Detection and Grading of Coronary Allograft Vasculopathy in Post Transplanted Heart Recipients Using Magnetic Resonance Imaging

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Awarding institution:
King's College London

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Detection and Grading of Coronary Allograft Vasculopathy in Post Transplanted Heart Recipients Using Magnetic Resonance Imaging

Dr Nathalie Dedieu

Thesis submitted to Kings College London for the Degree of Doctor of Philosophy (PhD)
ACKNOWLEDGEMENTS

This work is dedicated to all the children that have crossed my path, they always have been a source of inspiration; to those whose names and faces I will never forget and especially to those who made this thesis possible.

I would like to thank my supervisors, Professor Gerald Greil for his patience, guidance and advice and Professor Michael Burch for his support throughout my career in the UK, none of this would have ever been a reality without his help.

A massive thank you to Dr Tarique Hussain, words cannot express the depth of my gratitude and this thesis would have never been possible without him.

To all those, too many to name, at Great Ormond Street and Kings College London who have helped me and shared in their own way, the research journey with me.

Finally, I would want to dedicate this thesis to my family; to Rupert, to my Mum, to my brother Michael, to Astrid, Lara, Zoe and Eva and to Tatajo for their unconditional love and constant support.
PERSONAL CONTRIBUTION TO THE RESEARCH PRESENTED IN THIS THESIS

Chapter 1.1


Chapter 2.1


Chapter 2.2

Chapter 3

Detection of diffuse myocardial fibrosis and coronary late gadolinium enhancement using cardiac MRI as a marker of microvascular and epicardial disease in paediatric heart transplant. Submitted.

Chapter 4


Chapter 5.3


EXECUTIVE SUMMARY

Coronary allograft vasculopathy (CAV) remains the leading cause of death in paediatric heart transplantation. The diagnostic tools currently available are invasive and often lead to a late diagnosis, by which time the integrity of the graft is compromised.

This thesis explores Cardiac Magnetic Resonance Imaging (CMRI) as a reliable, non-invasive and radiation free tool for assessing CAV.

Chapter 1: In this chapter CMRI is introduced as a novel, safe, non-invasive and radiation free patient friendly tool in CAV detection. To confirm this, a review of the current state of the art of CAV diagnosis and management is provided as well as the relevant CMRI sequences.

Chapter 2: Coronary artery CMRI sequences applied to congenital heart disease and their potential in heart transplant patients are described in chapter two. We tested the use of an intravascular contrast agent combined with a specific MRI sequence as a tool to enhance the diagnosis of coronary abnormalities in congenital heart disease. The patient sample was unfortunately small, thus results have sometimes failed to reach statistical significance; however, they show a clear trend towards superiority in image quality, vessel length imaged and vessel sharpness when using an intravascular agent. Subsequently, these results have been confirmed in collaborative work, demonstrating improved coronary magnetic angiography when using an intravascular contrast agent in children with congenital heart disease.

Chapter 3: The core work of the thesis is presented in chapter three demonstrating, in thirteen patients, safe non-invasive and radiation free detection and quantification of CAV. Extracellular volume (ECV) as a marker of fibrosis in transplanted hearts correlates with intravascular ultrasound (IVUS) findings, the current gold standard of CAV assessment. ECV appears to be a promising marker of microvascular disease.
Late gadolinium enhancement (LGE) CMRI showed not only that coronary magnetic resonance angiography (CMRA) is more sensitive than conventional angiography in detecting epicardial disease but also that the degree of enhancement correlates with IVUS. Based on these results CMRI could be used as a standard screening tool in patients after heart transplantation. Patients with coronary vessel wall involvement can be identified non-invasively and radiation free using CMRA. IVUS can be reserved for patients with high suspicion of CAV and may be obsolete in the future for risk stratification.

Chapter 4: Alternative parameters for CAV assessment are presented in chapter four looking at wall motion abnormalities and strain defined by CMRI, using a newly available software, previously validated in congenital heart disease and other cardiac conditions.

Using feature-tracking-derived CMRI, wall motion abnormalities (WMA) were identified not only in all the patients who had angiographic disease but also in 9 additional patients. Our results correlated with both mean coronary artery stenosis on IVUS and graft survival on follow up. This pilot study demonstrates that wall motion abnormalities assessed with MRI allows discrimination between patients who have coronary involvement and those who do not. Accordingly, invasive investigations can be restricted to patients at risk defined by CMRI.

Chapter 5: Chapter five provides an overall discussion of the major findings of the thesis and proposes a multi-parametric CMRI sequence approach as well as a diagnostic algorithm that can safely and easily be applied to the paediatric transplant cohort in order to detect CAV earlier and non-invasively aiming towards improved outcomes in patients after heart transplantation.
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LIST OF ABBREVIATIONS

CAV: Coronary allograft vasculopathy

CAD: Coronary artery disease

CMRI: Cardiac magnetic resonance imaging

ECV: Extracellular volume

IVUS: Intravascular ultrasound

LGE: Late gadolinium enhancement

CMRA: Cardiac magnetic resonance angiography

LAD: Left anterior descending coronary artery

LCx: Left circumflex coronary artery

RCA: Right coronary artery

WMA: Wall motion abnormalities

MIT: Maximal intimal thickness

DSE: Dobutamine stress echo

Sm: Peak systolic motion velocity

TSm: Time to peak systole

CFR: Coronary flow reserve

ESE: Exercise stress echocardiography

FFR: Fractional flow reserve
SPECT: Single photon emission computed tomography

PET Positon emission tomography

MDCT: Multi-detector computed tomography

OCT: Optical coherence tomography

CMV: Cytomegalovirus

CNIs: Calcineurin inhibitors

MMF: Mycophenolate mofetil

PSIs: Proliferation signal inhibitors

CHD: Congenital heart disease

GdT: Gadofosveset trisodium

GdD: Gadopentetate dimeglumine

CNR: Contrast to noise ratio

SNR: Signal to noise ratio

BMC: Blood to myocardial contrast

IR: Inversion recovery

SSFP: Steady-state free precession

Gd-BOPTA Gadobenate dimeglunine

iNAV image-based navigator

GA: General anaesthesia

MOLLI Modified Look-Locker inversion recovery
SG: Stanford grade

PTLD: Post transplantation lymphoproliferative disease

EF: Ejection fraction

RWMA: Regional wall motion abnormalities

FT: Feature tracking

S: Strain

SR: Strain rate

LVEDV: Left ventricle end-diastolic volume

LVESV: Left ventricle end-systolic volume
CHAPTER 1 INTRODUCTION

1.1. DIAGNOSIS AND MANAGEMENT OF CORONARY ALLOGRAFT VASCULOPATHY IN CHILDREN AND ADOLESCENTS: LITERATURE REVIEW

1.1.1. DEFINITION AND PHYSIOPATHOLOGY

In children, coronary allograft vasculopathy (CAV) remains the main limiting survival factor after heart transplantation and the major cause of mortality after the first year post transplant ultimately leading to graft loss \(^1,2\). One elegant aetiological description of CAV is that of “immunologic mechanisms operating in a milieu of non-immunologic risk factors” \(^3\). The process is believed to start off as a response to endothelial injury in the graft, originated by a complex interaction of multiple donor and recipient factors. The resulting endothelial dysfunction, leads to altered endothelial permeability and subsequent intimal hyperplasia. The vascular remodelling is a consequence of the inflammatory response. The immunologic events constitute the original trigger and non-inflammatory events such as cytomegalovirus infection, ischemic time (reperfusion injury), increased donor age and classical cardiovascular risk factors (i.e. diabetes, dyslipidaemias, smoking and hypertension), perpetuate the inflammatory response and increase the endothelial injury \(^4\).

Typical lesions consist of diffuse intimal proliferation leading to the development of luminal stenosis and small vessel occlusion which then limits blood supply to the graft causing chronic vascular injury and ultimately myocardial ischemia \(^5\). The lesions develop earlier and quicker than atherosclerotic lesions. In addition, progression is often silent due to the lack of
ischemic symptoms from the denervated heart and often, the first clinical manifestation is an adverse cardiac event\(^6\).

Figure 1 Stenotic coronary artery: macroscopic aspect in a post mortem study of an explanted heart.
The real incidence of CAV among the paediatric population remains unknown, with a reported incidence varying between studies from 3% to 43%. According to an angiographic multicentre study, the incidence of CAV would be 2%, 9% and 17% at 1, 3 and 5 years. Looking at the incidence reported in studies that use intravascular ultrasound (IVUS), this is even higher, with 75% incidence of detectable intimal thickening at 5 years, with half of these representing at least mild disease. The most current angiographic data estimates the incidence of CAV in the paediatric cohort of 13% at 5 years, 25% at 10 years and 54% at 15 years.

According to the ISHLT registry, using angiographic definitions, 65% of recipients are free of CAV at 10 years, but after a diagnosis of CAV, the 2-year graft survival rate is less than 50%.

Age at transplantation has a strong influence on survival in paediatric recipients, with a 74% 8-year freedom of CAV in younger recipients (especially neonates and infants) compared to 56% in recipients older than 10 years.
As CAV lesions are preceded by endothelial dysfunction, it is essential to identify and characterize this as early as possible for targeted therapy and ultimately to improve patient survival.

1.1.2. DIAGNOSIS

The diagnosis of CAV is challenging. As a result of the denervation inherent to heart transplantation, patients fail to display classical clinical warning signs of angina \(^\text{11-13}\). The ability of early diagnosis is essential for the initiation and study of treatments but, unfortunately, the majority of the diagnostic techniques lack sensitivity or are invasive. A reliable and repeatable non-invasive method that detects CAV and its functional significance would have a huge impact on the follow up of heart transplant recipients. However, sensitivity and specificity of the currently available non-invasive tests remain limited.

Screening protocols vary among centres and the majority of units use a combination of diagnostic modalities, depending mainly on local preferences and expertise.

1.1.2.1. Angiography

For many years, until the introduction of IVUS, this has been the cornerstone of CAV diagnosis \(^\text{14,15}\). Despite its relatively low sensitivity \(^\text{16}\) and resulting delay in diagnosis, coronary angiography remains the most widely used diagnostic technique for CAV in the majority of transplant centres.

Angiography is known to underestimate the disease \(^\text{17}\). Adults series display a low sensitivity and negative predictive value. St Goar et al. found that 50% of patients with normal
angiographies had moderate to severe intimal thickening on IVUS\textsuperscript{16}. In a series by Tuzcu \textit{et al.} the sensitivity of angiography for CAV detection (defined by maximal intimal thickness >0.5mm) was 43%, specificity was however high with 95%.

Similarly, in a most recent paper, Gregory \textit{et al.}, using the same definition, showed a sensitivity even lower at 11% with a negative predictive value of 57%.

Defining CAV as mean intimal thickness >0.3mm, Stork \textit{et al.} found a sensitivity of 44% and a negative predictive value of 28% when compared to the IVUS data \textsuperscript{18}.

The main limitation of coronary arteries angiography arises from the fact that it assesses the vessel lumen. The contrast fills the patent lumen without direct visualisation of the vessel wall. By the time a filling defect appears and there is significant stenosis, the graft is already compromised. CAV tends to be diffuse and concentric affecting large and medium size vessels as well as the microvasculature \textsuperscript{19}. Typically there is initial vessel expansion: as the intima thickens, the external elastic membrane expands preserving initially the lumen area (Glagov-type positive remodelling) \textsuperscript{20-23}. This explains why the coronary angiography result can be normal in the presence of significant disease demonstrated by IVUS. Nevertheless, angiography is inexpensive, readily available across centres and findings have proven prognostic implications regarding graft survival and adverse cardiac events \textsuperscript{24,25}.

One of the largest experience in paediatric patients has been published by Pahl \textit{et al.} in 2005 and included multicentre data proceeding form the Pediatric Heart Transplant Study database. 2049 angiograms from 751 patients were analysed. The incidence of angiographic abnormalities at 5 years was and 17%. However, moderate-to-severe disease occurred in only 6% at 5 years.\textsuperscript{1} The use of IVUS in children is limited and published papers have shown a sensitivity of angiography compared to IVUS of between 18 and 30\% \textsuperscript{8,26}. 
In 2010, the ISHLT published new guidelines for CAV including a new classification in view to provide a more refined definition and prognostic value\textsuperscript{15}.

Table 1: ISHLT Consensus Grading for Coronary Allograft Vasculopathy (Mehra et al. 2010).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Not significant)</td>
<td>No detectable angiographic lesion</td>
</tr>
<tr>
<td>I (Mild)</td>
<td>Angiographic left main (LM) &lt;50% stenosis, or primary vessel with maximal lesion of &lt;70% , or any branch stenosis of &lt;70% ( including diffuse narrowing)</td>
</tr>
<tr>
<td>II (Moderate)</td>
<td>Angiographic LM 50-69% stenosis, a single primary vessel ≥70% stenosis, or isolated branch stenosis of ≥70%in branches of 2 systems</td>
</tr>
<tr>
<td>III (Severe)</td>
<td>Angiographic LM ≥70%, or 2 or more primary vessels ≥70% stenosis, or isolated branch stenosis of≥70% in all 3 systems, or mild/moderate angiographic disease with LVEF&lt;45% or evidence of significant restrictive physiology(i.e. symptomatic heart failure with echocardiographic E to A velocity ratio &gt;2 (&gt;1.5 in children), shortened isovolumetric relaxation time (&lt;60msec), shortened deceleration time (&lt;150msec), or restrictive hemodynamic values (Right Atrial Pressure&gt;12mmHg, Pulmonary Capillary Wedge Pressure &gt;25mmHg, Cardiac Index&lt;2l/min/m\textsuperscript{2})</td>
</tr>
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</table>
Figure 3: Left coronary angiography showing severe epicardial disease with multiple stenoses in left anterior descending artery and left circumflex artery.

