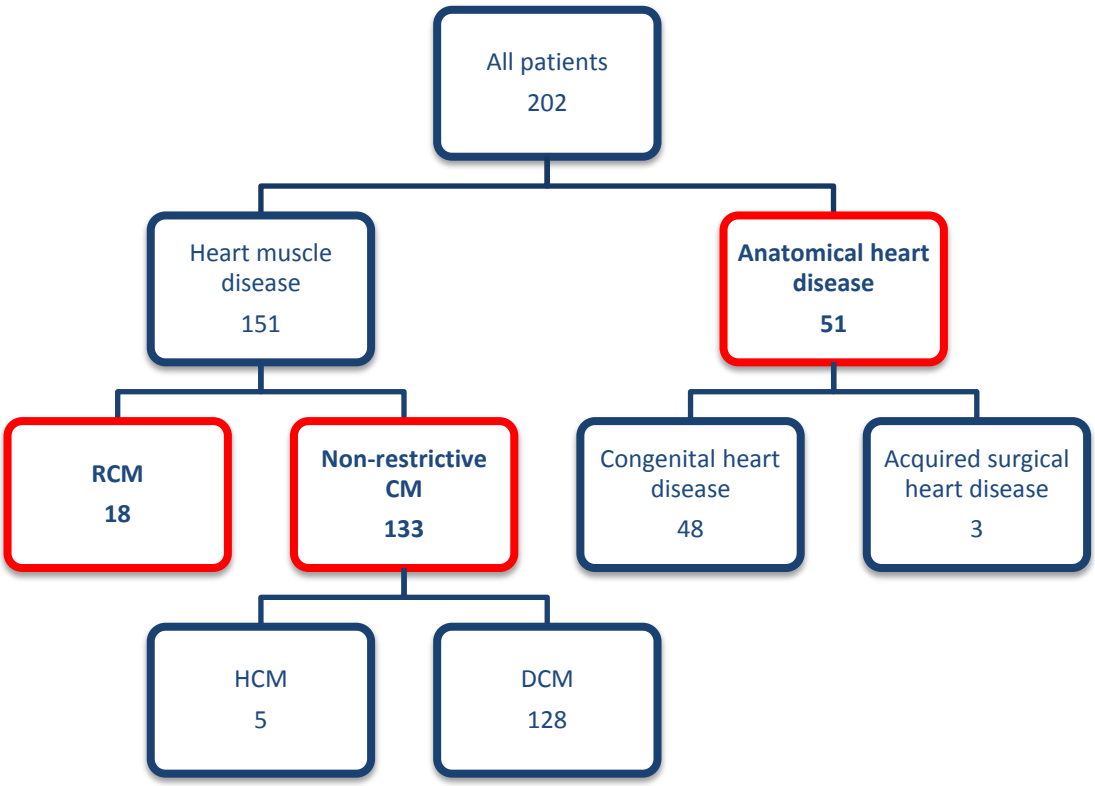

Figure 5.1 Study population divided by pre-transplant diagnosis. RCM – restrictive cardiomyopathy, HCM – hypertrophic cardiomyopathy, DCM – dilated cardiomyopathy. Numbers indicate the number of patients in each group. Red boxes indicate the final groupings used in analysis.

5.2.2 Local Transplant Protocol

Pre-transplant work-up involves formal catheter-based PVR measurements for any potentially high-risk patients, including those with suspicion of pulmonary hypertension on echocardiography, and all patients with restrictive cardiomyopathy. During the study period, 25 patients had a ventricular assist device prior to transplantation (all cardiomyopathies), and 38 patients had a period of extra-corporeal membranous oxygenation (ECMO); 9 patients overlap both groups, in that they had a period of ECMO followed by VAD. In total, 54 patients had some sort of mechanical support before transplantation.

5.2.2.1 Recipient Operation

Through a median sternotomy, cardiopulmonary bypass is initiated by venous drainage via cannulas in the SVC and IVC, and arterial return via an ascending aorta cannula at the level of the innominate artery. Body cooling is carried out to 32◦C, unless deeper hypothermia is requested for correction of complex heart defects (such as aortic arch reconstruction).

The aorta is cross-clamped. The heart is excised by dividing the right and left atrial walls close to the atrioventricular groove and the atrial septum, leaving a cuff of atrial wall to allow suture of the donor heart. For the bicaval anastomosis technique, the SVC and IVC are transected with a rim of atrial tissue on each. The rest of the right atrium is excised. The aorta and the main pulmonary artery are divided as close to their respective valves as possible. Subsequently, these vessels may be trimmed before being sutured to their counterparts of the donor heart. The donor heart is placed over the left side of the divided sternum, parallel to the remnant of the excised recipient heart. The donor heart is rotated to the left, so that its posterior surface faces anteromedially, such that the free walls of both recipient and donor left atria lie adjacent to each other. They are sutured to each other, using continuous polypropylene, starting at the base of the left atrial appendage of the donor heart and at a point close to the caudal end of the recipient left superior pulmonary vein. At a convenient stage, the donor heart is drawn down into the pericardium and the suture tightened. The suturing is continued around the superior and inferior walls of the left atrium onto the atrial septum. For the bicaval anastomosis, each vena cava is anastomosed individually. This technique requires meticulous attention to avoid purse-stringing or kinking of the SVC. Because of this risk, right atrial anastomosis may be another option of connection in small infants.

Aortic and pulmonary anastomoses are done in standard fashion. The left heart is meticulously de-aired and the cross-clamp is removed. Rewarming is started at this stage. The caval snares are released and the right heart is also de-aired. Monitoring lines are placed in the left atrium and the pulmonary artery. Right atrial and right ventricular pacing wires are inserted. When the myocardial function appears satisfactory, the patient can be weaned off the cardiopulmonary bypass.

5.2.2.2 Post-operative Care

Standard early post-operative care included inotropic support with milrinone, and low-dose epinephrine infusion, if required. Recipients that were suspected of having an elevated pulmonary vascular resistance were commenced on inhaled nitric oxide from the operating room, and this is weaned off during recovery. In recipients that developed renal failure manifested by oligoanuria, fluid overload or electrolyte imbalance, renal support in the form of continuous veno-veno hemofiltration was initiated in a timely fashion. Where conventional intensive care supports were ineffective and the circulation was failing, mechanical circulatory support was commenced, in the majority of cases with ECMO via either transthoracic or peripheral cannulation depending on patient factors and operator preference.

5.2.2.3 Immunosuppression

Over the study period, the immunosuppression regime evolved from induction with anti-thymocyte globulin to basiliximab, and standard maintenance triple immunosuppression from ciclosporin to tacrolimus, and azathioprine to mycophenolate mofetil, in addition to prednisolone, which – in the absence of significant rejection – is weaned over the first three months.

5.2.3 Study Outcome Measures

The primary outcome measures were (1) need for ECLS in the early post-transplant phase (defined as within 30 days of transplantation), and (2) graft loss, comprising death or re-transplantation. Secondary outcome measures were length of intubation, length of in-hospital stay, cerebrovascular accident, seizure (clinical or on selective EEG monitoring) and laboratory proven infection post-transplant.

5.2.4 Statistical Analysis

All statistical analyses were run using the entire data set, unless stated. The transplants were separated into those requiring ECLS immediately post-transplant ("ECLS group") and those without ("non-ECLS group"). Demographic and pre-transplant parameters were compared using two-sample Wilcoxon rank-sum (Mann-Whitney U) tests for continuous data, and Chi-squared tests for categorical. There was a small number of missing variables: ischemic time was missing in 2 patients, donor weight (and therefore donor:recipient weight ratio) in 4, and hours intubated in 3. Graft survival was analysed with Kaplan-Meier curves and the log rank (Mantel-Cox) test. Factors associated with the need for post-transplant ECLS were analysed with a multivariable logistic regression analysis. A p-value < 0.05 was considered significant. All analyses were performed using Stata v.12 (StataCorp LP, Texas, USA).

5.3 RESULTS

5.3.1 Need for ECLS

Of 202 orthotopic heart transplants in this study, 28 (13.9%) resulted in post-transplant ECLS; 24 were supported with veno-arterial extra-corporeal membranous oxygenation (V-A ECMO) only, 2 with right ventricular assist devices (RVAD) only, and 2 with V-A ECMO followed by RVAD. Primary indications for ECLS were primarily left ventricular failure (n = 2), right ventricular failure (n = 10), biventricular failure (n = 12), cardiac arrest (n = 2) and arrhythmia (n = 2). Of the 26 patients commenced on V-A ECMO, 8 had neck cannulation, 2 had neck and femoral cannulation, and 16 were cannulated trans-thoracically. A left atrial vent was used in 9.

5.3.2 Pre-transplant and Transplant Variables

Pre-transplant variables are shown in Table 5.1. There was no significant difference between the ECLS and non-ECLS groups with respect to age, sex, or VAD pre-transplantation. Re-transplantation tended to be more common in the ECLS group (7.1% vs. 3.4%), but the difference did not reach statistical significance. Patients requiring ECLS post-transplant were significantly more likely to have required ECMO prior to transplantation (35.7% vs. 16.1%, p = 0.01).

Variable	Non ECLS group (n = 174)	ECLS group (n = 28)	p
Age (years)	9.6 (0.1 – 17.9)	10.0 (0.7-17.0)	0.65
Female Sex	97 (55.7%)	14 (50%)	0.57
ECLS prior to HTx	28 (16.1%)	10 (35.7%)	0.01
VAD prior to HTx	21 (12.1%)	4 (14.3%)	0.74
Re-transplant	6 (3.4%)	2 (7.1%)	0.32

Table 5.1 Pre-transplant variables. Continuous variables are given as median followed by range in parenthesis unless stated; categorical variables are given as n followed by percentage of each group fulfilling the variable in parenthesis. ECLS – extracorporeal life support, VAD – ventricular assist device, HTx – heart transplantation.

Transplant variables are displayed in Table 5.2. There was no statistical difference between the groups with respect to donor or recipient weight or ratio (as a continuous variable), or ABO mismatch. However, it should be noted that patients requiring ECLS were more likely to have an extreme donor:recipient weight ratio (i.e. $\geq$ 3, when considering it as a categorical variable). In addition, those patients requiring ECLS post-transplant had significantly longer median ischemic time (4.15 vs. 3.74 hours, p = 0.02).

Variable	Non ECLS group (n = 174)	ECLS group (n = 28)	p
ABO mismatch	17 (9.8%)	2 (7.1%)	0.69
Recipient weight (kg)	23.1 (3.1 – 95.5)	30.9 (6.0 – 74.7)	0.27
Donor weight (kg)	50.0 (2.0 – 90.0)	55.0 (10.0 – 90.0)	0.22
Donor age (years)	16 (0.3-56)	21 (3-53)	0.08
D:R weight ratio	1.69 (0.65 – 3.73)	1.65 (0.70 – 4.00)	0.95
D:R weight ratio ≥ 3	15 (8.6%)	6 (21.4%)	0.04
Ischemic time (hours)	3.7 (1.1 – 7)	4.2 (1.5 – 8)	0.02

Table 5.2 Transplant variables. ABO mismatch and D:R weight ratio ≥ 3 values are given as n (percentage); all other values represent median (range). D:R – Donor:Recipient.

5.3.3 Post-transplant Morbidity

Those patients receiving ECLS post-transplantation had longer hospital stay, and were significantly more likely to require renal replacement therapy (82.1% vs. 22.4%, $p < 0.001$), suffer stroke (17.9% vs. 2.3%, $p < 0.001$) or acquire laboratory proven infection (21.4% vs. 2.9%, $p = 0.040$) prior to hospital discharge (Table 5.3). Two patients were re-transplanted directly from ECLS: one died 10 days after her second transplant; the second is alive at most recent follow-up, 10 years post-transplant.

Variable	Non ECLS group (n = 174)	ECLS group (n = 28)	p
Renal replacement therapy	39 (22.4%)	23 (82.1%)	< 0.001
Hospital acquired infection	14 (8.0%)	6 (21.4%)	0.04
Seizure	5 (2.9%)	1 (3.6%)	0.85
Cerebrovascular accident	4 (2.3%)	5 (17.9%)	< 0.001
Length of hospital stay (days)	6 (0 – 208)	21 (9 – 55)	< 0.001

Table 5.3 Post-transplant morbidity. Length of stay is given as median (range). All other data are n followed by (percentage).

5.3.4 Pre-transplant Diagnosis

Of 17 transplants undertaken for restrictive cardiomyopathy (RCM) during the study duration, 6 (35.3%) required post-operative ECLS (Table 5.4). This proportion was

significantly higher than that for dilated cardiomyopathy (DCM) (14 of 134 transplants – 10.4%) or anatomical heart disease (8 of 51 transplants – 15.7%) (Pearson's Chi-square = 7.99; p = 0.018).