Figure 4: Right coronary artery angiography demonstrating advanced disease as indicated by the arrows at several levels of the vessel.

1.1.2.2. Intravascular ultrasound (IVUS)

IVUS is more sensitive than angiography for early CAV detection and allows delineation of the vessel wall as well as measurement of intimal thickness. Even if it might provide an oversimplified picture of the disease process, the intimal thickening measured via IVUS
remains the most sensitive diagnostic modality available.\textsuperscript{15}

As mentioned above, Glagov-type positive remodelling occurs in response to the vessel wall disease. This serves to maintain initial lumen patency and the angiographic appearance of the vessel can therefore be normal despite significant CAV. This is particularly significant in the first year post transplantation. Later on in the disease process, constrictive negative remodelling of the vessel will occur and lead to the stenosis of the vessel.\textsuperscript{22}

IVUS parameters reported in the literature include: intimal thickness, mean intimal index (ratio of the mean intimal area to the sum of the mean intimal and luminal areas), total atheroma volume and percentage of atheroma volume. In 1995, Ricknbacher \textit{et al.} demonstrated that, in an adult cohort, moderate to severe intimal thickening diagnosed by IVUS was predictive of the future development of angiographically detectable disease (Table 2). This article describes CAV as being present when maximal intimal thickness is $\geq 0.3$mm. A further finding was that maximal intimal thickness (MIT) $\geq 0.3$ at 1 year was associated with a 4y survival of 73\% compared to 96\% within the group of MIT<$0.3$mm.\textsuperscript{27} Two more recent studies published in 2005,\textsuperscript{28} reported that a change of MIT $\geq 0.5$mm over the first year post-transplant was an independent predictor for subsequent angiographic development of CAV, for myocardial infarction and for all-cause death at 5-years post-transplant. Patients with a change in MIT $>0.5$mm had a 5-year incidence of 21 \% for death or graft loss, 46\% for all major adverse events and 65\% for the development of subsequent angiographic disease compared to 6, 17 and 35\% respectively for patients without a 0.5mm change.\textsuperscript{28}

Interestingly, however, intimal proliferation evaluated in IVUS does not always correlate with microvascular or small artery disease in biopsies specimens.\textsuperscript{15,29} Looking specifically at paediatric data, IVUS has not shown impact on prognosis and this probably relates to the limited number of studies, each with differing analysis methodology.

According to published data, the sensitivity increases with the number of vessels imaged.\textsuperscript{30} However, our experience in children suggests that this is not the case and multi-vessel
imaging increases risk without substantially altering sensitivity. Therefore, in our usual practice, we only image the left main and proximal left anterior descending. We use automatic pullback to enhance consistent sampling and identification of branch vessels that are used as landmarks in order to be able to compare serial investigations. We analyse 30 cross-section images taken at 1.5mm intervals and identified (as mentioned above) by branch points. Additionally, image analysis is performed during mid-diastolic rest period for consistency. In addition to maximal intimal thickness, mean intimal thickness, and mean intimal index, Stanford grading score (Table 2) and percentage of atheroma are recorded. We also use a semi-automatic interactive edge detection software (QIVUS) to improve reproducibility of measurements.  

Table 2: Stanford score (severity based on the localization of the most severe disease)


<table>
<thead>
<tr>
<th>GRADE</th>
<th>SEVERITY</th>
<th>INTIMAL THICKNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Minimal</td>
<td>&lt;0.3mm &amp; &lt;180 degrees</td>
</tr>
<tr>
<td>II</td>
<td>Mild</td>
<td>&lt;0.3mm &amp; &gt;180 degrees</td>
</tr>
<tr>
<td>III</td>
<td>Moderate</td>
<td>0.3-0.5mm OR 0.5-1mm &amp; &lt;180 degrees</td>
</tr>
<tr>
<td>IV</td>
<td>Severe</td>
<td>&gt;1mm OR 0.5-1mm &amp; &gt;180 degrees</td>
</tr>
</tbody>
</table>
Unfortunately, IVUS remains rather unused in clinical routine: the higher cost and potential morbidity added to the requirement of a trained operator, limits its use currently. This is particularly true in the paediatric population, where the size of the patient is an additional limitation. Nicolas et al., have reported feasibility in patients ≥ 10 kg but in our institution, we normally do not proceed in patients under 10 years of age 8,26.

1.1.2.3. Echocardiography

The usefulness and accuracy of several echocardiographic techniques, as diagnostic methods for CAV have been explored. Published data have shown disparate results but more recent reports involving dobutamine stress echocardiography have demonstrated greater prognostic value 32-34, 35-38.

Dobutamine stress echocardiography (DSE) allows assessment of wall motion, inducible ischemia and viability. Nevertheless, the sensitivity, specificity positive predictive value and negative predictive value vary significantly among these studies. Despite these limitations,
Spes et al. noted, in an adult cohort, that in patients with abnormal DSE, 90% had significant CAV by IVUS, but only 49% by angiography again demonstrating the relative insensitivity of angiography. Furthermore, they showed that a normal pharmacological stress echocardiography after heart transplantation has a high negative predictive value for any major adverse cardiovascular event. This suggests that if a strict DSE protocol is followed, a selective invasive angiography/ IVUS policy may be adopted. This was corroborated in a paediatric cohort by Pahl et al.

Some authors have pointed out that endothelial dysfunction might be the cause of abnormal wall motion detected by DSE and normal angiography.

In children, the variability when compared to angiography, is even higher than that showed in adults’ series. Sensitivity rates vary between 35 and 71%, specificity between 80 and 94%, positive predictive value between 45 and 91% and negative predictive value between 81 and 92%. If reliability within a given department is established, then it certainly appears to be an attractive option for children due to its non-invasive nature. However, it does require a good set up, effective sedation, expertise in images’ acquisition, expertise in interpretation and a standardised, reproducible protocol.

Sensitivity and specificity of stress echocardiography techniques can be improved by quantitative analysis using strain imaging. This modality can quantify regions of wall motion abnormality, (i.e. a reduction in peak systolic strain % will be seen in LV segments associated with inducible ischemia and accurate measurements of time to peak strain may also give information on regional wall motion abnormalities). Eroglu et al., demonstrated improved accuracy of DSE in adults using strain analysis.

Combined use of contrast-enhanced echocardiography with adenosine mediated hyperaemia in order to assess coronary flow reserve(CFR) has shown encouraging results in adults. Tona et al. demonstrated feasibility and prognostic value of coronary flow reserved measured by contrast enhanced echocardiography with good correlation with major acute
cardiac events. Severe CFR alteration was shown to precede acute cardiac event onset. On a more recent study, the same group, showed high sensitivity and specificity for this technique in the detection of significant CAV (defined by Media Intimal Thickness>0.5 in IVUS). Although these results are encouraging, more studies are needed to establish the reproducibility. Interestingly, a separate small study in adults showed that transoesophageal echocardiographic measurement of CFR impairment could identify CAV but it did not allow grading of severity. However, this approach will be more difficult to implement in children, owing to difficulty in imaging due to the small size of the coronary arteries and the need for sedation in many patients.

The application of tissue Doppler techniques to the transplant population is also worth mentioning. Dandel et al. showed the utility of power Doppler TDI for the diagnosis of CAV in adults. Systolic TDI parameters at basal lateral LV wall level showed the highest diagnostic accuracy. Peak systolic motion velocity (Sm) and time to peak systole (TSm) differed significantly between patients with and without CAV as identified by IVUS. Furthermore, with Sm >11 cm/s and TSm > 110 cm/s², angiographic disease can be excluded and, in the absence of any rejection, an Sm < 10 cm/s has a positive predictive value of over 97% for CAV (as detected by IVUS or angiography). The main limitations for the widespread use of this technique arise from the inter-observer and inter-departmental variability. These techniques have been applied to adult cohorts mainly and the available literature in the paediatric population is still very limited. One small retrospective study has shown that tricuspid annulus velocity was the best predictor of graft failure in pre-terminal patients. However, conventional echocardiographic parameters such as increase in tricuspid regurgitation severity and a reduction in left ventricular ejection fraction were also associated with increased mortality. Another recent study in a paediatric cohort showed poor correlation between TDI and haemodynamic parameters, highlighting the need for further confirmatory studies in children.

Exercise stress echocardiography (ESE) in adult patients was initially found to have
unacceptably low sensitivity for the detection of CAV\textsuperscript{32,49}. However, Chen et al. showed recently a sensitivity higher than 88\% with almost 92 \% specificity in detecting significant epicardial angiographic CAD among paediatric heart transplant recipients. The positive predictive value of ESE was 72.7\%, and the negative predictive value was 97.1\%\textsuperscript{50}. These results need wider confirmation prior to consideration as a screening tool.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Speckle-tracking echocardiographic analysis of myocardial deformation showing circumferential strain in a patient with CAV. On the top right image(B) we can appreciate the dyssynchrony (later contraction compared to the rest of the segments) and lower contractility of the green and purple segment corresponding to LCX territory. On the bottom right figure(D), we can appreciate how the same green and purple segments have significant lower contractility than the others (red corresponds to the maximum contractility and blue to the absence of it). The left superior panel (A) shows the colour coding for each of the segments. The left inferior panel(C) shows the time interval between beginning of QRS and maximal strain value.}
\end{figure}
1.1.2.4. Invasive Coronary Haemodynamics

CAV is a complex and diffuse process that leads to concentric luminal stenosis and occlusion of epicardial large and medium sized vessels. It also affects the intramyocardial microvasculature. Microvascular disease is present in heart transplant recipients early after transplant, even in asymptomatic patients and it is known to be associated with CAV, ischemia and death \(^{19,51}\).

Fractional flow reserve (FFR) is defined as the ratio of maximum flow in the presence of a stenosis to normal maximum flow. It is a lesion-specific index of stenosis severity that can be calculated by simultaneous measurement of mean arterial, distal coronary, and central venous pressure, during pharmacological vasodilation. FFR is a well-established tool to assess haemodynamic significance of coronary focal stenosis and has been recommended since 2010 by European Society of Cardiology for the physiological assessment of moderate coronary stenosis when functional information is lacking \(^{52}\) in atherosclerotic disease.

In such cases, pressure gradients and FFR are recorded throughout the length of the artery though a pull back of the wire during maximum pharmacologically-induced hyperaemia.

The Combowire® XT also allows simultaneous measurement of flow and pressure and FFR simultaneously to the coronary flow reserve (CFR).
Figure 7A: Resting pressure and flow recording in red the Aortic pressure, in yellow the distal Coronary pressure and in blue the pulse wave Doppler envelope.

Figure 7B: During hyperaemia note that the aortic pressure has decreased as well as the distal coronary pressure.

FFR: represents the ratio between the mean distal coronary pressure at a point past the stenosis and the aortic pressure during maximal hyperaemia.

CFR is the ratio between the hyperaemic blood flow and the resting myocardial blood flow.

In transplanted patients, the exact value of FFR to determine epicardial disease is difficult to establish and results have been inconsistent between series\textsuperscript{53,54}. In a publication by Hirohata \textit{et al.}, FFR improved as the microvascular disease deteriorated and therefore, due to the particular interaction between microvascular and epicardial disease that occurs in CAV, FFR might not be the best reflection of involvement of the epicardial arteries in this situation\textsuperscript{19}.

Coronary Flow Reserve (CFR) reflects the ability of the myocardium to increase blood flow in response to maximal exercise or stress. It is expressed by the ratio of the myocardial blood flow at peak stress, or maximal vasodilatation, to the flow at rest. Decrease in CFR, after Adenosine administration to achieve maximum vasodilation, in the absence of
significant epicardial stenosis (normal fractional flow reserve) indicates microvascular dysfunction.  

If the significance of decreased CFR is well established in the atherosclerotic population, the exact significance of CFR measurement in transplanted patients remains to be determined.

Using acetylcholine-mediated, endothelium-dependent, coronary vasodilatation measurement of CFR, Hollenberg et al. showed that endothelial microvascular dysfunction was more common in the group suffering adverse outcomes (death or angiographic evidence of CAV) than in those without adverse outcome. However, published data are not consistent between studies. In a larger cohort Kubrich et al., found no correlation between epicardial and microvascular disease: whilst microvascular dysfunction demonstrated by CFR was a predictor of outcome (death or adverse cardiovascular event) in the univariate analysis, it did not predict outcome in the multivariate analysis.

The paediatric population offers very limited data for CFR. In a small cohort, a decrease in CFR correlated with microvasculopathy seen in endomyocardial biopsy specimens. The invasive nature of Doppler wire flow measurements to determine CFR makes it an unattractive tool for children.

Several groups have presented data of CFR quantified by CMRI of the coronary sinus showing good correlation with PET or flow phantoms. More recently, Ishida et al. presented data on CFR as independent predictor of MACE in patients with known or suspected CAD. Kennedy et al. have translated this idea into the transplant population: they found that CFR determination by CMRI in the coronary sinus, was significantly decreased in patients with severe CAV and therefore, it may be a useful tool in non-invasively evaluating coronary allograft vasculopathy in heart transplant recipients.
1.1.2.5. Single Photon Emission Computed Tomography (SPECT)

SPECT is a useful clinical tool for myocardial perfusion imaging to detect and risk-stratify coronary atherosclerotic disease. Either exercise or pharmacological stress can be employed and, most commonly, one of the Tc-99m-labeled tracers is used. Numerous studies in adult population with coronary atherosclerotic disease have assessed the relative accuracies of stress imaging using nuclear cardiology techniques: for stress SPECT, sensitivity is around 87% with a specificity of 73% (compared to coronary angiography). Recently, it was recognized that some patients with non-critical coronary artery stenosis have abnormal stress perfusion imaging. This is due to microvascular and endothelial dysfunction causing abnormal flow reserve.

When applied to CAV, SPECT has a high negative predictive value in adults. When using Dobutamine stress and technetium-99m tetrofosmin, abnormal perfusion is associated to a risk ratio of 3.5 in predicting cardiac death. A reversible perfusion defect on stress SPECT is an independent predictor of mortality or graft loss and it seems that stress SPECT at one year post transplantation could be an early prognostic indicator.

In paediatrics, the experience with SPECT is largely anecdotal. The small size of the heart might be a limiting technical factor and the radiation related to the technique itself makes it a rather unattractive diagnostic tool.

1.1.2.6. Positron Emission Tomography (PET)

PET can be used for non-invasive assessment of myocardial perfusion measuring myocardial blood flow at rest and during stress. As well as myocardial perfusion reserve, perfusion of the epicardial arteries and the microvasculature can be determined. In
patients after heart transplantation, myocardial perfusion reserve measured with PET has been performed in a few studies \textsuperscript{80-82}. Wu \textit{et al.} found good correlation between IVUS and myocardial perfusion reserve even in the absence of angiographic lesions\textsuperscript{81}. Published data is very limited even in adults, related to the limited availability of the technique and the expertise required.

1.1.2.7. Multi-detector Computed Tomography (MDCT)

In the atherosclerotic population, MDCT has shown high sensitivity and specificity in the diagnosis of angiographic coronary arteriopathy and characterization of the stenotic disease \textsuperscript{83}. Recent studies also indicate that detection and characterization of the plaque is possible although challenging increasing its potential value as a diagnostic tool. \textsuperscript{84,85}.

The literature provides some data regarding the heart transplant population: Sigurds\textit{on et al.} used 16-detectorcomputed tomography to identify coronary stenosis and compared to angiographic disease (defined by luminal stenosis >95%). Sensitivity, specificity, positive and negative predictive values were 86%, 99%,81% and 99% respectively, unfortunately only a few subgroups of patients underwent IVUS \textsuperscript{86}.

Gregory \textit{et al.}, on the other hand, did use IVUS to compare 64-slice computed tomography results in 20 patients at 1 year post-transplant. They defined CAV as maximal intimal thickness >0.5mm and found that MDCT has a sensitivity of 70% and a specificity of 92% with a positive predictive value of 89% and negative predictive value of 77%. However, in this study, slightly less than 20% of coronary segments (mainly distal) could not be analysed due to poor image quality (probably in relation with elevated heart rate) \textsuperscript{87}.

Recent studies showed that dual source MDCT allows good image quality of vessel lumen \textsuperscript{88,89} and, when validated against IVUS, a high diagnostic accuracy \textsuperscript{90}.

A small study, just under 20 patients, demonstrated that a 64 slices MDCT, was superior to angiography for the identification of non-obstructive vessel wall disease. However, they did
not use IVUS for comparison. Schepis et al. compared 64 channels dual source MDCT with IVUS to look at vessel wall thickness. Defining CAV as intimal thickness >0.5mm on IVUS they established sensitivity, specificity, negative predictive value and positive predictive value of MDCT of 85%, 84%, 76% and 91% respectively.

Therefore, MDCT appears to be a useful tool for CAV screening. Although not as sensitive as IVUS, it is non-invasive and clearly superior to angiography. However, the elevated heart rate post-transplantation, especially in paediatric patients, compromises image quality and the need for potentially nephro-toxic contrast agent adds concern for heart transplant recipients, for whom renal impairment is a frequent comorbidity.

There is preliminary data available in children using MDCT compared to angiography and IVUS to identify coronary luminal stenosis, although the size of the series was very small and results would require further studies to be validated.

Again, the implied repeated radiation dosage, makes it a less attractive screening option in children.

1.1.2.8 Optical Coherence Tomography (OCT)

OCT is an intravascular high resolution imaging modality that measures reflected light waves intensity and converts these into a high resolution tomographic image. In CAD patients, OCT has been used to characterize plaque composition and differentiate between intimal hyperplasia, fibrous plaque, lipid-rich plaque or calcifications.
Figure 8: OCT images showing Intima hyperplasia. 
A: Transverse cut of the coronary.  
B: Longitudinal cut is shown.

Recent studies have evaluated the use of OCT in heart transplant recipients with promising results. The Optical coherence tomography for characterization of cardiac allograft vasculopathy after heart transplantation (OCTAV) study demonstrated, in 15 patients early post-transplant (with no angiographic evidence of CAV), that early quantification of intima-media ratio and characterization of the plaque is possible. There was no IVUS performed for comparison ⁹⁷. Garrido et al. compared OCT to IVUS in 21 patients, later post-transplant, and not only found good correlation with IVUS but also postulated that OCT offers better plaque characterization and less inter-observer variability ⁹⁸.

Cassar et al. compared OCT to IVUS and angiography in 53 patients, showing that OCT was superior to angiography but not to IVUS. IVUS and OCT were strongly correlated with 100% agreement ⁹⁹.
Further prospective and larger studies are needed to define the exact role of OCT in the diagnosis of CAV and, more importantly, to define its prognostic implications. Overall it appears promising but entails invasive approach and radiation in the cardiac catheterisation laboratory.

1.1.2.9. Cardiac Magnetic Resonance Imaging (CMR)

MRI coronary angiography in the context of CAD has proven its capacity to detect atherosclerotic plaque and proximal to mid-coronary artery stenosis \(^{100-102}\). Uribe et al. have demonstrated the feasibility and accuracy of MR coronary angiography in the detection of coronary anomalies in children, despite elevated heart rates with whole heart dual phase cardiac imaging \(^{103,104}\). Greil et al., have also previously shown the utility of coronary magnetic resonance angiography (CMRA) in patients with Kawasaki disease \(^{105}\).

These studies undoubtedly open the door for the application of CMRA in CAV including in paediatric cohorts. Unfortunately, when compared to MDCT, CMRA does not seem to be as sensitive or robust in the detection of coronary stenosis, although limited studies have been done. CMR offers several advantages: it provides functional information on myocardial characterization and contractility as well as wall motion performance; it allows quantitative measurements of ventricular volumes and it is radiation-free, which is especially valuable in a population already exposed to repeated x-ray angiography.

In conventional atherosclerosis, perfusion imaging has shown to be effective in detecting myocardial ischemia and to assess microvascular dysfunction as it detects downstream microvascular blood flow within the myocardium. The MR-IMPACT study demonstrated that MRI is superior to SPECT in identifying perfusion defects within the myocardium for atherosclerotic patients \(^{106}\). Perfusion stress with adenosine also provides prognostic data: a normal CMR stress perfusion scan showed 99% event free survival at 3 years \(^{107}\).

The use of adenosine for myocardium stress perfusion after heart transplantation has not
been widely reported. Nevertheless, Muehling et al. showed a reduced myocardial perfusion reserve in patients with CAV with good correlation between MRI and invasive measurements. Unfortunately, microvascular disease in this study could not be assessed. The authors also demonstrated that patients with CAV have a reduced myocardial perfusion even during rest conditions \(^{108}\).

In regards to CMR tissue characterization, Steen et al. showed that more than 80% of patients with severe angiographic CAV had a late gadolinium enhancement pattern suggesting subendocardial infarction with a distribution consistent with the angiographic pattern \(^{109}\). Furthermore, they were able to identify silent myocardial infarction in otherwise apparently event-free patients.

In a more recent publication, the same group looked at infarct-atypical myocardial involvement that they were not able to correlate with coronary angiographic pattern in the prior study. They found that within the 4 different patterns of infarct-atypical LGE-CMR, only the diffuse form was significantly higher in patients early post transplantation, however they could not establish a definite reason for the findings \(^{110}\).
Figure 9: Late Gadolinium Enhancement Scar imaging.
A: Typical infarct pattern Late enhancement with >75% transmurality.
B: Atypical pattern with diffuse pattern of late enhancement.

Hussain et al., have taken this technique further showing that high resolution late gadolinium enhancement (LGE) can be used to show vessel wall disease in CAV with good correlation with IVUS at 3T MRI scanners. LGE scores correlated well with the maximal intimal thickness and mean intimal index (Pearson coefficient 0.80 \( P<0.001 \) and 0.92 \( P<0.001 \), respectively). An enhancement diameter >7.5 mm gave promising sensitivity and specificity values of 86% and 93%, respectively, for the detection of significant CAV.
A recently published paper, evaluated in 48 transplanted patients both epicardial and microvascular disease concomitantly. The patients underwent coronary angiography, invasive coronary physiological assessment, IVUS and multiparametric cardiac MRI that includes, tissue characterization, perfusion analysis and tissue tagging. They found that cardiac MRI-based myocardial perfusion reserve was independently predictive of both epicardial and microvascular components of CAV and furthermore that diagnostic performance was significantly higher than angiography.¹¹¹

More studies are needed to establish MRI as a reliable non-invasive tool for CAV diagnostic but the latest data included in this thesis are encouraging.
RAPID PROGRESSION OF CAV WITHIN THE FIRST YEAR POST TRANSPLANT IS A STRONG INDICATOR OF SEVERE CAV, GRAFT LOSS AND MORTALITY. Therefore, prophylactic strategies are paramount and must be introduced early to improve long-term outcomes and prognosis. Similar to native coronary disease, primary prevention includes control of traditional cardiovascular risk factors such as hypertension, smoking, diabetes and hyperlipidaemia. This can be challenging, as many of these factors are also side effects of the immunosuppressive therapy. Tobacco should be avoided and care should be taken to avoid passive smoking in children. Transplant-specific risk factor modification includes prevention and aggressive treatment of sero-conversion of cytomegalovirus (CMV). In addition, it is essential to treat any episode of rejection early and aggressively.

1.1.3.1 Psychological care

Psychological support is crucial in transplanted children and their families throughout all the transplant journey: living with a reduced life expectancy when compared to peers is often complicated and, despite good quality of life, can be a source of distress for the recipients. In the context of CAV psychological support is especially important. Prevention is paramount and it is vital for the patients to be compliant with their antirejection therapy. However, it is well known that compliance declines in adolescence and cases of sudden death have been reported related to antirejection treatment discontinuation. Psychological support is essential to ensure good compliance. In cases of advanced CAV, the inevitability of graft loss can lead to severe depression that also requires psychological input.
1.1.3.2. Statins

Most transplant protocols nowadays include a statin, independently of the lipid level. Several studies have highlighted their benefits beyond lipid lowering effects\textsuperscript{114-116}, including reduced incidence of severe rejection episodes, reduced CAV progression and improved long term survival\textsuperscript{117-119}. Consensus guidelines unequivocally recommend statin therapy\textsuperscript{120}.

1.1.3.3. Cytomegalovirus (CMV)

CMV infection results in acceleration of CAV as the result of the host immune response. Aggressive treatment with ganciclovir reduces progression of CAV\textsuperscript{121} and the lack of prophylaxis is associated with increased lumen loss\textsuperscript{122}. Our institution, as with most of the transplant centres, uses acyclovir for viral prophylaxis during the first 3 months post-transplantation.

1.1.3.4. Vasodilators

A few reports indicate a potential role for vasodilators in preventing and slowing CAV progression. Calcium channels blockers and ACE inhibitors have been reported in the literature to be beneficial but large prospective trials are needed to determine their exact role\textsuperscript{123-125}. Most transplant institutions use both of these to treat hypertension, which develops frequently as a side effect of calcineurin inhibitor therapy.

1.1.3.5. Immunosuppression

Most of the data are from adult studies with limited evidence in the paediatric population.
1.1.3.5. Calcineurin inhibitors (CNIs)

Tacrolimus not only offers better protection against acute rejection compared to cyclosporine \(^{126-128}\), but it is also superior against CAV \(^{129}\). Moreover, Petrakopoulou et al. showed that tacrolimus is better than cyclosporine in the prevention of microvascular endothelial dysfunction \(^{130}\).

1.1.3.5.2. Mycophenolate mofetil (MMF)

MMF has demonstrated superiority to azathioprine in mortality and graft loss \(^{131}\). In the re-analysis of the same study, it also showed less intimal thickening and wider lumen area \(^{132}\). Finally, Kaczmarek et al., in 2006 demonstrated that MMF decreased CAV incidence \(^{133}\).

1.1.3.5.3. Proliferation Signal Inhibitors (PSIs)

Contrary to Calcineurin Inhibitors (CNIs) that block T-cell activation and proliferation by suppressing lymphokines production, PSIs inhibit T-cell and B-cell proliferation by impairing their response to growth promoting lymphokines \(^{134}\). In addition, PSIs have also a significant cytostatic effect on the immune system \(^{129,135}\). In 2003, Eisen et al. published the first data in favour of PSIs, using everolimus de novo after heart transplantation. They showed preservation of the coronary lumen at 1 year with significant lower incidence of CAV in the everolimus group compared to the azathioprine group \(^{136}\). A sub-study published in 2007 confirmed the results at 24 months \(^{137}\) and the same group has also shown reduced incidence of cardiovascular events in the everolimus group \(^{138}\). Nevertheless, despite the promising results of these studies, all of them compared PSIs to azathioprine, which is not used as first line therapy anymore and known to be associated with a higher rate of rejection than newer immunosuppressive agents.
Mancini et al., reported in a randomized study that sirolimus (as a secondary immunosuppressant) slows progression of CAV and reduces the incidence of clinically significant events, such as death or graft failure. Keogh et al., using randomized de novo treatment between sirolimus or azathioprine reported significantly reduced progression in intimal and medial proliferation at 6 months post-transplant and a reduction in the number of acute rejection episodes of around 50%. The effect was sustained at 2 years post-transplant using IVUS to quantify vessel wall proliferation.

Although a combined regime of CNIs and PSIs appears to be attractive in preventing and slowing CAV, serious concerns with this regimen relate to raised nephrotoxicity. PSIs have shown in several studies to increase side effects of CNIs, especially for nephropathy.