Diagnosis	Non ECLS group	ECLS group
Restrictive Cardiomyopathy (n = 17)	11 (64.7%)	6 (35.3%)
Non-restrictive cardiomyopathy (n = 134)	120 (89.6%)	14 (10.4%)
Anatomical heart disease (n = 51)	43 (84.3%)	8 (15.7%)

Table 5.4 Pre-transplant diagnosis groupings. Non-restrictive cardiomyopathy comprises dilated and hypertrophic cardiomyopathies; anatomical heart disease covers congenital heart disease and acquired valvar disease.

5.3.5 Multivariate Regression Analysis

On multivariate logistic regression analysis, factors associated with a need for post-transplant ECLS were RCM (odds ratio 5.12, C.I. 1.47-17.91), longer ischemic time (odds ratio 1.53, C.I. 1.05-2.23) and ECMO pre-transplant (odds ratio 3.59, C.I. 1.43-9.06) (Table 5.5). Although donor:recipient weight ratio as a continuous variable was not associated with post-operative ECLS, extreme ratio (taken as ≥ 3) was predictive of ECLS post-transplant (odds ratio 4.9, C.I. 1.45 – 16.09). Recipient and donor ages, surgical heart disease, and donor cardiac arrest were all non-significant.

Variable	OR	p	[95% C.I.]
ECMO pre-HTx	3.59	0.007	1.43-9.06
D:R weight ratio ≥ 3	4.9	0.009	1.45-16.09
Ischemic time (min)	1.53	0.03	1.05-2.23
RCM	5.12	0.01	1.47-17.91

Table 5.5 Multivariate analysis of predicators for post-operative ECLS. D:R – Donor:Recipient; RCM – restrictive cardiomyopathy; HTx – heart transplant

Graft survival was significantly higher in the non-ECLS group, with a five-year survival of 84.5% (95% C.I. 77.9-90.0%) vs. 57.7% (37.7-73.9%) (Figure 5.2; p = 0.0006 Log Rank). The most striking difference in post-transplant mortality was the high early attrition seen in the ECLS group, with a thirty-day mortality of 31.7% (16.7-52.2%) vs. 0%, and one-year mortality of 42.3% (36.1-73.3%) vs. 1.8% (0.6-5.2%). There have been no subsequent deaths seen in patients requiring ECLS post-transplant who survived to one year.

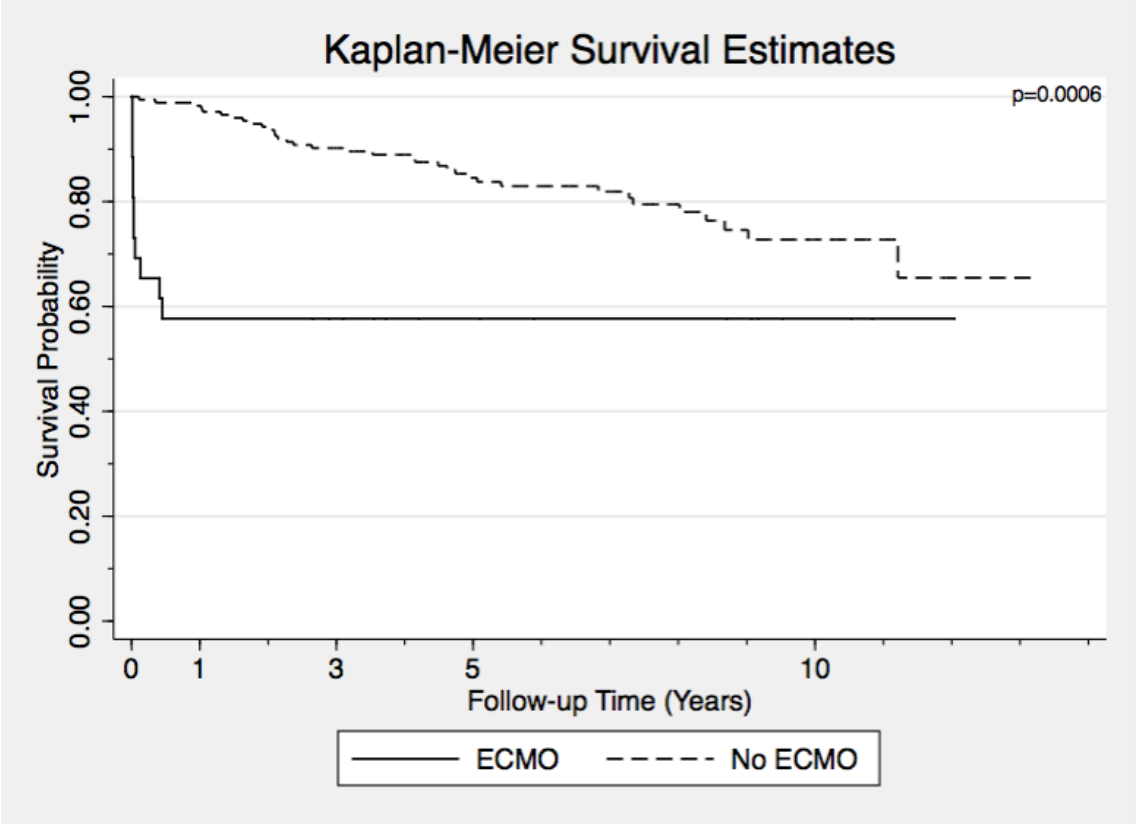

Figure 5.2 Kaplan-Meier curves demonstrating overall survival based on need for ECMO post-transplant.

5.3.6 Pre-transplant pulmonary vascular resistance data in patients with RCM

At Great Ormond Street, some patients with clinical concern over their pulmonary vascular resistance prior to transplantation undergo cardiac catheterisation in order to reduce the chances of a futile transplant. In this group, 12 patients with RCM had PVR studies performed prior to transplant, with reversibility of raised pulmonary pressure (tested with a combination of increased inspired oxygen fraction and inhaled nitric oxide) performed in 8. Of the 12 patients, 5 (41.7%) required ECLS immediately post-transplant.

Data for transpulmonary gradient and indexed pulmonary vascular resistance were analysed with the student's t-test, following testing for normality with the Shapiro-Wilk test. There was no significant difference between those requiring ECLS post-transplant

and those not with regards to TPG or PVRI, either at baseline, or with vasodilator testing (see Table 5.6).

	Non-ECLS RCM (n = 7)	ECLS RCM (n = 5)	p
TPG at baseline (mmHg)	10 ± 4.3	17.3 ± 7.9	0.17
PVRI at baseline (Woods.units.m^2)	3.9 ± 1.6	3.3 ± 1.5	0.51
TPG with vasodilation (mmHg)	9.3 ± 1.5	7.0 ± 4.4	0.86
PVRI with vasodilation (Woods.units.m^2)	2.6 ± 0.9	2.4 ± 1.2	0.26

Table 5.6 Transpulmonary gradient and indexed pulmonary vascular resistance. Data are presented as mean ± SD

As there are published and local thresholds at which TPG and PVRI would a classify a transplant as high-risk, they were also analysed at categorical data (with Pearson's Chi-square test), with cut-offs for TPG of 15 mmHg and PVRI of 6 Woods.units.m^2. All 12 patients had a PVRI of < 6 Woods.units.m^2 at baseline and with vasodilation; 3 patients had a TPG > 15 mmHg at baseline, but none had TPG > 15 mmHg with vasodilation. 1/7 patients with baseline TPG < 15 mmHg and 2/5 with baseline TPG >15 mmHg required ECLS post-transplant (p = 0.31).

5.4 DISCUSSION

This chapter reviews the use of ECLS as a rescue therapy following paediatric heart transplantation at a single institution over an 11-year period. During that time, ECLS was employed 28 times (14% of transplants), with approximately half of the patients surviving until 1 year. Conditional medium-term survival in patients surviving the first post-transplant year was excellent, with no later deaths in those 15 patients (median follow-up 6 years). Factors associated with a need for ECLS post-transplant were restrictive cardiomyopathy, pre-transplant ECMO, and longer ischemic time.

ECLS is an invasive, expensive therapy that carries with it a significant burden of morbidity and mortality, which has been increasingly employed in paediatric cardiac centres for over three decades.[138] In the post-heart-transplant setting, it can be utilized to give the donor organ time to recover from the peri-operative insults suffered during donor death, explantation and re-implantation, and in certain cases may be the only viable option to support a failing heart.[137, 139, 140] This chapter highlights the significant morbidity and mortality associated with its use; it is important to stress however, that in each of the 28 cases, conventional means of support had been exhausted, and in the absence of mechanical support, the demise of the patient was thought unavoidable.

The indication for ECLS post-treatment was in the majority of cases right ventricular failure, with or without concomitant left ventricular failure, a finding which reflects previous series.[137, 140-142] The explanation of this phenomenon is the sudden exposure of the donor graft to higher pulmonary vascular resistance (PVR) in the recipient. Conventional therapies to lessen the impact of pulmonary hypertension on the donor graft including inotropes (e.g. milrinone) and nitric oxide have certainly improved survival in such scenarios, and are now considered standard at many institutions, including Great Ormond Street;[143] if high PVR is suspected or known prior to transplantation, an atrial communication is created in the donor heart at implantation to act as a let-off valve in the case of high right-sided pressures. Prostacyclin, bosentan and sildenafil have also been used in this setting.[144, 145] However, despite these techniques in combination, a period of pulmonary hypertension post-transplant is sometimes unavoidable.

One group of patients considered at a higher risk for early graft loss due to high pulmonary pressures is those with restrictive cardiomyopathy,[146] where the development of pre-transplant pulmonary hypertension can be sudden and severe,[147] and may go unnoticed clinically. Furthermore, in the setting of worsening heart failure, it may not be discernable with standard echocardiographic measures such as tricuspid regurgitation jet velocity. In the course of the transplant programme at Great Ormond Street this patient group has proved problematic, with timing of listing for transplantation a particular conundrum. Over the 24-year history of transplantation here, the protocol has been modified such that all RCM patients have an atrial communication created in their donor hearts, and start milrinone and inhaled nitric oxide in theatre. However, this work indicates that – even with these measures – over one-third of RCM patients still require early mechanical support. The indication for ECLS post-transplant in the 6 patients in the RCM group was right ventricular failure in three, biventricular failure in two, and left ventricular failure in one. These proportions would be consistent with the hypothesis that high PVR immediately post-transplantation is relatively common in the RCM group, and has significant impact with graft function.

It is clear that the requirement for ECLS is much higher following transplantation than conventional cardiac surgery; at Great Ormond Street, for example, ECLS is required in approximately 2% of patients post-cardiac surgery,[148] as compared to the 14% in the current post-transplant data. This difference must be due – at least in part – to the operative substrate, with transplant patients often being in a worse clinical state, and with a higher rate of previous sternotomies. However, it is likely that the peri-operative insults peculiar to transplant surgery, including donor injury, explantation (including ischemia) and immunological factors post-implantation are also important factors resulting in the increased need for ECLS in this group.

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The influence of longer ischemic times on paediatric transplantation is a matter of on-going debate.[134, 149-151] With the growing imbalance between transplantation lists and donor rates, "marginal donors", including those geographically distant to the recipient with predictably longer ischaemic times, must be considered. These data suggest that those patients requiring ECLS post-transplant had ischemic times on average 30 minutes longer than those who proceeded without ECLS, a finding that reflects a recent paper from Colorado.[141] Interestingly, long-term outcome was not found to be affected by ischemic time in the latest registry figures from the International Society of Heart and Lung Transplantation.[134] In contrast, however, a recent and comprehensive review of paediatric transplantation in the U.S. concluded that an ischemic time of more than three-and-a-half hours was associated with a 30% increase in early graft loss; interestingly, in patients surviving to 6 months, there was no difference.[151] The propensity for those patients in our series receiving ECLS as a rescue therapy to have longer ischemic times fit well with those U.S. data, as does the excellent later survival in those patients surviving to hospital discharge.

Another consequence of fewer donors and longer waiting times is the increased use of mechanical support as a bridge to transplantation. These data indicate that patients on ECMO at the time of transplantation were more likely to require rescue ECLS; conversely, those receiving transplants after bridging with a ventricular assist device were not. One possible explanation for this difference is that those on ECMO pre-transplant bring with them the highest levels of co-morbidity, whereas a period of relative stability on a ventricular assist device may improve the substrate for transplantation in those highest risk patients. In particular, improved cardiac output for a sustained period with an LVAD may help to reverse pulmonary hypertension,[152, 153] and this effect may have helped avoid the need for post-transplant ECLS in those patients. In the setting of limited donor availability, and taking into consideration the substantial burden on health care systems of having children on ventricular assist devices, it is impossible to justify an "elective" period of LVAD support as a standard treatment if a donor organ was available, but it is encouraging that once stabilized, these patients have a smoother post-operative course.

It was not surprising that the ECLS group suffered a more intense post-operative morbidity than those not requiring ELCS, with obviously longer hospital stay. In addition, they showed higher rates of renal replacement therapy, infection and stroke. The increased requirement for dialysis probably relates at least in part to the period of low cardiac output that necessitated ECLS originally, combined with co-existent pre-renal impairment prior to transplantation. Infection is a common complication of ECLS, and for this reason our post-transplant antibiotic prophylaxis in patients on ECLS is extended to include antifungal cover and prolonged use of i.v. antibiotics. Despite these interventions however, it has not been possible to fully neutralize the increased susceptibility to infection of this group, and there were over twice as many laboratory proven infections in those on ECLS.

The rate of cerebrovascular accidents in the ECLS group was also predictably higher than the non-ECLS group, with 17.9% of patients suffering an event. This figure is roughly comparable to both the American ELSO registry figures for cerebrovascular events[154] and a specific post-transplant series from Colorado.[141]

The excellent medium-term survival in the sub-group of patients surviving ECLS post-transplant to hospital discharge is a potentially interesting finding. This may be nothing more than an effect of relatively small numbers, but it might also be hypothesized that a period of ECMO in the immediate post-operative phase may in some way protect against the high immunological insult that transplanted grafts receive during that time.

5.5 SUMMARY

In summary, these data indicate a good improvement with ECLS in short-term outcome for paediatric patients suffering from acute graft failure in the post-transplant phase – patients whose prognosis would otherwise be bleak. Encouragingly, survival through a period of mechanical support seems to be followed by excellent medium-term outcomes. This series indicates that restrictive cardiomyopathy, longer ischemic time and pre-transplant ECMO support are risk factors for the need for post-transplant ECLS - it is possible that aggressive interventions in the peri-operative phase of such patients (including attempts at normalizing high PVR) may further improve their short-term outcomes.

CHAPTER 6

TACROLIMUS IN PAEDIATRIC HEART TRANSPLANTATION: AMELIORATED SIDE-EFFECTS IN THE STEROID-FREE, STATIN ERA

Due to concerns over the side-effects of ciclosporin, tacrolimus is widely used in paediatric heart transplantation. However, tacrolimus therapy is also accompanied by potentially serious side-effects. This chapter examines the side-effect profile of tacrolimus in a large group of paediatric heart recipients. Data on renal function, diabetes, hyperlipidaemia and hypertension were collected by case-note review of 100 patients who had received at least 12 months' treatment with tacrolimus. 42 patients received tacrolimus from the time of transplant (de novo), and 58 were initially treated with ciclosporin (switch). Mean estimated glomerular filtration rate improved in the first six months post-transplant in the de novo group (66.7 to 84.6 mls/min/1.73m^2, p = 0.002). Conversely, it decreased in those initially treated with ciclosporin (82.1 to 68.8, p = 0.032), but improved after switch to tacrolimus (77.3 to 85.6, p = 0.006). 21% of patients exhibited glucose intolerance, and 2% had diabetes. 4.4% had borderline or elevated fasting cholesterol levels. Hypertension was seen in 67% at the point of switch from ciclosporin, which fell to 36% at latest follow-up (p = 0.001). These results present an encouraging outlook for this cohort of patients. The relatively low levels of complications shown may be due to early weaning of steroids, and concomitant statin therapy.

6.1 INTRODUCTION

Tacrolimus was first used in paediatric heart transplantation in 1989.[155] Since then, it has widely started to replace ciclosporin as primary immunosuppression, due to concerns over the latter's side-effects. Tacrolimus is thought to have at least as good efficacy as ciclosporin in preventing graft rejection,[156, 157] thereby prolonging graft survival. There remain, however, concerns over tacrolimus' own side-effect profile,[158] including renal dysfunction, glucose and lipid abnormalities, and hypertension – all of which have particular relevance to heart transplant patients: not only are they prominent stimuli for the development of later allograft vascular disease (and therefore risk factors for graft loss), but they are all considerations when deciding on suitability for re-transplantation, when the original graft fails. This study reviews the prevalence of unwanted effects in a large group of paediatric heart transplant recipients taking tacrolimus.

6.2 METHODS

6.2.1 Study Population

The notes from all heart transplant patients at Great Ormond Street Hospital were retrospectively reviewed up until 2006 (n = 226). Only those patients with a follow-up whilst receiving tacrolimus for at least one year were included; in the 126 patients excluded with a shorter tacrolimus period were six who had died within the first year. Included patients (n = 100) were then separated into two groups: those that were started on tacrolimus immediately post-transplant ("de novo") and those that were switched to tacrolimus having previously been on ciclosporin ("switch") **(Table 6.1)**. No patients have been switched from tacrolimus to ciclosporin at our institution. The local immunosuppression regime consists of basiliximab induction, followed by six months of weaning prednisolone. An antiproliferative agent (mycophenolate mofetil (MMF) or azathioprine) and a calcineurin inhibitor (tacrolimus or ciclosporin) are prescribed in all cases).

	Switch group	De novo group
Number of patients	58	42
Male sex (%)	24 (41)	19 (45)
Median age at transplant (years (range))	6.6 (0 – 16.0)	10.1 (0.3 – 17.3)
CM:CHD	44:14	33:9

Table 6.1 Patient characteristics. CM – cardiomyopathy; CHD – congenital heart disease.

6.2.2 Data collection

Data were collected from all included patients concerning post-transplant complications potentially related to tacrolimus use. Renal function was assessed by collection of serum creatinine levels at transplant, six months post-transplant (to assess improvement of acute renal failure following normalization of cardiac function) and at latest follow-up. In the switch group, creatinine levels at the point of changing from ciclosporin to tacrolimus were also recorded. Estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula.[159] Proportions of children with relative renal impairment were calculated according to the National Kidney Foundation guidelines.[160] Blood pressure measurements were taken at all follow-up visits, and a 24-hour ambulatory blood pressure test was performed on an annual basis. These values are used to direct anti-hypertensive treatment, and the requirement for such treatment was used for the present analysis. Fasting serum glucose measurements were used to assess post-transplant glucose intolerance (5.6-6.9 mmol/l) and diabetes (≥ 7 mmol/l),[161] and fasting cholesterol and triglyceride values used to assess lipid metabolism. A cholesterol of > 4.4 mmol/l was considered borderline, and a level of > 5.2 mmol/l was considered elevated.[162] Fasting samples were taken at the time of their annual review – a two day stay with fasting samples taken whilst fasted for cardiac catheterisation.

6.2.3 Statistical Analysis

Statistical analyses were run using SPSS 14.0 for Windows (SPSS Inc.). Following testing for normality using the Kolmogorov-Smirnov test, comparisons involving non-normally distributed data were performed using the Mann Whitney U test (triglycerides); normally distributed data were analysed with paired t-test (renal function) or unpaired t-test (cholesterol, glucose). Proportions of patients treated with anti-hypertensives, and with elevated glucose or cholesterol levels were analysed using Pearson's Chi-square test.

6.3 RESULTS

6.3.1 Renal Function

In those taking tacrolimus immediately after transplant (de novo), there was an improvement in eGFR from transplant to six months later (n = 42, mean 66.7 and 84.6 mls/min/1.73m^2, p = 0.002) (Figure 6.1). From 6 months to latest follow-up (mean 1.2 years), eGFR remained stable (84.6 to 89.6, p = 0.162). Conversely, in those taking ciclosporin during the initial post-transplant period (switch), eGFR worsened in the first six months from 82.1 to 68.8 (n = 32, p = 0.032), and remained stable (71.1 mls/min/1.73m^2) until switch to tacrolimus (mean 3.4 years, p = 0.614). From the point of switching from ciclosporin to tacrolimus until latest follow-up (mean 3.0 years), eGFR improved (n = 58, mean eGFR 77.3 to 85.6, p = 0.006).

eGFR was also employed to deduce the proportion of patients in different stages of renal impairment at latest follow-up.[160] Prior to transplant, 7 de novo patients and 3 switch patients had an eGFR < 30 (severe impairment or end-stage renal failure), and ten patients required continuous veno-venous filtration (CVVH) immediately post-transplant. At latest follow-up, of 42 de novo patients, 17 (40%) showed mild impairment (GFR 60-90), and 6 (14%) showed moderate renal impairment (GFR 30-60; mean follow-up 1.7 years). Of 58 patients originally taking ciclosporin (switch group), 30 (52%) showed mild impairment, and 6 (10%) had moderate impairment (mean follow-up 5.3 years). No patients from either group had an eGFR < 30 at the end of the study period.

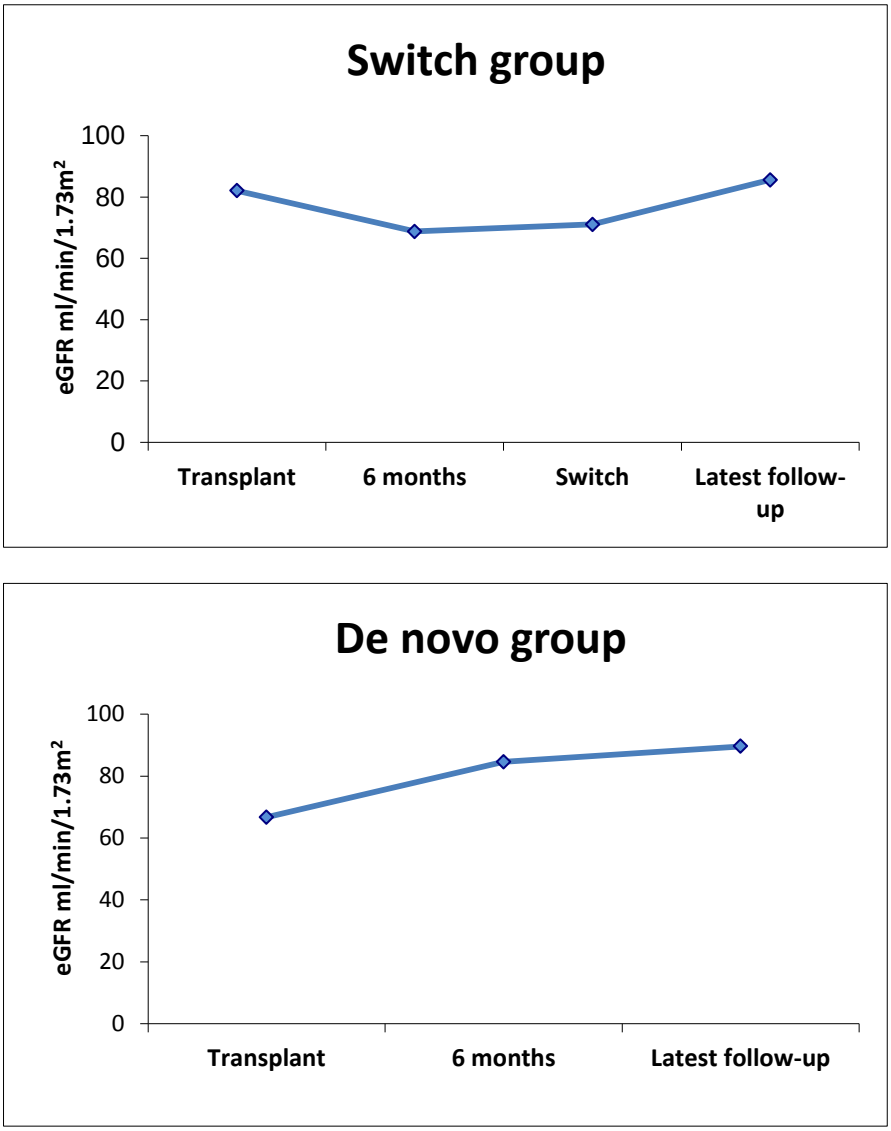


Figure 6.1 Change in renal function over time, expressed as eGFR. In the Switch group, renal function decreased immediately post-transplant, and recovered only after switching from ciclosporin to tacrolimus. Those patients receiving tacrolimus immediately post-transplant had no such fall in eGFR, but showed an improvement in renal function over the first 6 months post-transplant.

6.3.2 Blood pressure

Anti-hypertensive treatment was required by 23/42 (55%) of patients in the de novo group one year post-transplant, and 25/42 (60%) at latest follow-up (p = 0.659; mean follow-up 1.8 years) (Figure 6.2). In the switch group, 39/58 (67%) required anti-hypertensive treatment at the point of switch from ciclosporin to tacrolimus, which fell to 21/58 (36%) at latest follow-up (p = 0.001; mean 3.7 years following switch).