Raichlin et al. have published encouraging data with sirolimus-based immunosuppression, and even postulated that a CNI free regimen would be safe, well tolerated and associated with less CAV progression, coronary events and graft failure, when initiated beyond the first year (and within the first 2 years).

In a more recent study, the same group showed that early conversion to sirolimus attenuated plaque progression, improved overall survival, and increased freedom from cardiac events. However, the retrospective nature of the design and the differences in criteria for the therapy changes, make the results less generalizable. Moreover, a recent study reported that late conversion to PSIs is associated with necrotic plaque core and calcification of the plaque.

Hence, safety of early CNI withdrawal with PSI conversion remains uncertain, especially in the first year post-transplant with concerns also raised about acute rejection. Therefore, many continue to recommend against withdrawal of CNIs during the first 12m post transplantation.
Side effects from PSIs are not infrequent: anaemia, dyslipidaemia, increased incidence of bacterial infections, peripheral oedema, pericardial or pleural effusion, pneumonitis and delayed wound closure. They seem to be dose-related and reversed by discontinuation of the drug, although most can be controlled with dose adjustments 148.

PSIs have also been attributed with a reduction in CMV infections and an inhibition of Epstein-Barr virus-infected tumorigenic cell lines 149-151. In the paediatric population, PSI use is still limited to a rescue therapy for post-transplant complications such as CAV or renal impairment secondary to therapy.

1.1.3.6. Coronary revascularization

In contrast to native coronary disease, CAV is progressive and revascularization procedures are only palliative with no survival benefit 152,153. Moreover, the concentric, diffuse and distal nature of CAV precludes the majority of patients for revascularization procedures.

1.1.3.6.1. Percutaneous interventions

Percutaneous interventions in transplanted patients are characterized by good short term results but high restenosis rates 153-158. Unfortunately, stents do not offer better long-term results with a late re-stenosis rate around 70%. Drug eluting stents appear to have slightly better results with less restenosis 54,159. However, only the minority of CAV lesions are amenable for percutaneous revascularization as outlined above and stent angioplasty might only be an option in selected patients.

1.1.3.6.2. Bypass grafting

Surgical revascularization is associated with a very high mortality (up to 40%) and limited success and it is reserved to highly selected patients 158,160,161.
1.1.3.7. Re-transplantation

Re-transplantation is the only definitive treatment for CAV. Unfortunately it is associated with lower survival than with the primary graft \(^{162}\) (relative risk for 10 years mortality according to ISHLT 2012 data is 1.56) and the probability of CAV recurrence is higher (50% at 3 years) \(^{160,163}\).

The scarcity of donors, and prior antigen sensitization means that, in practice, re-transplantation occurs infrequently.

1.1.4. CONCLUSION

Despite a wide range of new diagnostic techniques, angiography remains, to date, the most commonly used diagnostic tool for CAV. Not only is it invasive, costly and radiation-prone but it also fails to identify the disease in its early phase. IVUS is the most sensitive technique but requires trained operators and it is, again, an invasive technique requiring ionizing radiation and remains unavailable in young children due to size limitations.

Overall, the available published evidence supports a role for MDCT or DSE as non-invasive screening tool to reduce the number of invasive angiograms (and IVUS). However, an accurate and reproducible non-invasive diagnostic tool is yet to be widely established. CMR offers anatomical, histological and physiological assessments and, in the future, it could be valuable in the detection and grading of CAV.

Early detection is paramount but remains challenging. It may allow us to identify those requiring modification in immunosuppression, such as early introduction of PSIs for those with more aggressive CAV.

Unfortunately, CAV remains the primary cause of graft failure after the first year post-transplantation and the only definitive treatment is re-transplantation.
1.2. Cardiovascular Magnetic Resonance Techniques Relevant to the Detection of Coronary Allograft Vasculopathy

1.2.1. T1 Mapping and ECV Detection

The application of T1 mapping for detection and quantification of diffuse fibrosis has been widely published, within multiples cardiomyopathies, infiltrative disease and aortic stenosis cohorts and has been shown to be effective in early diagnosis and risk stratification\(^{164-168}\).

Recently clinical studies using T1 mapping examined disease where fibrosis had a known underlying pathological role. Iles et al., studied a symptomatic heterogeneous heart failure population that included transplant recipients. They used post-contrast T1 mapping to demonstrate an inverse correlation of T1 values with percentage fibrosis on myocardial biopsy of transplanted hearts, as well as a reduction in T1 time with worsening diastolic function\(^{169}\). These results have been corroborated by several studies that also document inverse correlation between T1 time and biopsy findings and therefore offer histological validation\(^{170,171}\).

The measurements derived from the pre- and post-contrast T1 measurements represent the partition coefficient for gadolinium contrast, which, in conjunction with the blood haematocrit, allows an estimate of the extracellular volume fraction (ECV). This quantitative measurement of ECV correlates with fibrosis in the extracellular matrix.

ECV has also been shown to correlate with short-term mortality\(^{172}\) and histological collagen burden with low variability between scans\(^{173}\). However very few data are available in the transplanted population despite the non-invasive nature of the technique and its robustness.
1.2.2. Coronary and Myocardial Late Gadolinium Enhancement

MRI coronary angiography in the context of CAD has proven its capacity to detect atherosclerotic plaque and proximal to mid-coronary artery stenosis \(^{100-102}\). Previous publications from Kings’ College have demonstrated the feasibility and accuracy of MR coronary angiography in the detection of coronary anomalies in children despite elevated heart rates with whole heart dual phase cardiac imaging \(^{103,104}\).

These studies undoubtedly opened a door for the application of CMRI in CAV including in paediatric cohorts.

CMRI offers several advantages: it provides functional information on myocardial characterization and contractility as well as wall motion performance; it allows quantitative measurements of ventricular volumes and it is radiation-free, which is especially valuable in a population already exposed to repeated x-ray angiography.

Hussain et al., have shown that high resolution late gadolinium enhancement (LGE) can be used to show vessel wall disease in CAV with good correlation with IVUS at high field strength (3T) \(^{174}\). LGE scores correlated well with the maximal intimal thickness and mean intimal index (Pearson coefficient 0.80 [\(P<0.001\)] and 0.92 [\(P<0.001\)], respectively). An enhancement diameter >7.5 mm gave promising sensitivity and specificity values of 86% and 93%, respectively, for the detection of significant CAV.

In regards to CMR tissue characterization, Steen et al. showed that more than 80% of patients with severe angiographic CAV had a late gadolinium enhancement pattern suggesting subendocardial infarction with a distribution consistent with the angiographic pattern \(^{109}\). Furthermore, the authors were able to identify silent myocardial infarction in otherwise apparently event-free patients and able to disclose myocardial fibrosis in patients with absent or mild angiographic disease.
1.2.3. Detection of Wall Motions Abnormalities

Echocardiography speckle-tracking imaging is a well-described tool for the assessment of regional myocardial function. It is accepted that the obtained parameters provide important insights into systolic and diastolic function, ischaemia, myocardial mechanics and many other pathophysiological processes of the heart. Strain and strain rate provide valuable information on both global and regional systolic and diastolic function and their timing, therefore they can display cardiac dysfunction at an early stage of disease 175-177.

It is certainly non-invasive, widely available and easily applicable, but it is highly operator dependent and it requires patient cooperation. Furthermore, it relies on the quality of the acoustic windows, whereas this is not the case in an MRI environment.

In the transplant population, in order to identify CAV less invasively and in early phases, several groups have used dobutamine stress echocardiography (DSE) as it allows assessment of wall motion, inducible ischaemia and viability. Nevertheless, the sensitivity, specificity, positive predictive value and negative predictive value vary significantly among these studies 33-37,39. Despite these limitations, Spes et al. noted that in patients with abnormal DSE, 90% had significant CAV by IVUS, but only 49% by angiography again demonstrating the relative insensitivity of angiography 34. Furthermore, they showed that a normal pharmacological stress echocardiography after heart transplantation has a high predictive value for uneventful clinical course suggesting a high negative predictive value. Some authors have pointed out that endothelial dysfunction might be the cause of abnormal wall motion detected by DSE and normal angiography 178.

Using MRI for detection of cardiac performance with global and regional assessment of myocardial mechanics could be particularly helpful for the diagnosis of early wall motion abnormalities derived from fibrosis within the myocardium. TomTec® has developed an MRI software that allows quantification of myocardial function and deformation based on regular
CMRI images: Using routine cardiovascular magnetic resonance images, that have been acquired as part of a conventional cardiovascular MR examination, it analyses myocardial deformation based on proprietary tracking technology, calculating regional and global velocity, displacement, strain, and strain-rate parameters.

The only manual step needed for the software to process the data is the manual trace of a single endocardial contour. Subsequently the system automatically tracks the displacement of myocardial structures over the length of the complete heart cycle. The software tracks endo and epicardium, and describes left ventricular deformation in terms of longitudinal, radial and circumferential components. The software has been previously validated and is now FDA approved\textsuperscript{179,180}.

1.2.4. IMAGING CORONARY ARTERIES WITH MRI

Non-invasive, radiation free imaging of the coronary arteries is clearly desirable in adults and especially children, but CMRA imaging of the coronaries has proven to be difficult.

In a 2009 publication, Beerbaum \textit{et al.} describe how CMRA can be achieved in children and young adults (age range 2.6-25.8 years) with congenital heart disease that overcame significant challenges such as respiratory and cardiac motion as well as the proximity of coronaries to myocardium and epicardial fat\textsuperscript{181}.

In order to suppress cardiac motion, data was acquired at mid diastole when the heart is resting using a high temporal resolution cine imaging ECG-triggered sequence\textsuperscript{182}. Compensation for the respiratory motion has been achieved placing a navigator on the dome of the right hemi-diaphragm\textsuperscript{183} and the data is only accepted if the lung-diaphragm interface is within a 3 to 5 mm window placed in end-expiration. In order to suppress the signal from the epicardial fat, a small banded frequency selective pre-pulse is applied.
immediately before the imaging sequence \(^{184}\). Myocardial suppression was performed using a T2 pre pulse to generate contrast between blood and myocardium \(^{185}\).

However, imaging in small children especially younger than 6 years’ due to higher heart rates and smaller vessel size remained suboptimal.

A new protocol for coronary imaging was developed at Kings College London that proved to be successful in children older than 4 months \(^{104}\). The problem of cardiac motion at high heart rates was overcome by acquiring data during the cardiac rest period at the end of systole. The end systolic rest period remains relatively constant as opposed to the mid diastolic rest period that shortens significantly with higher heart rates.

General anaesthesia was used in this group of patients which allowed to compensate for respiratory motion with breath holds and regular breathing at the respirator with a very narrow navigator window (3mm). In accordance to other studies an intravascular gadolinium based contrast agent was used to improve signal within the coronary arteries \(^{186}\).

Subsequently a dual phase sequence was developed to acquire whole heart imaging during end-systole and mid-diastole during each cardiac cycle \(^{103, 187}\). Evaluating the coronary arteries using this sequence design different segments of the coronaries were optimally imaged during the systolic or diastolic rest period. Accordingly, dual phase coronary artery imaging proofed to be best CMRI coronary artery imaging method in paediatric patients \(^{103}\). However, coronary angiography remains challenging in paediatric patients despite advances in sequence design and the experience distributed to centres world-wide. Also scanning times of several minutes, lower spatial resolution and lower signal to noise ratio compared to MDCT made this technique not widely available.
CAV entails major implications for the prognosis of transplanted patients, therefore detection and early intervention has potentially a huge impact on the survival of a scarce but very vulnerable population.

This thesis aims to provide clinicians with a reliable tool that can detect and grade coronary allograft vasculopathy using MRI. Such a non-invasive tool has been sought by transplant teams for many years and is especially important in children.

This work investigates the application of novel MRI sequences that will detect microvascular disease as well as epicardial disease leading to identify those patients at increased risk of graft failure and death.

The hypothesis generated by this research is as follows: that with the integration of a comprehensive CMRI protocol to the clinical routine of transplanted patients we can discriminate between patients and select those risk and who, therefore, will require invasive assessment techniques. This has the potential to alter the follow up of heart transplant patients in the future.
2.1. CORONARY ARTERY IMAGING IN PATIENTS WITH CONGENITAL HEART DISEASE: IMPROVED IMAGE QUALITY USING AN INTRAVASCULAR CONTRAST AGENT AND SPECIFIC MAGNETIC RESONANCE SEQUENCE DESIGN

Dr Dedieu’s contribution to this paper as co-first author consisted of data analysis and manuscript drafting and editing.

2.1.1. ABSTRACT

Background: In patients with congenital heart disease (CHD) imaging of coronary artery origin and course can be crucial for preoperative planning. A novel intravascular contrast agent Gadofosveset trisodium (GdT) has demonstrated to be superior for angiography due to improved intravascular contrast compared to the currently used extravascular contrast agent Gadopentetate dimeglumine (GdD).

The aim of this study was to compare conventional post-contrast coronary magnetic resonance angiography (CMRA) using GdT or GdD to an optimized imaging sequence combined using only the novel agent, GdT.

Methods: Ten patients with CHD (age range 22 to 40 years; mean 31 years) were scanned at a 1.5 T clinical MR scanner (Achieva, Philips Healthcare, Best, The Netherlands) using a 32-element cardiac coil. An extravascular contrast agent (GdD) was administered first using a standard T2-prepared SSFP sequence. Within 72 hours, patients were re-scanned using
an intravascular contrast agent (GdT) and IR SSFP additional to the standard T2-prepared SSFP MR sequence. Image quality was graded form 0 (non-diagnostic) to 4 (best image quality). The left anterior descending (LAD), the left circumflex (LCX) and the right coronary artery system (RCA) were assessed separately. Contrast-to-noise ratio (CNR), Signal-to-noise ratio (SNR), blood-to-myocardial contrast (BMC) and image quality were analysed for the three techniques. A p-value ≤0.05 indicated statistical significance.