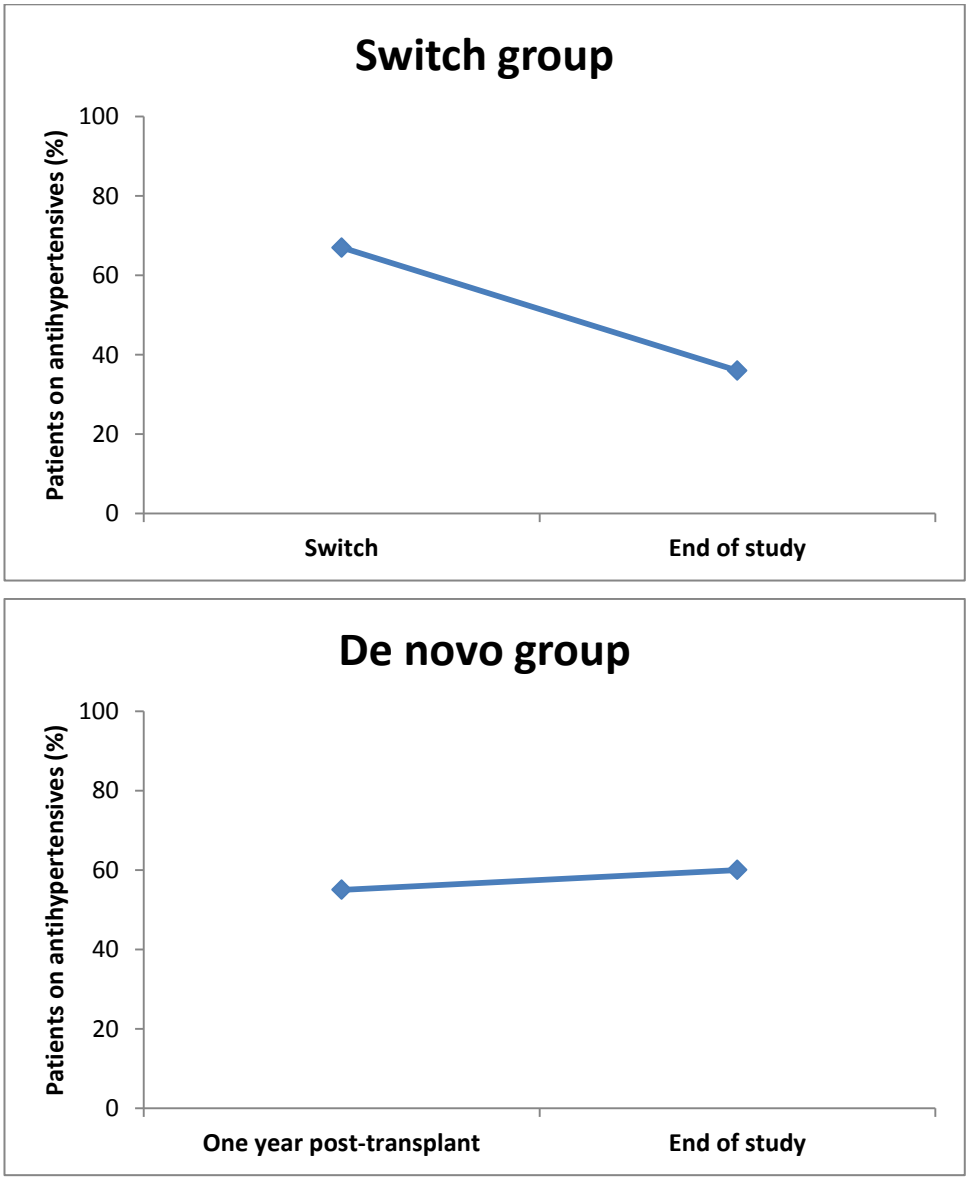


Figure 6.2 Anti-hypertensive use (as a surrogate marker for hypertension) in switch and de novo groups. The upper panel illustrates a reduction anti-hypertensive use when patients were switched from ciclosporin to tacrolimus.

6.3.3 Glucose metabolism

Data were available on 99 of the patients. There was no difference between the switch and de novo groups with regards to mean fasting blood glucose levels: de novo group 5.2 mmol/l and switch group 5.1 mmol/l ($p = 0.394$). Since clinically relevant values have defined cut-offs (normoglycaemic, glucose intolerant, and diabetic) the data was also analysed categorically. Again, there was no difference between the switch and de novo groups (Pearson chi-square: $\chi 2$ ($df = 2$, $n = 99$) = 0.936, $p = 0.626$, Table 6.2). One patient from each group had clinical diabetes mellitus, one of whom had a body mass index (BMI) of 33. Both patients require only oral hypoglycaemic treatment.

Fasting Glucose	Mean (SD)	< 6 mmol/l Normal	6-7 mmol/l Glucose intolerance	> 7 mmol/l Diabetes mellitus
De novo	5.1 (±1.4)	31 (72%)	11 (26%)	1 (2%)
Switch	5.2 (±0.8)	45 (80%)	10 (18%)	1 (2%)

Table 6.2 Fasting glucose in the de novo and switch groups. Values other than mean are n (%).

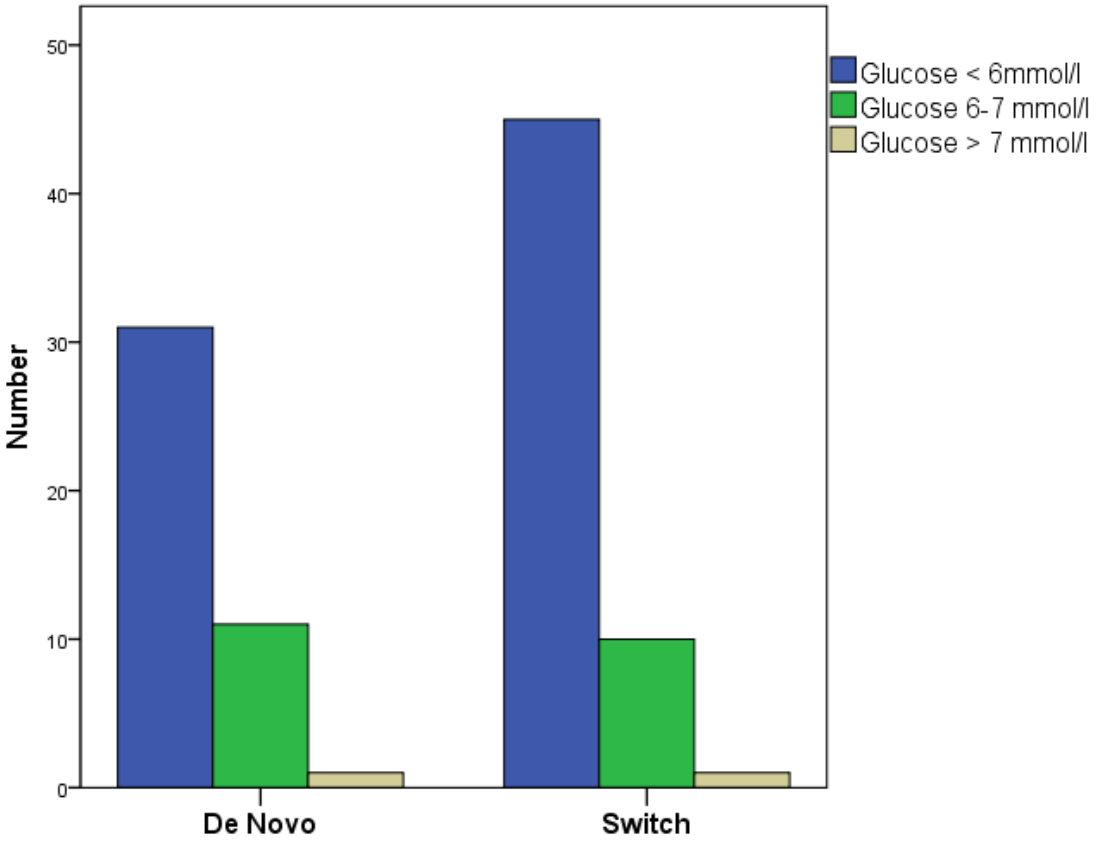


Figure 6.3 Glucose intolerance in switch and de novo groups.

6.3.4 Lipid metabolism

Data were available on 97 of the patients. There was no difference in the cholesterol or triglyceride levels between the de novo and switch groups (median follow-up time for this analysis was 3.1 years) (Table 6.3). Median fasting blood triglyceride level was 0.79 mmol/l in the de novo group, and 0.77 mmol/l in the switch group ($p = 0.873$). Mean cholesterol was 3.16 mmol/l in the de novo group, and 3.07 mmol/l in the switch group ($p = 0.522$). Again, as there are well-defined clinical thresholds with regards to cholesterol levels (borderline (> 4.4 mmol/l) and elevated (> 5.2 mmol/l)), data was re-analysed categorically, using Pearson's chi-square. 3/44 of the de novo group had borderline cholesterol levels (4.4-5.2 mmol/l) and 1/53 of the switch group had an elevated cholesterol level (> 5.2 mmol/l); all other patients had normal cholesterol values (< 4.4 mmol/l).

	De novo	Switch	p
Fasting triglycerides (mmol/l) (median, range)	0.79 (0.35-3.89)	0.77 (0.36-1.14)	0.87
Fasting cholesterol (mmol/l) (mean, SD)	3.16 ± 0.73	3.07 ± 0.68	0.52
Cholesterol category			
Normal (< 4.4 mmol/l)	41 (93%)	52 (98%)	0.22
Borderline (4.4-5.2 mmol/l)	3 (7%)	0 (0%)	
Elevated (> 5.2 mmol/l)	0 (0%)	1 (2%)	

Table 6.3 Fasting cholesterol and triglyceride levels.

39/40 (98%) of the de novo group and 43/50 (86%) of the switch group were known to be taking statins at the point of testing (Pearson's chi-square p = 0.06). The mean cholesterol level was 3.1 mmol/l in those on statins, and 3.4 in those not taking statin therapy (p = 0.28). All four of the patients with a borderline or elevated cholesterol level were on pravastatin. The median triglyceride level was 0.75 mmol/l for those on statins and 1.06 for those not on statins (p = 0.02). There was no correlation between statin dose (either absolute value, or dose/kg body weight) and triglyceride or cholesterol level.

6.3.5 Other parameters

During the follow-up period, 10 patients from the switch group, and 3 from the de novo group died. Within each group, 5 patients were diagnosed and treated successfully for post-transplant lymphoproliferative disease. No other malignancies were diagnosed within the entire cohort.

6.4 DISCUSSION

These data represent an encouraging review of the use of tacrolimus in a large cohort of paediatric heart transplant recipients. Whilst some patients did suffer from complications that may be linked to tacrolimus use, the overall incidence was not high. Moreover, those patients who had previously been taking ciclosporin exhibited an improvement in renal function and a decrease in the need for anti-hypertensive medication.

The estimation of renal function in heart transplant recipients is of obvious importance, but producing meaningful and accurate values is difficult, especially in the paediatric age group. I have attempted to overcome these problems by using the Schwartz formula to estimate GFR, and, by using analysis based on paired estimates in each patient, I have minimized the potential bias of pre-transplant renal impairment, poor muscle bulk and hyperfiltration injury. Previous comparative assessment of the renal side-effects of tacrolimus and ciclosporin using direct measurement and eGFR did not find one medication to be superior to the other.[163] This study suggests that, in contrast to ciclosporin, tacrolimus use post-transplant was compatible with a significant improvement in renal function associated with improved cardiac output in the first six months. I have also shown a similar improvement in renal function in those switched from ciclosporin to tacrolimus after at least six months post-transplant. In addition, de novo patients showed at least stability ineGFR after the first six months, which potentially corresponds to cessation of steroid therapy or lower levels of tacrolimus further out from transplant (mean tacrolimus level was 8.3 μg/l at 6 months, and 7.5 μg/l at one year).

It is important to remember, however, that average values across a cohort can mask a subsection with some degree of impairment. I therefore performed a secondary analysis of the proportion of patients with some degree of renal insufficiency, discovering that at latest follow-up, 47% of the entire group had mild impairment, and 12% moderate impairment (according to the National Kidney Foundation guidelines).[160] The potential aetiology of this injury is multifactorial: pre-renal failure occurs during low cardiac output prior to and immediately after transplant, as well as during cardiopulmonary bypass, and can be exacerbated with necessarily aggressive diuretic treatment in the early post-transplant phase. Clearly, renal function must continue to be watched closely in all recipients. However, it is encouraging that despite poor renal function prior to transplant, and the need for peri-operative CVVH in ten patients, none of these 100 patients currently have severe renal impairment, and that function improved in those switched from ciclosporin.

Hypertension remains an important issue post-transplant. The prevalence in this group (as defined by those requiring treatment) compares favourably to previous adult studies[164] and the International Society's paediatric report.[95] Again, although tacrolimus has been implicated, the aetiology of hypertension is multifactorial, and the GOS institutional policy of withdrawing steroid treatment within 6-months post-transplant may have had influence. It might also be expected that as time passes, not only will target tacrolimus levels diminish, but also the hypertensive effect of oversized donor hearts might decrease, and these two points may explain why the switch group (who were further out from transplant) had less requirement for anti-hypertensive treatment. It is encouraging, however, to notice that when patients were switched from ciclosporin they displayed a contemporaneous fall in the requirement for blood pressure medication, which may reflect the less severe hypertensive side effect profile of tacrolimus shown by Taylor et al. in adults.[164]

There is also concern over the potential diabetogenic effects of tacrolimus.[165] Again, it is not the sole potential aetiological agent, with steroid treatment also culpable.[166] The overall level of post-transplant diabetes here is lower than other published series,[165] which may partly be the result of the withdrawal of steroids by six months. One of the two patients with diabetes in this sample had a BMI of 33 at the time of diagnosis, which would certainly have accelerated her diabetes; she is now adequately controlled with oral antihyperglycaemic therapy. None of the 100 patients in this cohort currently require insulin. However, it is again prudent to stress that a significant proportion of these patients – in this case just over one-fifth – showed glucose intolerance (even in this relatively short follow-up period), and it is probable that some of them will go on to require antidiabetic therapy in the future.

The results concerning lipid metabolism show a similar pattern, with the vast majority of patients within the normal range. Previous studies have also concluded that hyperlipidaemia may be less common in patients on tacrolimus than on ciclosporin.[167, 168] The transplant team at Great Ormond Street now routinely prescribes pravastatin (which may not only lower cholesterol levels, but also reduce coronary allograft vasculopathy),[169] and these results should of course be viewed in this light. All four patients with abnormal cholesterol levels were being treated with sub-maximal levels of pravastatin, and we anticipate that the subsequent increase in dose will result in a lowering of their values.

The influence of statin therapy on the side-effect profile of tacrolimus may not be limited to its lipid lowering effects, but also via modulatory influences on the renal system. Statins have been shown to slow decline of GFR and decrease proteinuria[170] in chronic kidney disease, and have also been linked to decreased renal failure in heart transplant patients.[171] Similarly, early reduction in steroid exposure may also be helping to moderate the side effects of tacrolimus by reducing renal injury. Prolongation of renal function in this manner may be linked to the decrease in hypertension and diabetes in this cohort.

6.5 CONCLUSION

In summary, this chapter records post-transplant complications in a sizeable cohort of paediatric heart transplant recipients. Despite its retrospective nature, the large population studied have provided valuable insight into the four areas discussed (renal function, hypertension, and lipid and glucose metabolism), which have all previously been linked to tacrolimus use.[158] In this group, the prevalence of these disorders was relatively low compared to other studies, and those switched from ciclosporin showed a contemporaneous decrease in side-effect profile (although it is impossible to be certain that the switch was the major factor). These results suggest that, in the current era of steroid-free immunosuppression and widespread use of statins, the side-effect profile of tacrolimus appears to have been partly ameliorated.

CHAPTER 7

PERIPHERAL ENDOTHELIAL DYSFUNCTION AND CYTOMEGALOVIRUS REPLICATION IN PAEDIATRIC HEART TRANSPLANTATION

This chapter investigates a potential role of cytomegalovirus (CMV) on cardiac allograft vasculopathy (CAV) – the major limiting factor to the long-term success of paediatric heart transplantation. CMV has been shown to be a significant risk factor for the development of CAV. Previous work has demonstrated CMV DNA in leukocytes in the absence of direct allograft infection, suggesting that vascular changes may not be limited to the allograft. Systemic arterial endothelial function was assessed using high-resolution ultrasound to determine brachial artery flow mediated dilatation (FMD) in 50 paediatric heart transplant recipients (aged 8 to 17 years, 27 male). Patients were separated into two groups according to CMV status: those without evidence of CMV replication post-transplant (n = 38, 19 male); and patients with evidence of viraemia post-transplant (n = 12, 8 male). No patient had detectable viraemia at the time of study. FMD was significantly impaired in patients with CMV replication post-transplant (6.64 ± 1.12%; mean ± S.E.) compared to those without (9.48 ± 0.56%; p = 0.02). This difference remained after adjustment for age, time since transplant and medication. Pre-transplant recipient and donor CMV status and traditional CMV risk were not associated with FMD. This chapter shows therefore that CMV replication following cardiac transplantation is associated with chronic endothelial dysfunction in the

systemic circulation in children. The implication for both systemic and coronary vascular health is examined in the following chapter.

7.1 INTRODUCTION

Although short- and medium-term results are improving, long-term survival for paediatric transplant recipients is limited by cardiac allograft vasculopathy, an accelerated form of obliterative coronary disease. Human cytomegalovirus (CMV) is associated with increased risk of post-transplant coronary vasculopathy.[172, 173] A previous study from Great Ormond Street has shown CMV serology to be an important factor in allograft vasculopathy of paediatric recipients.[174] Coronary endothelial dysfunction is associated with CMV infection[175] and the later development of transplant vasculopathy.[176] Recent work has shown that low-level systemic CMV infection is common after transplantation,[177] although infection of the heart itself is rare.[178] It is therefore possible that the coronary vascular disease attributed to CMV may be driven by the consequences of low grade systemic infection – rather than infection of the transplant organ – which may have broader vascular consequences. However, the effect of post-transplant CMV on the systemic vasculature is unknown. This chapter tests the hypothesis that CMV replication could affect systemic endothelial function after paediatric cardiac transplantation.

7.2 METHODS

7.2.1 Study population and design

All children over the age of eight years who had previously had a heart transplant returning to Great Ormond Street Hospital for their annual review between October 2004 and August 2006 were invited to take part in the study. Exclusion criteria were: coexisting vascular disease, patient or parent refusal, active intercurrent systemic illness, and clinical or echocardiographic evidence of heart failure (left ventricular fractional shortening < 30%). Fifty-two patients were invited, two of whom were excluded due to lack of parental consent. The remaining fifty patients (aged 8-17 years, 27 male) were enrolled, and were separated into two groups based on CMV status: group 1 (n = 38, 19 male) – those without evidence of CMV replication post-transplant (negative polymerase chain reaction [PCR] and no change in CMV IgG post-transplant); and group 2 (n = 12, 8 male) – patients with evidence of post-transplant viraemia (positive CMV PCR and/or seroconversion from negative to positive CMV IgG). No patient from either group was viraemic at the time of assessment. All patients were free from angiographic evidence of CAV, and were assessed for conventional vascular risk factors (hypertension, smoking, diabetes, lipid profile) and transplant and medication history.

Characteristics	**No CMV replication**	**CMV replication**	**p**
Number of patients	38	12	-
Age (years)	14.5 ± 0.4	13.1 ± 0.6	0.07
Male sex	19 (50)	8 (67)	0.32
Heart rate (median, b.p.m. (IQ range))	91 (79 – 104)	89 (84 – 97)	0.75
Systolic blood pressure (mmHg)	113 ± 2	110 ± 3	0.53
Diastolic blood pressure (mmHg)	71 ± 1	65 ± 3	0.06
Cholesterol (mmol/l)	3.12 ± 0.11	3.09 ± 0.21	0.88
Triglycerides (mmol/l)	0.88 ± 0.06	1.01 ± 0.14	0.29
HDL (mmol/l)	1.02 ± 0.05	1.03 ± 0.07	0.96
LDL (mmol/l)	1.73 ± 0.11	1.59 ± 0.19	0.55
Glucose (median, mmol/l (IQ range))	5.10 (4.80 – 5.78)	5.20 (4.40 – 6.00)	0.59
hs CRP (median, mg/l (IQ range))	0.86 (0.25 – 3.55)	0.45 (0.11 – 0.70)	0.10
Creatinine (μmol/l)	67.7 ± 2.8	70.6 ± 8.0	0.66

Table 7.1 Baseline characteristics of study participants. HDL – high-density lipoprotein, LDL – low density lipoprotein; hs CRP – high-sensitivity C-reactive protein, IQ range – interquartile range. All blood tests are fasting values. Values are expressed as mean ± S.E.M. or frequency (percentage), unless otherwise specified.

7.2.2 Conventional and transplant-related risk factors

There were no smokers or diabetics amongst the cohort. There was no significant difference between the groups with respect to age, sex, heart rate, blood pressure, or blood glucose or lipid levels (Table 7.1). Renal function – measured by serum creatinine and estimated glomerular filtration rate (data not shown) – was similar in both groups.

Transplant-related variables and drug use were comparable between the groups (Table 7.2). A double-therapy immunosuppressant regime of calcineurin inhibitor and a purine synthesis inhibitor was used except when the latter was not tolerated, when single agent therapy was used. No patients were receiving steroids at the time of the scan. 11 patients were donor-positive, recipient-negative for CMV IgG at transplant (seven in group 1, four in group 2). Of these, 6 received CMV prophylaxis (valganciclovir 450mg o.d.) for 3 months post-transplant (three in group 1, three in group 2).

Characteristics	**No CMV replication**	**CMV replication**	**p**
Age at transplant (median, years (IQ range))	11 (6 – 15)	9 (7 – 13)	0.55
Donor age (years)	19.3 ± 2.0	21.7 ± 4.3	0.57
Ischemic time (mins)	196 ± 11	156 ± 18	0.08
Episodes of acute rejection (median (IQ range))	0 (0 – 1)	0 (0 – 1)	0.64
Years since transplant (median, (IQ range))	3.63 (1.40 – 7.97)	2.68 (1.58 – 5.07)	0.67
Statin use	34 (90)	9 (75)	0.33
Double immunosuppression	34 (90)	10 (83)	0.62

Table 7.2 Transplant related variables. Values are expressed as mean ± S.E.M., or frequency (percentage), unless otherwise specified.

7.2.3 Peripheral endothelial function measurements

Endothelial dysfunction was studied as previously described.[179] Briefly, the subjects rested supine for 10 minutes, having abstained from caffeine and fatty food for four hours prior to the scan. The right brachial artery was then imaged using high-resolution ultrasound (Prosound SSD-5500, ALOKA, Japan). Forearm ischemia was induced by inflating a blood pressure cuff to 200mmHg for five minutes; reactive hyperaemia followed cuff deflation. Changes in brachial artery diameter were measured offline using an automated edge detection system (Brachial Tools, Medical Imaging Applications, Iowa) and calculated as a percentage change from baseline diameter. Blood flow was measured continuously using a pulsed-wave Doppler signal. Maximal increase in blood flow within 15 seconds of cuff release was expressed as a percentage change from baseline flow (± standard error). Endothelium independent vasodilator response to 25µg sublingual glyceryl trinitrate (GTN) was then measured and expressed as a percentage change in diameter from baseline. Blood pressure was initially taken after resting for five minutes and also after flow meditated dilatation (FMD) and GTN studies using an Omron M5-I sphygmomanometer. All studies were performed in a temperature-controlled vascular laboratory by a single trained operator, who was blinded to all clinical history of the patients, including CMV variables.

7.2.4 CMV Measurements

Blood for CMV analysis was taken prior to transplant, at monthly clinic visits post-transplant in the first year, and annually thereafter. DNA was extracted from 200µl of EDTA whole blood using QI Amp Blood Mini Kits (Cat no. 80204, www.qiagen.com) according to the manufacturer's instructions. CMV DNA was quantified in whole blood by a method developed by Garson J. and Tedder R. at University College Hospital, London (personal communication) that was standardized against samples distributed by the European Quality Control for Molecular Diagnosis programme (info@qcmd.org).

The mastermix for the reaction consisted of 10µl of Taqman universal mastermix (ABI Biosystems Cat No 4304437) for tests run on the ABI 7000 and 7300 machines, or 10µl of Taqman fast Master Mix (ABI Cat No 4352042) for tests run on the ABI 7500 machine. 1µl (10pm/µl) CMV forward primer GCA TGC GCG AGT GTC AAG AC; 1µl (10pm/µl) reverse primer GTT ACT TTG AG(CT) GCC ATC TGT TCC T; 2µl (10pm/µl) probe FAM- TGC GCC GTA TGC TGC TCG ACA- TAMRA; 1µl of nuclease free water and 10µl of DNA template. Cycling conditions were as follows: ABI 7000, ABI 7300: 1 cycle 50˚C for 2 minutes, 1 Cycle 95˚C for 10 minutes, 45 cycles at 95˚C for 15 seconds and a final step at 60˚C for a minute; ABI 7500 fast machine: 1 cycle 5˚C for 20 secs followed by 45 cycles at 95˚C for 3 seconds and a final step of 60˚C for 30 secs. Viral loads were obtained by reference to ten CMV (strain AD169 culture in human embryo lung fibroblasts) standards that were included in each run, ranging from 100 to 2 million copies/ml extract. Assays were considered valid if the correlation coefficient for the standards was > 0.99 and the gradient between -3.3 and -3.6. CMV IgG was measured by a Vidas machine (Biomerieux).

7.2.5 Statistical analysis

Data were analysed with SPSS 13.0 for Windows (SPSS Inc.). Two-group comparisons of means were performed with the unpaired Student *t*-test for normally distributed data (age, cholesterol, triglycerides, creatinine, blood pressure, donor age, ischemic time, flow mediated dilatation and dilatation to GTN) and the Mann-Whitney U test for non-normally distributed data (age at transplant, time since transplant, episodes of acute rejection, heart rate, serum glucose, and hsCRP), following testing for normality using the Kolmogorov-Smirnov test. Multiple-group comparisons were conducted using a one-way ANOVA (CMV risk stratification). Comparisons of proportions were calculated using Fisher's exact test (sex, medications). Linear regression with multiple predictors was performed to explore the relationship between CMV status and FMD adjusting for age, time since transplant, and medication.