Results: The intravascular contrast agent GdT showed significantly improved CNR and BMC when combined with an optimized IR SSFP sequence. There was a trend towards being able to image longer segments of the LAD and RCA and towards improved vessel sharpness but the level of significance was not reached. As expected, coronary artery diameter and image quality, were not significantly different since we imaged the same individuals.

Conclusion: The combination of a novel intravascular contrast agent (GdT) and the addition of an adapted inversion recovery whole heart sequence design seems to provide improved contrast of the coronary artery system compared to currently available extravascular contrast agents and conventional sequence design.

2.1.2 INTRODUCTION

Major improvements in the last decades in CHD diagnosis and treatment have led to an increased patient survival up to approximately 85% to adulthood \(^{188}\). Consequently, with this continuously growing and aging population, physicians are facing new diagnostic challenges. Knowledge about coronary anatomy can be essential for long-term follow-up of these patients. Examples are patients after surgical repair of transposition of the great arteries \(^{189,190}\) and patients requiring a pulmonary homograft or transcatheter valve implantation to exclude abnormal course of the coronaries \(^{104,188,191-193}\). Imaging of coronary arteries with echocardiography is challenging in young children and becomes extremely
difficult in older patients due to poorer acoustic windows. Catheter-based angiography is invasive, costly and entails radiation, which carries the risk of cancer especially in patients who need regular follow-up exams including cardiac imaging \(^{189,190,194}\). Therefore, MRI as a radiation free non-invasive diagnostics tool represents a great advantage for these patients.

Classically the standard MR angiography (MRA) for assessment of arterial and venous anomalies in congenital heart disease is performed using a 3-dimensional (3D) single-or multiphase first-pass MRA sequence with an extracellular contrast agent without cardiac gating during at least one breath hold. Unfortunately, this entails limited spatial resolution related to the limited capacity of breath holding of sick patients or limited compliance in young children or infants. Moreover, this approach generally does not include ECG gated sequences or respiratory motion compensation and is therefore quite prone to artefacts.

Whole heart coronary MR angiography (CMRA) has been previously used in adults and children with congenital heart disease \(^{104,195}\). This free-breathing high spatial resolution three-dimensional sequence allows acquisition of extensive cardiac data via a navigator-gated ECG-triggered T2-prepared SSFP sequence. Good diagnostic accuracy (up to 84% according to Tangcharoen et al.) \(^{104,196}\) and extensive details on cardiac anatomy, volumes as well as function assessment, via a single and easy to apply sequence have been demonstrated \(^{103,181,197,198}\).

The use of a conventional extravascular contrast agent like Gadopentetate dimeglumine (GdD) has been widely described in MR angiography \(^{197,199}\). However, first pass techniques carry limitations due to the fast diffusion of GdD into the extravascular space. For visualization of small structures such as coronary arteries, this technique would benefit from increased and longer blood pool contrast compared to the extravascular tissue or fluid \(^{197,200}\).

The novel intravascular contrast agent, Gadofosveset trisodium (GdT) has the potential to increase intravascular contrast to improve image quality and therefore diagnostic performance for the coronary arteries \(^{201}\).
Furthermore IR SSFP provides enhancement of contrast between tissues with different T1-relaxation times through the addition of a 180° inversion pre-pulse \(^{202}\), with a determined tissue signal being specifically nulled by selecting an optimal inversion time. Originally this technique was developed and used to detect myocardial infarction \(^{203,204}\) but it has since then been extended to other cardiac diagnosis *i.e.* cardiomyopathies, myocarditis, amyloidosis, Anderson-Fabry disease etc.

The aim of this study was to compare conventional post-contrast CMRA using GdT or GdD to an optimized imaging sequence combined using only the novel agent, GdT.

**2.1.3. MATERIAL AND METHODS**

The study was approved by the local ethics committee (Guy’s and St. Thomas’ NHS Research Ethics Committee, London, England) and was registered with the United Kingdom Medicines and Healthcare Products Regulatory Agency.

Ten patients with CHD agreed to take part in the study (eight men, two women, age range 22 to 40 years, mean 31 years: table 3).
Table 3: Patients characteristics.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at MRI (years)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>30</td>
<td>TGA (Mustard procedure)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>28</td>
<td>Coarctation</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>26</td>
<td>Coarctation</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>40</td>
<td>PAPVD</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>36</td>
<td>VSD with infundibular stenosis</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>22</td>
<td>TA (Fontan procedure)</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>28</td>
<td>TOF</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>34</td>
<td>Coarctation</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>34</td>
<td>Coarctation</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>29</td>
<td>Coarctation</td>
</tr>
</tbody>
</table>


Written consent was obtained and medical history was assessed in all patients. Only patients with a history of CHD who were scheduled for a routine MR imaging examination were included in this study. Patients with significant renal dysfunction were excluded (defined as estimated glomerular filtration rate [eGFR] < 30 ml/min/1.73 m²). All patients had follow up clinical appointments after the scan and at 1-year post MRI.
MRI technique

Patients were scanned on 2 occasions at a 1.5 T clinical MR scanner (Achieva, Philips Healthcare, Best, The Netherlands) using a 32-element cardiac coil. During the first scan, gadopentetate dimeglumine (Magnevist®; Bayer Schering Pharma, Berlin, Germany) was administered at a dose of 0.2 mmol per kilogram of body weight (maximum volume, 40 mL) and patients were scanned using a standard commercially available respiratory navigator–gated and ECG-triggered T2-prepared SSFP sequence. During the second scan (within 72 h), a dose of 0.03 mmol per kilogram of body weight gadofosveset trisodium (Vasovist®; Bayer Schering Pharma, Berlin, Germany) was injected and all patients were re-scanned using a IR preparation- pre-pulse SSFP sequence additional to the standard T2-prepared SSFP MR sequence.

The optimal inversion time to suppress extravascular tissue was determined by a Look-Locker sequence. In this study, it was used to determine the optimal inversion time (approximately 260–280 msec) to minimize signal from extravascular tissue (Table 4).

Table 4: MR imaging parameters for contrast-enhanced 3D sequences.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T2-prepared SSFP</th>
<th>IR SSFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetition time /echo time</td>
<td>4.7/2.4</td>
<td>4.5/2.1</td>
</tr>
<tr>
<td>(msec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flip angle (degrees)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Bandwidth (Hz/pixel)</td>
<td>542</td>
<td>542</td>
</tr>
<tr>
<td>Field of view (mm)</td>
<td>340 x 340</td>
<td>340 x 340</td>
</tr>
<tr>
<td>Voxel size (mm) *</td>
<td>1.4 x 1.4 x 1.4</td>
<td>1.4 x 1.4 x 1.4</td>
</tr>
<tr>
<td>Acceleration factor for SENSE</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Acquisition time (sec)</td>
<td>240 - 255</td>
<td>230 - 248</td>
</tr>
<tr>
<td>Navigator efficiency (%)</td>
<td>52 ± 8</td>
<td>53 ± 9</td>
</tr>
</tbody>
</table>

Images analysis

Image processing, reformatting and analysis were performed with commercially available software (ViewForum, Philips Healthcare, Best, The Netherlands), as well as a specialised custom-made coronary analysis software ("Soapbubble")\(^{189,190,195\ 104,188,191-193}\). Two experienced investigators analysed the images independently (DL with 8 years and ND with more than 2 years’ experience in cardiac MRI). Consensus reading was performed in case of disagreement.

Signal to noise and contrast to noise: (SNR and CNR)

For quantitative image analysis, regions of interest (ROIs) were defined in areas of myocardium, the intra-aortic blood-pool close to coronary ostia, and in a region anterior to the chest wall, where no respiration-induced motion artefacts were visually identified. Signal-to-noise (SNR) was defined as the mean signal intensity found in a ROI divided by the standard deviation found in the ROI anterior to the chest wall\(^{189,190,194}\) (Equation 1).

SNR was evaluated using the following formula:
\[
SNR = \frac{S_{\text{Mean}}}{SDEV_{\text{Mean}}} \tag{1}
\]

\(S_{\text{Mean}}\) in [1] denotes the mean signal intensity in the user defined region of interest and \(SDEV_{\text{Mean}}\) relates to the standard deviation of the mean of the signal intensity anterior to the chest (parallel imaging was used: sensitivity encoding for fast MR imaging [Philips specific])\textsuperscript{104,191-193,195}.

At the same anatomical level, SNR was also quantified in the blood-pool of ascending aorta. SNR of the muscle signal was determined in the muscle of the LV anterolateral wall at the level of proximal RCA.

Contrast to noise ratio (CNR) was defined as the difference of the mean signal intensities in two user specified ROIs divided by the standard deviation found in the ROI anterior to the chest wall \textsuperscript{104,190,194,196} (Equation 2). The CNR between blood and muscle was defined as:

\[
\text{CNR} = \frac{S_{\text{Mean,Blood}} - S_{\text{Mean,Muscle}}}{0.5 \times (SDEV_{\text{Mean,Muscle}} + SDEV_{\text{Mean,Blood}})} \tag{2}
\]

**Blood to myocardium contrast: BMC**

BMC was defined as the mean signal intensity of the blood divided by the mean intensity of the myocardium in the previously described chosen ROIs.
Vessel sharpness, length and diameter measurements

For the quantitative image analysis of coronaries vessel wall sharpness, length and area a custom-made analysing tool (“Soap Bubble”) was used \(^{104,181,195,197}\). As previously described by Botnar et al. \(^{195,197,199,206}\) vessel sharpness can be obtained using a Deriche algorithm \(^{104,196,197,200}\). Briefly, this algorithm allows the calculation of an edge image by using a first-order derivative of the input image; the local value in a Deriche image, which represents the magnitude of local change in signal intensity. A vessel sharpness of 100% refers to a maximum signal intensity change at the vessel border. A lower edge value is consistent with inferior vessel sharpness. For the identification of the vessel edges along the path, a semiautomatic vessel tracking algorithm is used.

Each one of the main coronary arteries (LAD, LCX and RCA) were analysed for the use of the extracellular (T2-prepared SSFP) contrast agent and intracellular (T2-prepared SSFP and IR-SSFP) contrast agent.

Visual scoring of the quality of the coronary images:

For qualitative image analysis, after coronary reformat using Soap Bubble software, image quality was graded for the 3 sequences for each of the main coronary vessel from 0 (non-diagnostic) to 4 (best image quality) \(^{181,197,201}\) (Table 5).

Table 5: Image quality assessment (from McConnell et al.) \(^{197,199,202}\).
<table>
<thead>
<tr>
<th>Score</th>
<th>Grading</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non diagnostic</td>
<td>Coronary artery not visible</td>
</tr>
<tr>
<td>1</td>
<td>poor</td>
<td>Coronary artery visible with markedly blurred borders / edges</td>
</tr>
<tr>
<td>2</td>
<td>good</td>
<td>Coronary artery visible with moderately blurred borders / edges</td>
</tr>
<tr>
<td>3</td>
<td>very good</td>
<td>Coronary artery visible with mildly blurred borders / edges</td>
</tr>
<tr>
<td>4</td>
<td>excellent</td>
<td>Coronary artery visible with sharply defined borders / edges</td>
</tr>
</tbody>
</table>

**Statistical analysis:**

For statistical comparison, non-parametric methods using statistics software (IBM SPSS Statistics Version 20) were applied. In light of the small sample size we checked the preconditions for parametric testing, using Kolmogorov-Smirnov tests. Since variables were not normally distributed, we conducted the non-parametric Wilcoxon tests for dependent variables. A p-value ≤0.05 was considered to indicate statistical significance.

**2.1.4. RESULTS**

Ten patients completed the study protocol (Table 3: patient characteristics) without any complications. All indications for MRI were part of the clinical follow up, and all patients agreed to undergo a second scan for research purposes. One of these patients had an abnormal origin of the LCX. None of them experienced any side effects of the MRI scan as well as contrast agent administration.
Quantitative analysis

SNR, CNR and BMC

The use of IR SSFP with GdT resulted in significantly improved CNR and blood to myocardial contrast (BMC) when compared with the conventional T2-prepared SSFP sequence using GdT (p=0.002 for CNR and BMC) as well as GdD (p=0.002 for CNR and BMC). Furthermore, GdT improved SNR significantly using T2-prepared SSFP (p=0.039) and IR SSFP (p=0.0098) when compared to GdD.

Figure 11: Graph showing qualitative analysis of the images with SNR, CNR and BMC displayed for the different sequences (T2-prepared SSFP for GdD, GdT and IR SSFP GdT), p-values are indicated above the column graphs.
Vessel length and vessel diameter

We did not find any statistically significant difference in vessel diameter when comparing the two contrast agents and three different imaging protocols. This indicates that, as expected, neither the contrast agent nor the sequences let to a difference in vessel diameter within each of the scanned individuals.

Despite not reaching statistical significance, there was a trend towards increased length of LAD or RCA imaged when using IR SSFP with GdT.

Figure 12: Graph showing vessel length for each of the coronary arteries and each of the contrast agent and sequence combination.
Vessel sharpness

Mean vessel sharpness was measured (GdD T2-prepared SSFP, GdT T2-prepared SSFP, GdT IR SSFP respectively) for LAD (0.44, 0.43 and 0.45), LCX (0.39, 0.42 and 0.48) and RCA (0.46, 0.46 and 0.48). Once again these results seem to indicate a trend in favour of IR SSFP with GdT for the LAD and RCA compared to the conventional T2-prepared SSFP sequence but did not reach statistical significance).

Figure 13: Graph showing vessel sharpness for each of the coronary artery and each of the contrast agent and sequence combination.

Qualitative image analysis

Image quality was scored for the RCA, left main, LAD, and LCX artery according to McConnell et al. 197,200,203,204 as stated in table 5. Mean image scoring (GdD T2 prepared
SSFP, GdT T2-prepared SSFP, GdT IR SSFP respectively) was for LAD (3.3, 3.35 and 3.05) for LCX (1.95, 3.0 and 2.7) and for RCA (2.3, 3.2 and 2.7) respectively and did not reach statistical significance.

Figure 14: Image rating score for each of the coronary artery and each of the contrast agent and sequence combination based on McConnell et al classification as shown in table 5.

2.1.5. DISCUSSION

The present study evaluated the impact of an intravascular contrast agent on whole-heart MR angiography for characterization of origin and course of coronary arteries as well as the identification of possible coronary abnormalities. Additionally, IR SSFP in combination with GdT was evaluated. Whole heart MR angiography enables visualization of the majority of cardiovascular structures of the thorax, assessing simultaneously venous and arterial anomalies including the coronary artery system with a free breathing acquisition. If the data are acquired isotropically, the complete coronary artery system can be reformatted from
a single whole heart dataset by post-processing software applications. Currently, a whole heart T2-prepared SSFP sequence is the most commonly used approach\textsuperscript{189,202,207-210}.

Whole heart CMRA does have some disadvantages these include the following: the acquisition time can be fairly long; the compensation for breathing artefacts depends on adequate navigator efficiency and finally, the contrast between vascular structures and surrounding tissues or fluids is often reduced leading to reduced imaging quality.\textsuperscript{104,188,191,193,203,204} In that context, intravascular contrast agents may provide a significant advantage compared to extravascular contrast agents. Since GdD is an extravascular contrast agent its binding capacity to albumin is relatively low. Conversely, GdT has a non-covalent, transient, reversible but much stronger binding to albumin, that results in a prolonged intravascular enhancement\textsuperscript{189,194,205,211-214}.

Thus, GdT's non-covalent binding to albumin not only contributes to slower renal excretion but also increases T1 relaxivity up to 6 to 10 times when compared to GdD\textsuperscript{104,195,214-217}. The intravascular contrast staying in the blood pool for a longer amount of time allows characterization of all cardiac segments and structures, including arterial and venous phases, with a reduced requirement for precise timing of the contrast bolus. Recently published data has shown that intravascular agents are superior in defining anatomical structures in patients with congenital heart disease\textsuperscript{206,218}.

Although, Raman \textit{et al.} demonstrated in a recent study that GdT was associated with an increase in CNR (24\%) between arteries and myocardium as well as an improvement in improved image quality when compared to GdD, the differences between the 2 groups were not as significant as in our cohort\textsuperscript{161}. It is worth mentioning that this study was conducted on a 3T scanner while our study was performed on a 1.5T scanner. However these results were
similar to those published by Wagner et al. \textsuperscript{219} where GdT whole heart MRA showed a slightly better image quality.

In our study CNR and BMC were significantly better for GdT using the IR SSFP sequence that allows a suppression of fluids and extravascular tissue in the presence of the short T1 values of the blood. As GdT provides short T1 values of the blood for several hours, CNR and BMC values increased significantly when combining GdT and IR-SSFP (Figure 11).

We would have also expected a higher image quality in terms of image rating for IR SSFP when compared to T2-prepared SSFP sequences \textsuperscript{205,218}. In our study the LCX seemed to benefit most from higher intravascular contrast and the IR SSFP sequence (Fig. 14). As image quality is mainly influenced by respiratory and cardiac motion as well as heart rate variability, but not intravascular contrast, techniques to suppress respiratory and cardiac motion will predominantly influence image quality. Respiratory motion remains a significant impediment in image quality and speed. The standard 1D navigator used assumes a constant linear relationship between cardiac and diaphragmatic motion, which is incorrect and not only increases scan time but, on occasion, leads to residual motion artefacts. The recent introduction of 2D and 3D self-navigating result in improved coronary artery imaging and shorter scanning times \textsuperscript{200-202,220,221}. These new navigator techniques in combination with GdT and IR-SSFP might therefore offer a significant improvement in imaging the coronary artery system and need to be evaluated in future studies.

Regarding vessel length, we did not find a statistically significant difference between the two contrast agents (fig 12). However, when using GdT in combination with both IR SSFP and T2-prepared SSFP, the trend is favourable for the intravascular agent for the LAD and RCA. As expected, vessel diameter did not differ between the three groups since we compared the coronary arteries in the same individuals.
Vessel sharpness did not significantly improve with GdT. However, there was a tendency for better vessel wall sharpness of the GdT-IR-SSFP combination (Fig 13). Similar results were reported by Makowski et al.²⁰³,²⁰⁴,²¹⁸ with better identification and definition of the anatomy of the great vessel using this technique. It is very likely that we could not reach statistical significance due to the small sample size as well as the much smaller vessel size.

Figure 15: Representative reformatted images of the RCA in the same patient using GdD with a T2-prepared SSFP sequence (a), GdT with a T2-prepared SSFP (b) and GdT with IR SSFP (c).

Figure 16: Representative reformatted images of the LCX in the same patient using GdD with a T2-prepared SSFP sequence (a), GdT with a T2-prepared SSFP (b) and GdT with IR SSFP (c).
2.1.6 CONCLUSION

A single injection of an intravascular contrast agent in combination with an IR SSFP sequence showed significantly improved CNR and BMC. Image quality was not significantly different, which could be addressed in the future with novel motion compensation techniques for cardiac and respiratory motion.

Using this technique, we also imaged more vessel length of the LAD and RCA and improved vessel sharpness for all coronaries, but unfortunately statistical significance was not reached.

2.2. CONTRAST ENHANCED CORONARY MAGNETIC RESONANCE ANGIOGRAPHY USING RESPIRATORY IMAGE BASED NAVIGATION IN PATIENTS WITH CONGENITAL HEART DISEASE:

IMPROVED CORONARY MAGNETIC RESONANCE ANGIOGRAPHY USING GADOBENATE DIMEGLUMINE IN PAEDIATRIC CONGENITAL HEART DISEASE (SILVA VIEIRA ET AL.)

Dr Dedieu’s contribution to the paper included data analysis and manuscript editing\(^\textsuperscript{222}\).

2.2.1. INTRODUCTION

Three-dimensional whole-heart coronary magnetic resonance angiography (CMRA) is a well-established technique to assess coronary anatomy and cardiovascular morphology in patients with congenital heart disease\(^\textsuperscript{104,206,223}\). In the paediatric population, the desire to minimise radiation makes it an attractive modality to image any suspected coronary abnormality.
CMRA is typically acquired during free breathing using an electrocardiographic SSFP readout and T2-preparation pulses to generate contrast between blood and myocardium. It is also frequently combined with a fat-suppression technique to eliminate the signal from epicardial and mediastinal fat\textsuperscript{206}. Traditionally, a respiratory navigator positioned on the diaphragm has been used to suppress respiratory motion artefacts by only accepting data acquired in a predefined respiratory gating window\textsuperscript{224}.

In paediatrics, non-contrast enhanced CMRA coronary imaging remains challenging and the published experience is limited\textsuperscript{104,223,225}. In fact, despite ongoing advances in CMRA, numerous factors such as high heart rates, irregular breathing; small diameter of the coronary arteries; and poor contrast between the blood pool and extravascular structures can result in lengthy and suboptimal imaging acquisitions. These are also prone to respiratory and cardiac motion artefacts. Recently, respiratory image-based navigator (iNAV) techniques have demonstrated to improve image quality in patients with congenital heart disease\textsuperscript{226,227}. A further challenge related to paediatric CMRA compared to adults, is the relative lower spatial-resolution and SNR of the images which decreases diagnostic accuracy.

Contrast-agents are conventionally given to assess myocardial perfusion and viability, which increases the SNR of SSFP sequences and thus improves image quality of CMRA\textsuperscript{228,229}. Further improvement may be achieved by replacing the T2 preparation pulses with an inversion-recovery (IR) pulse. This introduces heavy T1- weighting and thus is beneficial with the administration of a T1- shortening contrast-agent\textsuperscript{218}. In addition, signal from pericardial fluid can be suppressed due to its long T1. In some subjects, high signal from fluid within the pericardial recesses with T2-prepared approaches can obscure the proximal coronary arteries.

Gd-BOPTA is a second-generation contrast agent, with higher relaxivity compared to non-specific Gd-chelates due to binding to blood albumin, and consequently slower total blood
clearance and longer plasma half-life, resulting in a higher and prolonged intravascular signal. The use of high-relaxivity contrast-agents has been shown to improve coronary imaging in adult patients, however the data in paediatric patients is still limited \textsuperscript{218,229,230}.

2.2.2. METHODS

This study was performed in children older than 2 years with congenital heart disease who were referred to our department for clinical indication of CMRI under general anaesthesia. Forty children were prospectively enrolled and all of them scanned at a 1.5 T (Achieva, Philips Healthcare, Best, The Netherlands).

The whole-heart CMRA scan consisted of an ECG-triggered 3D-SSFP sequence. The acquisition had a coronal orientation which was chosen to exclude the chest wall and minimize respiratory motion artefacts. Data acquisition was synchronized with the ECG to coincide with the longest quiescent cardiac phase. The optimal trigger delay time and acquisition window were determined from an axial high-temporal resolution four-chamber cine. Single-phase studies were acquired and the longest rest period of the heart coinciding with the late-systolic or diastolic-phase images was determined by evaluating the movement of the right coronary artery (RCA) \textsuperscript{231}.

The conventional pre-contrast coronary MRI sequence used a fat-suppression pre-pulse and T2-preparation pre-pulse to suppress signal from the myocardium and improve the blood-to-myocardium contrast (sequence A). Subsequently, contrast (Gd-BOPTA) was administered as a bolus by hand injection followed by a saline bolus \textsuperscript{229}. The post-contrast CMRA scan (sequence B) was performed approximately 5–8 min after the injection of Gd-BOPTA (0.2 mL per kilogram of body-weight) This allowed the circulating contrast material to stabilize in the blood-pool and thereby avoid significant changes in the inversion-time during the post-contrast scanning. For the post-contrast CMRA scan, an IR approach was used to null signal from the myocardium. The optimal inversion-time for nulling the myocardium was
determined using a Look-Locker sequence prior to the post-contrast CMRA.

For respiratory motion compensation, both sequence A and B used a recently developed image-based navigator (iNAV) that was used to directly track and correct the respiratory motion of the heart. In addition, respiratory gating with a constant efficiency of 50% was used to limit data acquisition to end-expiration.

Coronary reformatting and quantitative analysis of vessel length, diameter and wall sharpness was performed using a dedicated software ("Soap-Bubble", Philips Medical Systems, Best, The Netherlands). This custom-made validated tool facilitates multiplanar reformats of CMRA datasets, while also providing vessel length and diameter for objective quantitative comparison. Furthermore, the local vessel wall sharpness can be obtained by means of a Deriche algorithm which is the basis of a semi-automated vessel-tracking tool to identify the vessel borders along the path. Coronary image quality was determined by the same scoring system previously described by Mc Connell et al. (table 5). Analysis was performed by two independent experienced readers blinded to the study results or details of the sequences used to report the findings.

2.2.3. RESULTS

Both sequence A (pre-contrast) and sequence B (post-contrast) had similar acquisition durations. Furthermore, there was no significant difference in the HR during both sequence acquisitions. The mean vessel length was significantly longer with sequence B (p <0.001) for a similar average vessel diameter.

There was substantial intra and inter-observer agreement for the qualitative coronary scores given by the two independent readers.

CMRA after Gd-BOPA administration and acquired with sequence B resulted in significantly higher SNR and CNR compared to sequence A, (both p<0.001) (Figure 17).
Overall, higher coronary vessel wall sharpness (p=0.001) and qualitative scores (p<0.001) were achieved with sequence B. The number of non-diagnostic coronary segments was significantly lower for sequence B (p=0.002).

The same trigger delay was used for both sequence On bivariate analysis, there was no correlation between the resting trigger delay selected and the coronary vessel wall sharpness for both sequence.

Finally, there was no correlation between patients' variables such as age, BSA or HR and coronary vessel wall sharpness.

Figure 17: CMRA reformatted images of 3 selected patients. Superior panels sequence A (pre-contrast). Inferior panels sequence B (post contrast). Left hand side LAD in a patient with Tetralogy of Fallot. Centre LCX in a patient with double outlet left ventricle, Coarctation of the aorta and ventricular septal defect. Right hand side RCA in a patient with transposition of the great arteries.
2.2.4. DISCUSSION

For this study, children were imaged using two self-navigated SSFP sequences. In both, a novel self-navigation approach based on a recently developed 2D iNAV was used\(^{220}\). In contrast to a conventional diaphragmatic navigation approach, the new iNAV sequence allows direct estimation and correction of the respiratory induced bulk cardiac motion and diaphragm-heart hysteresis, which allows improved image quality and does not require any dedicated planning for the navigator setup, nor any additional post-processing steps\(^{220,232}\). The only difference between the two sequence, was that sequence B used an IR pre-pulse instead of a T2prep and was acquired shortly after administration of Gd-BOPTA.