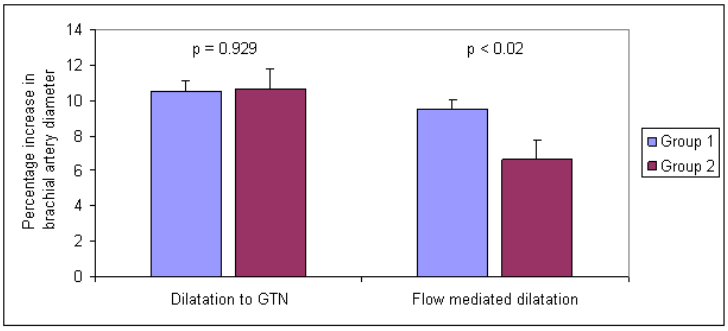
The study was approved by the Great Ormond Street Hospital Ethics Committee. Informed consent was obtained from all patients' parents, and consent/assent was taken (as appropriate) from the patients themselves.

7.3 RESULTS

7.3.1 Vascular function

No patients were CMV PCR positive at the time of the brachial scan. The mean time from last positive PCR to scan was 23 months, and only two patients had a positive PCR within the 6 months prior to the scan. Brachial artery flow mediated dilatation (FMD) was significantly reduced in patients with previous active CMV viraemia post-transplant compared to patients without evidence of replication (6.64 ± 1.12% vs. 9.48 ± 0.56%, p = 0.02; Figure 7.1). This difference remained significant when adjusted for age, time since transplant, and medication (p = 0.02). In contrast, response to GTN was comparable between the two groups (10.68% and 10.55%, respectively), indicating that the difference in FMD was not due to differences in smooth muscle function. Baseline arterial diameter (2.96mm ± 0.079 vs. 3.23 ± 0.12, p = 0.094) and flow (reactive hyperaemia 489 ± 41% vs. 437 ± 128%, p = 0.618) were also similar in groups 1 and 2.

The patients were also analysed according to their donor CMV status, recipient pre-transplant status and traditional CMV risk stratification[180] (Table 7.3). None of these three variables was predictive of FMD. There was no correlation between maximum CMV PCR detected or duration of PCR positivity and FMD.



Brachial artery diameter in response to glyceryl trimitrate (GTN) and increased flow. Group 1 (n=38) no post-transplant CMV replication, Group 2 (n=12) postivie CMV PCR or seroconversion post-transplant

Figure 7.1 Flow mediated dilatation and GTN response.

Criterion	**Value**	**n**	**FMD**	**p**
Donor CMV status	Positive	19	9.03 ± 0.89	0.91
	Negative	29	8.90 ± 0.67	
Recipient CMV status	Positive	15	8.82 ± 0.72	0.84
	Negative	33	9.06 ± 0.70	
Traditional risk stratification	High-risk	11	9.46 ± 1.40	0.92
	Intermediate-risk	16	9.00 ± 0.69	
	Low-risk	20	8.95 ± 0.85	

Table 7.3 Mean % FMD ± S.E.M., grouped by pre-transplant CMV variables. High-risk: donor CMV-positive/recipient CMV-negative; intermediate-risk: recipient CMV-positive; low-risk: donor and recipient CMV-negative. N.B. The donor CMV status was not available in two cases. The recipient pre-transplant status was not available in two cases.

7.4 DISCUSSION

This chapter presents the first study to link CMV and systemic vascular dysfunction following heart transplantation. It demonstrates that children with evidence of CMV replication following heart transplant (as shown by positive PCR or seroconversion) have significantly worse systemic endothelial function. Although impaired endothelial dysfunction has been described in children with symptoms of acute viral illnesses,[181] these patients had neither signs of infection nor evidence of CMV DNA in their blood at the time of vascular study, suggesting a more chronic association between CMV and vascular function. The relationship between active CMV replication and reduced FMD remained after adjustment for multiple potential confounding variables. The young age of the cohort minimises the impact of many of the potential confounders for systemic vascular disease such as smoking, hypertension and diabetes. In addition, there was no relationship to known risk factors such as inflammation (hsCRP), lipids, or blood pressure. This abnormality in endothelial function may have important implications for the later systemic vascular health of young transplant recipients, and may reflect changes in the coronary circulation of relevance to the pathogenesis of CAV.

While systemic vascular health is clearly important for children after transplantation, it is coronary vasculopathy that limits their long-term outcome.[89, 182] The pathogenesis of this disease is complex and multifactorial, and currently incompletely understood. However, studies have highlighted the relationship between CMV and allograft vasculopathy in adults[172, 173, 175] and, more recently, in children.[174] One of the hallmarks of allograft vasculopathy is endothelial dysfunction.[176] Although the mechanisms by which CMV may cause vasculopathy are unknown, it is likely that dysfunction of the endothelium is important. CMV has been shown to infect endothelial cells,[183] and is related to coronary vasomotor dysfunction in heart transplant patients.[175] This work now extends these findings to the systemic vasculature.

In previous adult studies, CMV-negative recipients of CMV-positive hearts have shown an increased cardiovascular risk.[184, 185] In this study, no relationship was seen between systemic endothelial function and donor or recipient CMV status, the traditional markers of risk of CMV disease. CMV prophylaxis has reduced the risk of coronary disease in this group.[186] However, in the majority of patients, no prophylaxis is given, and CMV infection involving leukocytes remains common.[177] Evidence is accumulating that graft vasculopathy may be driven by the consequences of low-grade systemic infection and reduced by its suppression.[186] Pre-transplant CMV seropositivity (and consequent lack of aggressive prophylaxis) appears associated with coronary lumen loss.[187] This is consistent with our previous work on CMV and graft vasculopathy in children,[174] and with our current findings, where viral DNA was documented in patients CMV IgG positive prior to transplantation. That subclinical CMV infection may have a systemic endothelial effect is further illustrated by our finding that eleven of the twelve children with post-transplant CMV replication did not go on to develop overt CMV disease, yet nevertheless showed reduced systemic endothelial function.

A recent study by Tu et al has investigated the immune response to subclinical CMV infection in recipients who were CMV IgG positive at transplant.[188] This showed that patients with CMV-specific CD4 T-cells in the first month post-transplant showed less coronary artery lumen loss than those without. These "early responders" were also found to have low/undetectable levels of CMV by PCR (mean < 40 copies/$1x10^5$ polymorphonuclear cells (PMN), compared to > 250 copies/$1x10^5$ PMN in those without the early response). Although the present study was not designed to explore T-cell responses, and the study populations are different in terms of CMV status at transplant, it is probable that there is overlap of our group without detectable virus and the early responder group of Tu et al. I would hypothesize that similar immune mechanisms may be working in my study, protecting against systemic vascular injury. A follow-up project investigating immune responses and systemic arterial function is required to explore this theory in detail. However, both my results and the work by Tu et al demonstrate that subclinical CMV infection is associated with later vascular abnormalities and that those without evidence of viral replication have a more favourable vascular outcome.

The precise mechanism by which prior CMV replication causes on-going endothelial dysfunction is unclear. It is possible that low level CMV viraemia (undetectable by current assays) or persistent infection of leukocytes or endothelial cells affects the vasculature directly. Alternatively, either of these mechanisms may impair endothelial function indirectly through chronic immune activation. It is also possible that these observations are a lasting consequence of a prior endothelial insult, sustained at a time of higher viral load.

Quantification of endothelial function using ultrasound assessment of brachial artery FMD is a well-validated, non-invasive method. Recently, the first large, prospective study has shown FMD to be predictive of future cardiovascular events in adults.[189] The prognostic value in younger patients remains unproven, as the cardiovascular endpoints typically present decades later. FMD is also predictive of coronary endothelial function in adults,[190] raising the possibility that depressed FMD in these children reflects impaired coronary endothelial function relevant to the progression of CAV. Certainly, a non-invasive marker of transplant coronary vasculopathy would be diagnostically attractive, particularly in the paediatric age group. Furthermore, as FMD is a dynamic measurement, it may be useful as a therapeutic marker in clinical trials assessing the response to CMV infections in the transplant population.

All children in this study had angiographically normal coronary arteries, although a more sensitive assessment of the coronary vasculature – such as intravascular ultrasound – would have been useful. Although this technique is limited by the size of coronary arteries in young children, it is possible in older children and adolescents. The monitoring of CMV viral load was limited to outpatient attendances and it is possible that some patients could have experienced low level CMV replication which was not detected; this was in part accounted for by checking for seroconversion in those patients who were CMV negative at transplantation.

7.5 CONCLUSION

This chapter reveals for the first time that CMV replication following cardiac transplantation is associated with chronic endothelial dysfunction in the systemic circulation in children. The implication for both systemic and coronary vascular health remains unknown, however. This is explored in the next chapter.

CHAPTER 8

CYTOMEGALOVIRUS CAUSES FUNCTIONAL AND MORPHOLOGICAL CHANGES IN SYSTEMIC AND CORONARY VASCULATURE OF PAEDIATRIC HEART TRANSPLANT RECIPIENTS BUT NOT IN HEALTHY CHILDREN

Cytomegalovirus (CMV) is thought to be an important risk factor for post-transplant vascular disease, particularly in young recipients. This chapter reports the effects of CMV on the coronary and systemic arteries of paediatric heart transplant recipients. In addition, the systemic arteries of normal children were investigated, to elucidate whether CMV changes occur purely in the immunocompromised post-transplant setting. 37 paediatric heart transplant recipients (9.3-17.3 years, mean 14.2) and 69 healthy controls (6.3-17.5 years, mean 12.2) were investigated. Coronary intimal-medial area of transplant recipients was measured with intravascular ultrasound. Systemic vascular health was assessed in both groups with brachial flow mediated dilatation (FMD) and carotid intimal thickness (cIMT). Comparisons were made using the Student's t-test. 19 (51%) transplant recipients and 21 (30%) controls were CMV IgG-positive at investigation. Mean time from transplant was 4.7 years (range 1 – 12.4 years). Coronary intimal-medial area was increased in CMV-positive compared to CMV-negative transplant recipients (23.9% vs. 17.2%; $p = 0.01$). cIMT was also increased (0.50mm v 0.47mm, $p < 0.05$), and FMD reduced (6.9 vs. 9.3%; $p < 0.05$). In contrast,

there was no difference between CMV-positive and negative healthy controls with regards to cIMT (0.44 vs. 0.45; p = 0.26) or FMD (9.5 vs. 9.4%; p = 0.87). This chapter represents evidence linking CMV and coronary allograft vasculopathy in paediatric transplant recipients. Moreover, CMV-positive patients exhibit morphological and functional impairment of their native arteries. CMV does not inflict such damage in immunocompetent hosts. These findings strengthen the proposal for therapy against CMV post-transplantation.

8.2 INTRODUCTION

With surgical and intensive care modifications improving short-term survival, cardiac allograft vasculopathy (CAV) has become the major limiting factor to the success of adult and particularly paediatric heart transplantation.[87, 88] While 15-year survival after transplant might be considered an acceptable outcome after adult transplantation, it represents the prospect of death or re-transplantation as a young adult for paediatric recipients.

Cytomegalovirus (CMV) is a herpes virus which has a predilection for harmful interaction with endothelial cells,[191] and has been associated with morphological changes in the coronary vasculature after heart transplantation.[172, 173, 175] However, the importance of CMV in adult allograft vasculopathy is not certain.[192] Conventional risk factors such as smoking, hypercholesterolaemia, obesity, hypertension and diabetes have been implicated in transplant coronary disease and pre-existing (donor) coronary disease from older donors has also been identified as a risk factor. Paediatric transplant recipients, generally devoid of these confounders, are a more homogeneous population to study the effects of CMV on vasculopathy.

Work from Great Ormond Street Hospital has previously shown that CMV is an important risk factor for later mortality after paediatric heart transplantation and is associated with an increased incidence of angiographic coronary disease.[174] However, angiography is a crude assessment of transplant coronary disease, and intravascular ultrasound is generally accepted to be a more sensitive technique,[193-195] although it has been used in few paediatric studies.[196, 197] The previous chapter has also shown that CMV infection has a systemic endothelial effect in paediatric transplant recipients,[198] but concomitant systemic structural arterial damage has not been assessed in this population.

Therefore, in this chapter, I have extended those initial studies into the role of CMV and paediatric allograft vasculopathy by using intravascular ultrasound. In the same children, I have investigated disease beyond the graft/recipient interaction by assessing the carotid intima/media thickness and brachial flow-mediated dilatation. Furthermore, as the impact of CMV seropositivity on the systemic vasculature of immunocompetent children is unknown, I have investigated the carotid and brachial arteries of healthy non-transplanted children, thus exploring whether CMV-modulated changes are limited to the immunosuppressed state.

8.2 METHODS

8.2.1 Study Population and Design

All heart transplant recipients over the age of nine years at Great Ormond Street Hospital were invited to take part in the study at the time of their annual coronary angiography. Only patients who were at least one year post-transplant were included, in order to minimize the effects of donor disease, and allow post-transplant changes to occur. Exclusion criteria were: patient or parent refusal; active intercurrent systemic illness; active CMV replication as measured by PCR for CMV DNA; and clinical or echocardiographic evidence of heart failure (left ventricular fractional shortening < 30%). A double therapy immunosuppressant regimen of calcineurin inhibitor or sirolimus and a purine synthesis inhibitor was used except when the latter was not tolerated, when single agent therapy was used. No patients were receiving steroids at the time of the scan. In total, 37 transplant recipients (22 male, aged 9.3-17.3 years, mean 14.2) and 69 aged-matched healthy controls (32 male, age 6.3-17.5 years, mean 12.2) were included, and divided into two groups based on CMV IgG status at the time of their investigations (19 transplant recipients (51%) and 21 controls (30%) were CMV-positive).

8.2.2 Coronary intimal thickness measurements

Intravascular ultrasound (IVUS) was performed during routine post-transplant coronary angiography. After maximal dilatation with intracoronary glyceryl trinitrate (GTN) (150μg), a 3.5F 30-Mhz IVUS catheter (Boston Scientific, Natick, MA) was advanced over a 0.014" coronary guidewire into the distal left anterior descending (LAD) coronary artery through a 6F guide catheter. A suitable mid/distal LAD coronary artery segment was identified and an automated pullback at 0.5 mm/second was performed. Images were acquired using a Galaxy 2 IVUS machine (Boston Scientific). Further analysis of digitally stored procedures was performed offline in accordance with the American Consensus statement for intravascular ultrasound,[199] and as described previously.[200] Off-line, a segment of the coronary artery was identified between two identifiable branch points. Suitable cross-sectional images were selected approximately 1 mm apart (60 frames) by a single operator blinded to the clinical and CMV status of each patient. A minimum of fifteen digitised images were then analysed with semi-automatic edge detection software (QIVUS, Medis Medical Imaging Systems, Netherlands) for the area of the external elastic membrane (EEM) and the internal lumen area (ILA). Intimal-medial area was selected as the most appropriate parameter to assess the diffuse coronary disease seen in the transplant population, and was calculated as a percentage of the EEM area occluded by intimal-medial thickening, thus: (EEM-ILA)/EEM x 100%. A mean value for intimal-medial area was calculated for the coronary segment imaged.

8.2.3 Carotid measurements

Participants rested supine for 10 minutes before undergoing high resolution ultrasound scanning (7.5MHz transducer; Prosound SSD-5500, ALOKA, Japan) of right and left common carotid arteries. Arteries were imaged longitudinally, 1 cm inferior to the bifurcation. For IMT, a recording was taken of zoomed images triggered to the R wave of the ECG. Distension of the common carotid artery was measured using an echo-tracking subsystem installed in the colour Doppler system.[201] Ipsilateral brachial blood pressure was taken immediately after carotid measures using an M5-I sphygmomanometer (OMRON Corp., Japan), and analysis was performed offline. IMT was defined as the distance between the leading edge of the intima and the media-adventitia interface, and was measured using ultrasonic calipers. Three measurements were taken in both the right and the left common carotid arteries, and a mean IMT calculated. For distension analysis, a minimum of 3 waveforms were selected using proprietary analysis software (ALOKA), and the distension calculated as an average of the difference between three peaks and troughs.[201]

8.2.4 Brachial artery endothelial function

Endothelial dysfunction was studied as previously described,[179] and is recorded fully in section 7.2.3.

8.2.5 Statistical analysis

Data were analysed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). Two-group comparisons of means were performed with the unpaired Student *t*-test for normally distributed data (percentage intimal-medial area, carotid intimal thickness, age, cholesterol, triglycerides, creatinine, eGFR, heart rate, blood pressure, donor age, ischemic time, time since transplant) and the Mann-Whitney U test for non-normally distributed data (episodes of acute rejection, serum glucose and hsCRP), following testing for normality using the Kolmogorov-Smirnov test. Comparisons of proportions were calculated using Fisher's exact test (sex, medications). Linear regression with multiple predictors was performed to explore the relationship between CMV status and vascular changes adjusting for age, time since transplant, and medication.

The study was approved by the Great Ormond Street Hospital Ethics Committee. Informed consent was obtained from all patients' parents, and consent/assent (as appropriate) was also taken from the patients themselves.

RESULTS

8.2.6 Conventional and transplant-related risk factors

There were no smokers or diabetics amongst the transplant cohort. There was no significant difference between the CMV-positive and negative groups with respect to age, sex, heart rate, blood pressure, renal function (serum creatinine and estimated glomerular filtration rate (eGFR) by the Schwartz formula[159]) or blood glucose or lipid levels (Table 8.1).

Parameter	**CMV IgG Negative**	**CMV IgG Positive**	**p**
Number of patients	18	19	-
Age (years)	14.4 ± 0.5	13.9 ± 0.6	0.57
Male sex	10 (56)	12 (63)	0.74
Heart rate (b.p.m)	90 ± 3	89 ± 3	0.85
Systolic blood pressure (mmHg)	112 ± 3	114 ± 3	0.58
Diastolic blood pressure (mmHg)	69 ± 3	66 ± 2	0.36
Glucose (median, mmol/l (IQR)	5.1 (4.9 – 6.1)	5.0 (4.7 – 5.5)	0.16
Cholesterol (mmol/l)	3.14 ± 0.14	2.91 ± 0.14	0.26
Triglycerides (mmol/l)	0.78 ± 0.07	0.79 ± 0.07	0.94
hs CRP (median, mg/l (IQR)	0.45 (0.22 – 2.22)	0.24 (0.1 – 0.71)	0.28
Creatinine (μmol/l)	64 ± 4	67 ± 4	0.60
Estimated GFR (ml/min/1.73m^2)	92 ± 7	87 ± 5	0.59

Table 8.1 Baseline characteristics of transplanted patients. All blood tests are fasting values. Values are expressed as mean ± S.E.M. or frequency (percentage), unless otherwise specified. Key: hs CRP – high-sensitivity C-reactive protein, IQR – interquartile range.

Transplant-related variables and medications were comparable between the groups (Table 8.2). 5 (26%) of the patients who were CMV IgG positive at the time of investigation had been CMV IgG negative at the time of transplant. Mean time from transplant to investigation was 4.7 years (range 1 – 12.4 years).

Parameter	**CMV IgG Negative**	**CMV IgG Positive**	**p**
Age at transplant (years)	9.8 ± 1.1	9.2 ± 4.8	0.73
Donor age (years)	20.5 ± 3.1	20.7 ± 3.8	0.97
Ischemic time (mins)	206 ± 17	173 ± 15	0.17
Episodes of acute rejection (median (IQ range))	0 (0 – 1)	0 (0 – 1)	0.52
Time since transplant (years)	4.7 ± 0.8	4.7 ± 0.7	0.99
Statin use	17 (94)	18 (95)	0.74
Primary immunosuppression (tacrolimus:sirolimus:ciclosporin)	17:1:0	17:1:1	1.0
Additional immunosuppression (MMF:azathioprine:none)	6:10:2	9:7:3	0.60
Double immunosuppression	16 (89)	16 (84)	0.68

Table 8.2 Transplant related variables. Values are expressed as mean ± S.E.M., or frequency (percentage), unless otherwise specified.

8.2.7 Vascular measurements – transplant recipients

Coronary artery intimal thickness (as measured by percentage intimal-medial area) was significantly greater in CMV IgG positive patients compared to those who have remained CMV IgG negative (23.9% vs. 17.2%, $p = 0.01$) (Table 8.3). There was a similar discrepancy in mean carotid artery thickness (0.50 mm vs. 0.47 mm, $p < 0.05$). In addition, flow mediated dilatation of the brachial artery was impaired in those patients who were CMV IgG positive (6.9% vs. 9.3%, $p < 0.05$). In contrast, dilatation to GTN (a measure of endothelium-independent arterial function) was similar between the groups (10.6% vs. 9.8%, $p = 0.68$), confirming that the difference in FMD was endothelium-mediated. All differences remained significant when adjusted for age, time since transplant, and medication with a multivariable logistic regression model.

Parameter	**CMV IgG Negative (n = 18)**	**CMV IgG Positive (n = 19)**	**p**
Coronary intimal index (%)	17.2 ± 1.3	23.9 ± 2.1	0.01
Carotid intimal thickness (mm)	0.47 ± 0.01	0.50 ± 0.01	< 0.05
Brachial flow mediated dilatation (%)	9.28 ± 0.94	6.89 ± 0.72	< 0.05
Brachial GTN dilatation (%)	9.84 ± 1.13	10.55 ± 1.31	0.68

Table 8.3 Vascular parameter results for heart transplant patients.

8.2.8 Vascular measurements – healthy children

There was no significant difference in cIMT between CMV IgG positive and negative healthy children (0.44mm vs. 0.45mm, p = 0.26) (Table 8.4). FMD was also similar between these two groups (9.5% vs. 9.4%, p = 0.87).

Parameter	**CMV IgG Negative (n = 48)**	**CMV IgG Positive (n = 21)**	**p**
Carotid intimal thickness (mm)	0.44 ± 0.01	0.45 ± 0.01	0.26
Brachial flow mediated dilatation (%)	9.4 ± 0.46	9.5 ± 0.82	0.87

Table 8.4 Vascular parameter results for healthy control children

8.3 DISCUSSION

This chapter demonstrates the deleterious effects of CMV IgG positivity on the coronary arteries of paediatric transplant recipients by the sensitive method of IVUS. It also shows systemic functional and morphological damage in the brachial and carotid arteries of these same transplant recipients. These systemic effects indicate that CMV is not reliant on donor-host interactions for its vascular pathogenicity. However, these changes were not seen in control subjects, implying that the vascular disease is a post-transplant event, related to CMV replication in the immunocompromised state.

Cardiac allograft vasculopathy remains the most common cause of death after 5 years post-transplant in adults,[87] and 3 years in children.[88] Previous studies have concluded that CAV is less prevalent in paediatric than adult patients;[202] indeed, the registry report of the International Society of Heart and Lung Transplantation reported that almost three-quarters of patients remained free from CAV at 8 years post-transplant.[203] In reality, this is likely to be a significant underestimate based primarily on limited angiographic surveillance, and IVUS is now accepted as a much more sensitive tool for the diagnosis of vascular changes.[193, 204] In contrast, a minority of adult patients will be completely free of vasculopathy on IVUS at 10 years post-transplant.[87] Despite this, 20 year-survival is a realistic hope for some patients following transplantation, which could be considered a good outcome for an adult where the mean age at transplant is over 50 years.[87] For a paediatric recipient, however, this represents death or re-transplant as a young adult. Clearly CAV remains a great concern for those involved in paediatric transplantation.

Paediatric patients provide a relatively homogeneous population to study CAV. The confounding effects of other risk factors for transplant coronary disease, such as diabetes, hypertension, smoking and elderly donors, are minimized.[203] Furthermore, there is acceleration of vascular disease post-transplant from a relatively low background level, which is quantifiable with the sensitive technique of intravascular ultrasound. The previous demonstration at Great Ormond Street of a link between CMV and graft vasculopathy used only angiography.[174] It is important that detailed IVUS investigation (using measurement of intimal-media percentage area for the first time in a paediatric series) has confirmed this hypothesis.

The current methodology differs somewhat from the few substantive paediatric IVUS papers and assessments of CMV in transplantation.[196, 197] This study used a measurement of intima/media percentage area to represent the severity of diffuse transplant coronary disease. Furthermore, I have chosen not to include patients with positive viral cultures or CMV DNA by PCR in the blood at the time of the study, as acute infection can alter peripheral vascular testing.[181] Instead, I assessed chronic subclinical CMV replication by dividing our population by CMV IgG status. I believe it is the chronic exposure to CMV which is key to vascular disease. Indeed, protection against subclinical CMV replication via T-cell immunity has been shown to reduce CAV,[188] as has anti-CMV prophylaxis,[186] and the prevention of vasculopathy by everolimus has been linked to its anti-CMV effects.[205]

In addition to the morphological changes seen in the coronary arteries, this chapter has also demonstrated a similar thickening of the carotid arteries of CMV-positive transplanted children. Carotid IMT is known to be an indicator of systemic atherosclerosis and coronary artery disease in non-transplant patients,[206, 207] and has been used previously to demonstrate an atherosclerotic tendency in paediatric transplant recipients,[208] but this is the first time that CMV has been implicated. This morphological change is reflected in the functional impairment demonstrated by the contemporaneous brachial artery FMD results.

There is obvious overlap between the FMD investigations of this and the previous chapter. In Chapter 7, analysis by CMV IgG alone showed a trend towards worse FMD for IgG positive patients, but this failed to reach clinical significance. I hypothesised there that that study was not sufficiently sensitive to illustrate a statistical difference on IgG alone, and post-transplant replication acted as a filter to select those patients that would have a more severe vascular phenotype. In this chapter, with the patients being more closely matched, it appears that CMV IgG positivity alone is enough to demonstrate that difference.