Gd-BOPTA is a second-generation gadolinium contrast-agent, with a more lipophilic structure compared with conventional contrast agents which results in a weak and reversible interaction with serum albumin. This slows its extravasation out of the vascular space and increases its relaxivity compared to other agents, thus rendering a higher intravascular signal and improvement in diagnostic CMRA\(^{229,230,234}\). The T1 shortening effects and prolonged intravascular time of Gd-BOPTA, together with the changed magnetization preparation scheme resulted in the higher SNR. As a matter of fact, sequence B was specifically designed to benefit from the prolonged intravascular half-life of Gd-BOPTA and to increase the blood-to-background tissue contrast by means of an IR approach to null signal from the myocardium. That was also demonstrated by a significantly higher CNR. Because Gd-BOPTA resulted in a higher and stable intravascular signal, it also allowed isotropic high-spatial resolution imaging to be performed within a clinically feasible scan time of about 5 min, while also permitting dynamic vascular imaging with a single contrast injection. Adding the contrast to the novel sequence design resulted in a significant improvement in coronary visualisation independent of age, BSA and HR, all those known to have detrimental effects on image quality. This improvement was also noted in all coronary territories and it was independent of the vessel imaged or the resting cardiac phase chosen. Despite the fact that the mean vessel length obtained with sequence B was significantly
higher than that of sequence A, both had similar mean vessel diameters. Although counterintuitive given the normal angiographic tapering of the coronary arteries, the post-contrast images had higher vascular signal and a better delineation of the wall, as demonstrated by a higher vessel wall sharpness. Because the vessel border was less clearly visualized before contrast injection, we hypothesize that signal loss due to partial volume artefact and noise resulted in underestimation of the true lumen in sequence A despite having the same spatial resolution as sequence B 235.

This proposed sequence optimized for Gd-BOPTA may also enable tissue characterization with the same patient preparation as with extracellular gadolinium-based contrast agents and a single contrast bolus, with no need for dedicated or cumbersome mixed double contrast protocols (as perfusion scan or delayed enhancement followed by an intravascular agent for vascular imaging), a known limitation of “blood-pool” contrast agents 228. Importantly, no heavy venous enhancement was seen with sequence B, which has been described to complicate interpretation of coronary imaging with blood pool contrast agents 236.

2.2.5. CONCLUSION

The association of Gd-BOPTA with an IR 3D-SSFP sequence design was shown to result in improved coronary imaging visualization in children with high HR and within a clinically acceptable scan time. This non-invasive approach may eventually replace invasive cardiac catheterisation for diagnostic coronary imaging and preoperative planning in paediatrics.
3.1. ABSTRACT

While coronary allograft vasculopathy (CAV) remains one of the leading cause of death post transplantation it also remains challenging to diagnose, especially in the early phases and generally involves invasive techniques.

In this study we prospectively enrolled 13 patients, 9 of whom were males, aged between 11 and 17.5 years with a median age of 14 years. The study patients underwent cardiac magnetic resonance imaging (CMRI) with tissue characterisation and late gadolinium enhancement in addition to their usual biannual review workup.

Extracellular volume (ECV) was calculated from T1 mapping values pre and post gadolinium injection, at base, mid cavity and apex level. The patient’s haematocrit was taken at the time of the scan.

Late gadolinium enhancement imaging was performed to detect coronary disease as well as myocardium scarring and compared to IVUS and angiography findings.

Median age at Transplant was 6.8 years (0.5 to 12 years) and median time post-transplant at MRI was 7y (1 to 13.5 years). Mean ECV was 0.028±0.03;0.29±0.04 and 0.29±0.03 at base, mid cavity and apex level. All but one of those with coronary involvement either on IVUS or
LGE angiography had ECV>0.3, whereas conventional angiography displayed coronary
disease in only 2 patients. IVUS intimal thickness correlated with ECV at apex and mid-
ventricular level r=0.72(p=0.04) and r=0.8 (p=0.006). IVUS Stanford grade was also
significantly associated the degree of LGE in the MRI coronary angiogram(p=0.01).

Only 2 patients demonstrated myocardial scaring.

Conclusion: CMRI appears a non-invasive, radiation free, useful and valid tool for early
detection of CAV. ECV correlates well with IVUS findings. Further studies, especially in
paediatric patients, are needed to further explore the association of ECV with transplant
outcomes however these preliminary results are encouraging.

3.2. INTRODUCTION

In children, CAV remains the main limiting survival factor after heart transplantation and the
major cause of mortality after the first year post transplant leading ultimately to graft loss 1,2. One elegant aetiological description of CAV is that of “immunologic mechanisms operating in
a milieu of non-immunologic risk factors” 3. The process is believed to commence as a
response to endothelial injury in the graft, with a complex interaction of multiple donor and
recipient factors. The resulting endothelial dysfunction, leads to altered endothelial
permeability and subsequent intimal hyperplasia. As the intimal disease progresses in
severity, there is also an increase in fibrosis in the media and advent and an accumulation
of extracellular matrix 3.

CAV, alongside, rejection seems to represent the major cause of fibrotic remodelling of the
myocardium 237,238 and limits long term graft function and survival 239.

Microvascular disease is known to be present early after heart transplant, even in
asymptomatic patients and it is known to be associated with CAV, ischemia and death \textsuperscript{19,51}. Palmer \textit{et al.}, found proliferative arteriolar occlusion 10 months post-transplant in biopsy specimens with minimal angiographic changes \textsuperscript{240}.

Early diagnosis appears important as new medical therapies for CAV are introduced. A method that would detect and potentially monitor graft fibrosis could be of clinical use in the follow up of heart transplant recipients.

Recently clinical studies using T1 mapping examined disease where fibrosis had a known underlying pathological role. Iles \textit{et al.}, studied a symptomatic heterogeneous heart failure population that included transplant recipients. They used post-contrast T1 mapping to demonstrate an inverse correlation of T1 values with percentage fibrosis on myocardial biopsies of transplanted hearts, as well as a reduction in T1 time with worsening diastolic function \textsuperscript{169}. These results have been corroborated by several studies that also document inverse correlation between T1 time and biopsy findings and therefore offer histological validation \textsuperscript{170,171}.

The measurements derived from the pre- and post-contrast T1 measurements represent the partition coefficient for gadolinium contrast, which, in conjunction with the blood haematocrit, allows an estimate of the extracellular volume fraction (ECV). This quantitative measurement of ECV correlates with fibrosis in the extracellular matrix. ECV has also been shown to correlate with short-term mortality \textsuperscript{172} and histological collagen burden with low variability between scans \textsuperscript{173}. However very few data are available in the transplanted population despite the non-invasive nature of the technique and its robustness.

3.3 AIMS

The aims of this study were to use CMRI as a non-invasive radiation free technique in a paediatric population post heart transplant, firstly to compare fibrosis detected using T1
mapping and ECV measurements to IVUS findings; secondly to compare contrast enhanced coronary angiography to IVUS findings in assessing coronary epicardial disease and finally to detect typical or atypical myocardial scars using myocardial late gadolinium enhancement.

3.4. METHODS

3.4.1. Study protocol and population

Patients older than 10 years undergoing annual review after heart transplantation were invited to take part in the study. Written and informed consent was obtained from the patient, parent or legal representative. Patients were excluded if they had any contra-indication to MRI (e.g. contra-indicated metallic implant or device) or if they had any contra-indication to intravenous gadolinium-based contrast agent (e.g. end-stage renal failure). All patients required general anaesthesia (GA) for angiography and IVUS, but all CMR studies were performed without GA or sedation. This study was approved by the institutional review Board (Research Ethics Committee reference number 09/H0713/53 for the Paediatric cohort and 13/EE/0046 for the adult). Paediatric patients underwent their CMRI the day before their routine conventional X-Ray angiography and IVUS.

All the scans were performed at a 1.5 Tesla clinical MR scanner (Philips healthcare, Best, NL). Images were obtained using a 32-element cardiac phased–array receiver coil. During the scan, a 0.2mmol/kg body weight of a Gadolinium-based contrast medium (Gadovist®, Bayer Schering Berlin Germany) was injected intravenously.

Longitudinal survival and graft survival (i.e. if listed for re-transplantation) was recorded and a survival analysis performed.

3.4.2. Magnetic resonance images acquisitions

After an initial survey to obtain coil sensitivity and patient position references, a short axis, pseudo 2, 3 and 4 chambers for images planning and rest period to plan image acquisition
window are acquired using balanced steady state free precession (SSFP) two dimensional cine MR sequence.

Then a T1 mapping sequence at mid ventricular, apex and basal level, were acquired followed by intravenous contrast injection.

After contrast injection, a whole heart navigator-gated free breathing and cardiac –triggered T2 prepared 3D SSFP, a short axis stack cine images as well as 2,3 and 4 chambers balanced SSFP 2D cine images were performed \(^{179,180,241}\). Fifteen minutes post contrast injection, T1 mapping sequences were repeated in the same fashion to obtain post contrast values. Finally a Look Locker was run every 2 heart beats, with specific patient’s inversion time to null muscle signal, previous to myocardial scar imaging and then an every heart beat Look Locker to null blood signal before the vessel wall imaging \(^{242}\). For this purpose, a whole heart, free breathing, navigator gated, cardiac triggered fat suppressed T1 weighted, 3D gradient-echo inversion recovery sequence is used \(^{243}\).

3.4.3 Magnetic resonance image analysis

Images analysis was performed using ViewForum (Philips) for volumetrics analysis. OsiriX (version 3.9.1) with an in house plug-in, was used for measurements of T1 times. Regions of interests were drawn manually on the MOLLI images in short axis at mid ventricular, apex and base level within the interventricular septum and the blood pool. Regions of interest were confined to the central two thirds of the interventricular septum myocardium and were manually adjusted on each image of the MOLLI sequence to compensate for inter-image variability. Blood T1 times were measured in the centre of the ventricular cavity avoiding trabeculations or papillary muscles.

A blood sample for the haematocrit value was obtained when the patient was cannulated for IV access.
Figure 18: T1 mapping image in short axis pre contrast injection. Region of interest drawn within the interventricular septum and blood and finally T1 mapping image in the same patient post contrast injection.

ECV was calculated according to the equation published in 2012 by Wong et al.\textsuperscript{172}.

\[
ECV = (1 - \text{hematocrit}) \times \frac{(T1\text{\text{ post}} - T1\text{\text{ pre}})}{(T1\text{\text{ blood post}} - T1\text{\text{ blood pre}})}
\]

A cut off value of ECV >0.30 was used based on a recent publication of normal ECV values at 1.5T in healthy myocardium\textsuperscript{244}. Of note the same MRI scanner was used for the study and that publication.

Contrast enhancement data analysis were also carried out using Philips ViewForum. Two independent operators, blinded to IVUS and angiography results, conducted image analysis once the study was finished. LGE coronary MRA images were analysed using OsiriX (version 3.9.1.). LGE was defined as areas in the vessel wall with a signal intensity >2 SDs higher than normal non-enhancing vessel wall. The descending aorta was used as the reference for normal vessel wall. With the coronary MRA used as an overlay roadmap, coronary vessel wall enhancement in the left anterior descending coronary artery was qualitatively evaluated as none, mild, moderate or severe enhancement independently by two operators as for scar imaging. For contrast enhanced myocardium MRI data analysis, images were analysed and
classified as described by Steen ‘s group into typical or infarct-atypical forms of LGE involvement: (a) lesions at the right ventricle (RV) insertion, (b) intramural, (c) epicardial and (d) diffusely enhancing areas omitting the sub-endocardium.  

![Diagram of cardiac regions](image)

Figure 19: From Braggion Santos et al. Clin Res Cardiol (2014) 103:57–63.

3.4.4. IVUS images acquisition and coronary angiography

Conventional coronary angiography and IVUS were undertaken usually on the day after the CMR scan as part as clinical routine follow up. Catheterization is normally performed under general anaesthesia for children and adolescents, according to institutional policy, IVUS was performed in the left anterior descending coronary artery with automated pullback at 0.5 mm/s (temporal resolution of 30 frames per second). A suitable length of vessel was imaged to allow analysis of at least 30 cross-sectional images evenly spaced (at ≈1.5-mm intervals in mid-diastole) over the same segment of the left anterior descending coronary artery that was analysed at the previous annual review (identified by branch points). Mean percent area stenosis, Maximal intimal thickness (MIT) and Stanford grade were recorded.
Mean percent area stenosis is the ratio of the mean intimal area to the sum of the mean intimal and luminal areas. In addition, a semiautomatic interactive edge-detection software (QIVUS Clinical Edition, Medes Medical Imaging Systems) was used to improve reproducibility of measurements\textsuperscript{31,188,245}.

Coronary angiography images were analysed after catheterization by the operator and any abnormalities were confirmed subsequently by a second experienced independent observer.

3.5. STATISTICAL ANALYSIS

Statistical analysis was performed using STATA (version 13 StataCorp. 2013. TX). Data are presented with mean ± standard deviation or median with range. Continuous variables are presented as mean ± standard deviation after testing for normality. The correlation between continuous variables measured using IVUS and ECV was calculated using Pearson’s correlation coefficients. Comparisons between groups using continuous variables were performed using Student’s t-tests. A Wilcoxon rank sum test for the continuous variables (based on the variables distribution) was used to compare IVUS Stanford grade with qualitative late gadolinium enhancement in CMRI.

Additionally, a Kaplan-Meier survival curve was constructed using a temporal variable of survival time since MRI scan with censure points of death or listing for re-transplantation.

A level of significance of p-value <0.05 was used.