Although systemic impairment is likely to be less important clinically to this group of patients than coronary changes, the relevance of damage to native arteries should not be ignored. Firstly, the ability of CMV to cause vascular injury without additional donor-host immune interactions is important in understanding the pathogenesis of viral-mediated vascular damage post-transplant. It also leads to speculation that similar damage may be occurring in non-transplant immunosuppressed states. Furthermore, non-invasive investigations of systemic arteries may potentially identify a group of low vasculopathy risk patients and decrease the frequency of coronary surveillance required to check for CAV. With one of the goals of transplant programmes being a resumption of as normal a lifestyle as possible, this would be of obvious benefit.

The control group had an expected incidence of CMV seroconversion,[209] and age-matched the transplantation group well; the increase in CMV-positivity in the transplantation group was due to the typical increase in seroconversion post-transplant. I found no difference in measures of arterial structure and function in the systemic arteries of the control group, suggesting that in CMV-positive immunocompetent children, viral activity is insufficient to produce such accelerated injury. Direct comparison of transplanted and healthy children is debatable, but it is interesting that CMV IgG-negative transplant recipients have values for brachial FMD and cIMT that are similar to non-transplant controls. One interpretation of these results is that – without the added insult of CMV replication – transplanted children do not suffer systemic vascular injury.

These results confirm the need to target CMV replication post-transplantation. What remains in doubt is the optimal approach for combating the effects of CMV in solid organ transplantation. With no vaccine currently available, therapeutic strategies are split between pre-emptive and prophylactic options.[210] The possibility of indefinite anti-viral therapy has been explored in lung transplantation.[211] However, current therapies are expensive and potentially harmful.

I was unable to randomise the transplant patients with respect to CMV, for obvious reasons. The cross-sectional nature of this paper also limits insight into how the vascular health of these patients will be affected in the long-term by CMV. However, these findings are strengthened by the fact that paediatric transplant patients are a relatively homogeneous group less confounded by the risk factors for coronary disease seen in adult patients, and using a single centre experience further limited the potential for bias.

8.4 CONCLUSIONS

These findings emphasize the importance of CMV infection in paediatric heart transplantation. The earlier angiographic and physiological data has been extended through the application of accurate quantitative analysis using extracorporeal and intravascular ultrasound. This chapter has implicated subclinical CMV replication in coronary artery vasculopathy and, beyond the donor organ-recipient interaction, in systemic vascular changes and endothelial dysfunction. Each of these findings could prove detrimental to long-term outcome. Normal children, however, showed no systemic changes after CMV IgG seroconversion, implying the increase in vasculopathic CMV activity is a phenomenon of the immunosuppressed state.

CHAPTER 9

CONCLUSIONS AND POTENTIAL FUTURE DIRECTIONS

9.1 OVERVIEW

My aim when setting out on this body of work was to look at various aspects of the heart transplant journey for children, to understand better the factors that control success of the procedure. A successful heart transplant programme is the result of the culmination myriad components, each of which effects the patient directly, and – through interactions with other components – more indirectly, the product of which, we hope, is a healthy graft that lasts for as long as possible. Through dedicated research and clinical teams throughout the history of transplant medicine, these components have been studied, understood and improved, and have facilitated the successful care with which we provide our patients today.

The study of the entirety of paediatric heart transplantation would be beyond the scope of a single thesis. For this reason, I selected specific areas within the transplant sphere with which the Heart Transplant Team at Great Ormond Street had particular issues. I designed the project with a broad aim in mind – to look at children at various stages of their transplant life (namely pre-transplant, peri-transplant and post-transplant), to appreciate areas where changes have enhanced the outlook for these patients, and recognise factors that require further study and improvements. It was crucial, however, that this broad aim was broken down into specific, discrete and answerable questions.

The focus of Chapter 3 was the effect of pre-transplant diagnosis on post-transplant outcomes. It was designed to challenge the accepted teaching that patients with congenital heart disease had worse outcomes than those transplanted for cardiomyopathy. This teaching had several potential justifications: patients with congenital heart disease were more likely to have had previous operations, associated anatomical abnormalities (particularly vascular), and multi-organ dysfunction than their cardiomyopathic counterparts. However, the team at Great Ormond Street had felt that in the most recent era, our results for transplantation for congenital heart disease had greatly improved. The hypothesis of the work, therefore, was that the difference in outcomes for these two groups was largely due to a relatively high early attrition seen in the immediate post-operative period in congenital patients, which could be overcome with increasing experience in operating on this heterogeneous group. (Experience in the more homogeneous cardiomyopathy group was gained quickly by many centres around the globe). For this theory to stand up, I had to show that although historically transplantation for congenital heart disease had worse outcomes than for cardiomyopathy, the discrepancy had disappeared in the recent era. Since the crucial factor here was increasing surgical and intensive care experience, rather than a specific improvement (an evolution, rather than a revolution) an arbitrary time point of the Millennium was chosen to divide the programme into two approximately equal parts. Chapter 3 does indeed indicate that results of transplantation for congenital heart disease are now – at our institution – equal to those for cardiomyopathy, a result which is not yet echoed throughout the world of heart transplantation. I attribute the relative success in these patients of the Great Ormond Street heart transplant unit to the willingness to take on these difficult patients, the result of which has been the elusive but crucial experience in performing transplantation in this setting.

Chapter 4 moved chronologically onwards in the transplant journey, to assess the impact of peri-operative induction immunosuppression in our patients. The specific issue here was to review the success of the change in policy to administer basiliximab prior to cardio-pulmonary bypass. The basis of this decision was that – as a monoclonal antibody – concerns over washout during bypass of the effect of basiliximab were unfounded, and that perhaps added protection in the immediate post-operative phase might be beneficial (when the potential for immunological stimulation and damage might be considered highest). This work showed that post-operative administration of basiliximab in our patients had had no protective effect against early rejection when compared to no induction or anti-thymocyte globulin, but that pre-bypass delivery reduced post-transplant rejection and early mortality, thereby justifying the change in clinical protocol.

The purpose of Chapter 5 was to examine the reasons and risk factors associated with the need for extra-corporeal life support in the early post-operative period. Although increasing surgical and intensive care expertise has greatly reduced early mortality following heart transplantation, a significant proportion of patients still require mechanical support to bridge them through this challenging time. My work highlighted the relatively frequent need for ECLS (compared to conventional cardiac surgery), but illustrated the excellent medium-term survival for the patients who survived to hospital discharge, supporting its use. Importantly, I was able to identify restrictive cardiomyopathy, longer ischaemic times and extreme donor:recipient weight mismatch as risk factors for the need for ECLS. These factors are now used to guide our peri-operative care, particularly the use of nitric oxide and other pulmonary vasodilators, as well as the creation of an inter-atrial communication during surgery.

Whereas much of the development of heart transplantation has been directed at improvements in early mortality, the final section of my thesis was designed to focus on the long-term health (both cardiac and non-cardiac) of our patients. This is especially important for paediatric heart transplant recipients, whose grafts can only be expected to survive for around twenty years. The preservation of the graft is therefore paramount, along with the avoidance of other organ damage, which would preclude them from re-transplantation in the future. Chapter 6 looked at the side effect profile of tacrolimus, noting reductions in renal impairment and hypertension in patients following a switch from ciclosporin. The overall incidence of serious chronic side-effects in the study population was low, which may be also attributable to the early weaning of prednisolone in the majority of patients.

Chapters 7 and 8 examined the effects of cytomegalovirus on vascular health in transplant patients. Chapter 7 concluded that systemic vascular health in transplant recipients who had exhibited post-transplant CMV replication to be significantly impaired compared to those who had not. This laid the foundation for the additional study in Chapter 8 probing those effects further. By measuring intimal thickness measurements with intravascular ultrasound, for the first time in paediatric patients it was possible to link CMV with quantifiable coronary vascular damage post-heart transplant. I also looked at the systemic arteries of non-transplant children. This healthy, immunocompetent group exhibited no such discrepancy in vascular health based on CMV status, confirming the changes seen in the transplant population had occurred post-operatively. Since these studies have concluded, the transplant team at Great Ormond Street have moved towards earlier treatment of CMV replication, in an attempt to limit the effects of CMV post-transplant.

9.2 FUTURE WORK

Of course, there are important components of the transplant journey that were not the primary considerations of this thesis. Pre-transplant health, mechanical bridging to transplantation, organ harvesting and preservation, operative technique and re-transplantation are only some of the areas where further study is required. The following, however, are suggestions for possible future study that leads on from my work directly.

9.2.1 Mechanical rescue therapies for early graft failure

This thesis has outlined some of the risk factors for early graft failure and need for ECLS post-transplant. Although long-term outlook for those surviving to hospital discharge is excellent, there remain unsolved problems around this field. The proportion of patients needing ECLS is much higher in transplant than in conventional cardiac surgery – and predictably so. Protective steps, such as pulmonary vasodilatation and interatrial communications have certainly helped, but with such a significant mortality and morbidity burden associated with ELCS, further prevention is required. Perhaps earlier pre-transplant mechanical support, particularly in the case of a failing (or even potentially failing) right heart, might result in a reduction in mortality. With increasing expertise – not just in paediatric ECMO, but also paediatric ventricular assist devices – future studies may be able to demonstrate additional survival benefit in this challenging sub-group of patients.

One important study might look at the reversibility of high PVR whilst waiting for transplant on medical therapy (such as milrinone) compared to mechanical support. With more and more patients remaining as inpatients for three months or more, the results of PVR testing in the catheter laboratory at the point of listing and 3 months later could be compared, and any reduction further compared to improvements in immediate and long-term post-transplant outcome. It may also be possible to take direct measurements on the operating table prior to the commencement of cardiopulmonary bypass at the time of transplant, to define more accurately any improvements in pulmonary vascular physiology.

9.2.2 Immunosuppression in paediatric heart transplantation

Until the holy grails of either immune tolerance to transplanted grafts, or even implantation of non-immune organs are found, immunosuppression will continue as the double-edged sword of transplant medicine. Unfortunately, not only do today's agents have unwanted side-effects (e.g. hypertension, renal impairment), but their immunosuppressive effects leave the transplant recipient vulnerable to infection and post-transplant malignancy. In addition, under-immunosuppression brings with it potentially devastating rejection. Chapters 4 and 6 examined the use of immunosuppressive drugs in paediatric heart transplantation, but these studies, as well as current clinical practice, are limited by the inability to closely and accurately monitor immune function in our patients. Rather, we rely on crude surrogate markers (e.g. levels of drug metabolites) to direct immunosuppressant use. A possible solution to this would be the development of cost-effective, quick, reliable assays of immune function that would facilitate the highest protection against rejection with the lowest side-effect profile.

One potential study would be to compare the results of such an assay to the traditional method of trough tacrolimus level in being able to predict rejection. If results were encouraging, a cross-over trial of immune assay vs. tacrolimus levels in preventing rejection in new transplant recipients (using their clinically indicated one, three and six-month biopsies as outcome measures).

9.2.3 Cytomegalovirus and heart transplantation

The final two chapters of this thesis linked CMV with vascular impairment post-transplant, in particular with cardiac allograft vasculopathy, which remains the most important factor limiting long-term success in heart transplantation. However, it remains unclear why some patients with CMV remain relatively free of coronary problems, whilst others succumb to rapidly progressive, severe disease. One crucial unsolved riddle is the mechanism by which CMV causes these changes. My work has been fundamental to the development of an on-going study at Great Ormond Street probing the mechanism of CMV in transplant vasculopathy, looking at the number and function of various parts of the immune system, including T-lymphocytes, smooth

muscle cells, and circulating endothelial cells, in order to understand whether it is the virus itself, or the innate immune response to the virus, that is driving the vascular changes. Understanding this will be pivotal in developing management strategies, potentially prior to transplantation and certainly post-transplantation, to limit the effects of this common virus.

Once the results of these cellular studies are known, applying them to clinical practise through translational research will be crucial. The next stage might involve investigating the effects of anti-viral medication (e.g. ganciclovir) or even a CMV vaccine in controlling the cellular and molecular changes seen in patients who CMV levels have risen, and, of course, comparing to clinical CMV outcomes, including the systemic and coronary vascular changes investigated in Chapters 7 and 8.

9.3 CONCLUSION

Paediatric heart transplantation remains the only long-term solution for end-stage heart failure in children. Although the principles of operative technique, intensive care management and immunosuppression have been largely standardised, there remain significant unanswered questions in transplant medicine, and this thesis has attempted to investigate some of these.

Already, the research presented here has proved useful, both at Great Ormond Street and at other units, by challenging accepted teaching, justifying current practices, and changing others. In addition, it forms the basis for current on-going work, and I hope it will lay the foundation for other studies in the future. It is only through persistent investigation that we can hope to provide our patients with the best possible outlook in this challenging field.

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