3.6 RESULTS
Table 6: Patients characteristics.

<table>
<thead>
<tr>
<th>Patients characteristics</th>
<th>Patients (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male /Female</td>
<td>9/4</td>
</tr>
<tr>
<td>Body Surface Area (m²)</td>
<td>1.6±0.8</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>89±14.8</td>
</tr>
<tr>
<td>Age at Transplant (years)</td>
<td>6.7±4.7</td>
</tr>
<tr>
<td>Age at MRI(years)</td>
<td>14.3±2</td>
</tr>
<tr>
<td>Time since Transplant (years)</td>
<td>7.5±5.3</td>
</tr>
<tr>
<td>Ischemic time (min)</td>
<td>192±46.5</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>20±13.9</td>
</tr>
<tr>
<td>CMV status: Positive/ Negative</td>
<td>5/8</td>
</tr>
<tr>
<td>VO2max in exercise test (ml/kg/min)</td>
<td>35.8±9</td>
</tr>
<tr>
<td>MRI LV EF(%)</td>
<td>70.2±6.6</td>
</tr>
<tr>
<td>Haematocrit(%)</td>
<td>0.36±0.05</td>
</tr>
<tr>
<td>Cholesterol(mmol/L)</td>
<td>2.82±6.6</td>
</tr>
<tr>
<td>Diagnosis :Cardiomyopathy/Congenital Heart Disease/Complete Heart Block</td>
<td>10/2/1</td>
</tr>
<tr>
<td>Patients with previous episodes of rejections</td>
<td>2(15%)</td>
</tr>
<tr>
<td>Antihypertensive therapy</td>
<td>8(62%)</td>
</tr>
<tr>
<td>Statins</td>
<td>13(100%)</td>
</tr>
<tr>
<td>Tacrolimus only</td>
<td>3(23%)</td>
</tr>
<tr>
<td>Tacrolimus + Mycophenolate mofetil</td>
<td>10(77%)</td>
</tr>
<tr>
<td>Steroids</td>
<td>2(15%)</td>
</tr>
</tbody>
</table>
Fifteen patients were recruited, however the first two patients were scanned at a 1.5T Achieva Philips scanner and therefore were not included in the study. Thirteen paediatric patients (69% males) median age 14 years (range 11 to 17 and a half years) completed the protocol at the 1.5T Ingenia Philips scanner and underwent their CMRI the day before their bi-annual review with IVUS and coronary angiography. All of them fulfilled the inclusion criteria, however one patient did not complete the full study as he become claustrophobic and the study was abandoned after T1 mapping, he did not undergo the late gadolinium enhancement imaging.

Median age at transplant of the cohort was 6.8 years (range 0.5 to almost 12 years). At the time of MRI, median time since transplant was 7 years (range 1 to 13.5 years) (Table6).

Mean ECV in our cohort were respectively 0.28 ± 0.03, 0.29 ± 0.04 and 0.29 ± 0.03 at base, mid ventricular and apex level. The majority of patients had an ECV within normal values; however, patients with coronary involvement, either in angiography, IVUS or in CMRA late gadolinium, had an ECV > 0.3 except one.

Three patients did not undergo an IVUS, in one case, the coronary ostium was too small for the IVUS catheter, and, in the other two, the disease was angiographically so advanced that the physician in charge of the procedure felt the IVUS was not necessary nor clinically appropriate.

The majority of patients in the study had Stanford grade 3 or more coronary disease: Six patients presented with grade 3 and four with grade 4 coronary disease. Only one patient had Stanford grade 1 disease and one had Stanford grade 2.

Despite not undergoing IVUS, those two with severe angiographic disease were assumed to have Stanford grade 4 coronary disease and were included in the analysis. In patients with...
more than mild disease: Stanford grade 3 or more, ECV was significantly higher than in those with grade 1 or 2. Even if the p value did not reach statistical significance for ECV calculations at base level (p=0.07), it did at mid ventricular and apex level with respective p values of 0.003 and p=0.01 showing that higher ECV is associated with higher coronary disease on IVUS as shown in Figure 20.

![Figure 20: Box plot showing correlation between ECV and IVUS.](image)

In our cohort, IVUS maximum intimal thickening for the whole group was 0.53 (SD 0.26), and maximum IVUS stenosis 27.75 (SD 7.19). Both appear to correlate with ECV values at base, mid ventricular and apex level, however, statistical significance is only reached for IVUS intimal thickness at mid ventricular and apex value with a coefficient of correlation and p values or r=0.72, p=0.04 and r=0.86, p=0.006 respectively (Fig 21).
Conventional coronary angiography displayed epicardial coronary disease in only two patients of the cohort, the physician in charge of the procedure decided on clinical grounds not to proceed with IVUS on those two patients. However, CMR angiography with late gadolinium showed severe enhancement in three patients, including those two identified with conventional angiography and moderate enhancement in another three patients. The rest of the cohort displayed none or mild enhancement on late gadolinium images. This shows once more the poor sensitivity of angiography and the late diagnosis that it entails. When coronary plaque via late gadolinium was compared to plaque measured in IVUS via intimal thickness it was statistically significant for both mean and maximal intimal thickness with p values equal to 0.02 and 0.03 respectively (Fig 22).
IVUS Stanford grade (SG) was significantly associated with the degree of MRI coronary enhancement ($p=0.01$). All the patients that presented with moderate or severe coronary enhancement with late gadolinium did also have SG 3 or 4.

It is striking, however, that in one patient, there is a huge disparity between T1 mapping, (particularly ECV results which are within normal ranges) and angiography results, as it showed severe disease. In this patient as well, there was enhancement in the coronaries in accordance with the angiographic findings. Of note, in short axis stack 2D images this patient showed subjectively wall motion abnormalities suggestive of RCA territory infarct.

Only 11 patients underwent myocardial late gadolinium enhancement, one, as mentioned before, abandoned the study due to claustrophobia after T1 mapping and in another patient due to the inability to breath-hold adequately.

Only two patients presented a myocardial scar, both atypical diffuse as per Steen classification (Figure 19 and 23$^{110}$, one of them being the patient with the most severe CAV
of the study who died 2 months after undergoing the study protocol. The other patient who displayed a scar within the myocardium, despite having a normal angiography, had SG grade 3 on IVUS and moderate late gadolinium enhancement on his CMRA. The numbers are unfortunately too small to reach any statistical significance but once again it reinforces the value of CMRI in the diagnosis of CAV.

![Angiographic disease and corresponding coronary LGE images with myocardial scar.](image)

The immunosuppressant regime was based on Unit policy and consisted for ten of the patients of double immunosuppression with tacrolimus and mycophenolate mophetil, two of those receiving additionally steroids, due to prior episodes of rejection however, in both of these patients, biopsy at time of the study showed no evidence of rejection. One of these two patients was the early death in the study. The other patient remains well. Her ECV was higher than normal but IVUS, angiography and late gadolinium enhancement were negative.

Three patients were on monotherapy with tacrolimus. All the patients received statins and 8
of them (62%) were also on an antihypertensive agent (Table 6).

Only 2 patients underwent biopsy at the time of the CMRI and none of those showed any evidence of rejection. Five patients (38%) were CMV positive.

There was no correlation between ECV and the use of antihypertensive therapy, CMV status, donor age, age at transplant or ischemic time.

Mean VO2max in the cohort was 35.8g/min, interestingly Vo2 max values correlate inversely with ECV at base level with a correlation coefficient of –0.75 and p=0.01.

Mean length of clinical follow up after MRI was 3.08 years (range 2m to 4 years).

Two patients have died and one has been listed for re-transplantation in the study period. All of these adverse outcomes were due to CAV disease. At the time of their MRI study, 2 patients had angiographic disease, one died shortly after her MRI and one was listed for re-transplantation 6 months after his study. The additional patient died after 30 months, while already transitioned to adult services, as shown in figure 24.

Figure 24: Survival Curve of the cohort.
3.7. DISCUSSION

CAV alongside rejection represents the major cause of fibrotic remodelling of the myocardium \(^{237,238}\) and limits long term graft function and survival \(^{239}\). CAV affects both epicardial and microvascular vessels \(^{246}\), leading to recent research now focusing on microcirculation \(^{246-249}\). It is now well known that microvascular dysfunction is independently associated with the onset of epicardial CAV and higher risk of death \(^{247}\). CMRI derived ECV is a validated measure of fibrosis in native hearts but has also been validated in transplanted hearts in the adult population \(^{166}\).

Recently the Toronto \(^{250}\) and Pittsburgh \(^{251}\) groups have published encouraging data validating ECV as a quantification of fibrosis in a paediatric transplant cohort.

In our study we found a good correlation between ECV and IVUS data. In all the patients presenting IVUS disease class 2 or more, ECV was > 0.30 (limit cut-off established by the literature \(^{244}\). Unfortunately, IVUS data are not available for all the patients.

In one of those patients, where IVUS was not performed, ECV was >0.30, but there was no evidence of angiographic disease. This patient had previously post transplantation lymphoproliferative disease (PTLD) and was diagnosed a few months after undergoing his MRI with Murphy stage IA late polymorphous Epstein Barr virus negative post-transplant lymphoproliferative disease, therefore the high ECV could be explained by some degree of inflammation and oedema related to the lymphoproliferation. It is well known that any inflammatory processes lead to increased ECV and T1 native values \(^{252}\).

Correlation would probably have been stronger if all patients would have undergone IVUS.

It is known that epicardial intimal thickening is associated with microvasculopathy and poor outcome \(^{43}\), as it is also well established that mild intimal thickening \(\geq 0.5\) mm predicts cardiac events in transplanted in patients even in the absence of angiographic coronary disease \(^{253}\).
One patient showed mild elevation of her ECV despite normal IVUS and angiography. Of note, she had a history of recurrent rejection and this could explain an increase in collagen, although the biopsy at the time of the annual review was negative. There was no fibrosis in the biopsy, which may represent sampling bias and the fact that specimens are only taken from the right ventricle free wall.

Nevertheless, one of the patients with severe angiographic disease, especially in the RCA territory, had an ECV within normal ranges. Due to the severity of the disease on angiography an IVUS was not performed. As opposed to the rest of the cohort, his T1 mapping results were completely discordant. The patient did not display alteration in any T1 times or in ECVs however LGE was seen in the coronary imaging in accordance to the angiographic findings. There were wall motion abnormalities in the short axis cine MRI but no scar was identified in the late myocardium enhancement.

Only 12 patients had LGE imaging as one abandoned the study due to claustrophobia as previously mentioned.

In previously published work Hussain et al., demonstrated that results of coronary MRA with late gadolinium enhancement were significantly superior at 3T compared to 1.5 T. As stated in their publication imaging quality improves with higher filed strength $^{174}$. In our study, motions artefacts and the decreased image quality at 1.5T would have made quantitative analysis complicated, therefore we chose to focus on qualitative analysis, which has been fairly robust. As a matter of fact, we have demonstrated significant association between the degree of MRI enhancement and the SG on IVUS ($p=0.01$) and with IVUS max intimal thickness ($p=0.02$ and $p=0.03$).

Coronary vessel wall enhancement was present in both of the patients with severe
angiographic disease and the findings were concordant in severity and location. MRI also demonstrated severe enhancement in another patient where the angiography was normal and moderate enhancement in another 3 patients. All those had SG grade 3 or 4 on the IVUS and all but one of these patients had high ECV.

Of the 11 patients that had myocardial LGE imaging, only one had angiographic evidence of CAV. This patient had severe myocardial fibrosis and epicardial coronary disease and in accordance to the rest of findings, myocardial LGE imaging did show a myocardial scar in left circumflex territory that correlates with the angiography (Figure 22).

Another patient presented with atypical diffuse myocardial scar, this patient did not have angiographic disease but had moderate LGE enhancement suggesting lack of sensitivity of angiography. This is different to other published data. In a series of post-transplanted patients published by Steen, they found a significant proportion of LGE CMRI lesions, up to 50% non-ischaemic and 37% ischaemic respectively, even in patients with mild evidence of epicardial disease on angiography 109. Subsequently Butler et al. published a series of 38 patients where 50% had evidence of LGE (79% of the lesions were non ischaemic and 21% ischaemic) 254. The Manchester group have also published a series of 48 adults patients and showed 49% atypical infarcts and additionally 9% of typical infarcts 111. Our prevalence is significantly lower, however this could be related to the small number of patients included in our study, or it could reflect our paediatric population. We are dealing with younger hearts (median age of donor in our study was 14 years) and fewer associated coronary risk factors in both donors and recipients. It is important to note, however, that the quality of the images was frequently suboptimal and the sequence required 11 breath holds which in some children might be challenging compared to the free breathing sequences.

Systolic function was preserved until CAV was at a late stage. Even in the patient who died two months after her CMRI and displayed the most severe disease, LV systolic function was preserved in MRI. Furthermore none of the functional MRI data seem to correlate with ECV
in concordance with the other 2 paediatric publications as well as in several adults ones
250,251,255.

3.8 LIMITATIONS

There are various limitations in our study. The most important is the sample size. There were
only thirteen paediatric patients and therefore the results need to be interpreted with caution.
This should be considered as a pilot study. The same protocol has been applied to an adult
population post heart transplant and will hopefully confirm our findings. However, while ECV
has shown promising correlation with IVUS data, not all patients had IVUS data available.

3.9 CONCLUSION

Our preliminary data demonstrate potential for radiation-free non-invasive assessment of
transplanted heart in paediatric patients. ECV correlates well with IVUS Stanford Grade, as
does late gadolinium enhancement of the coronary arteries. This suggests that CMRI would
allow earlier diagnosis compared to conventional angiography and that could be used as a
non-invasive radiation free tool in the paediatric population with a similar sensitivity to IVUS.
Further studies are needed to explore the association of ECV with outcomes post heart
transplantation.
CHAPTER 4 DETECTION OF WALL MOTION ABNORMALITIES USING CARDIAC MAGNETIC RESONANCE IMAGING IN CHILDREN POST HEART TRANSPLANTATION

4.1 ABSTRACT

Background: Coronary allograft vasculopathy (CAV) remains one of the main limiting factors for survival in children after heart transplantation.

Aim: In this chapter we explore the incremental value of routine cine Cardiac Magnetic Resonance (CMR) for evaluation for the detection of CAV using qualitative and quantitative analysis of regional and global myocardial function and strain.

Methods: This was a prospective imaging, biomarker validation trial. 22 patients (11 male), aged between 10 and 17 years (median 14 years) post heart transplantation were prospectively enrolled and underwent CMR in addition to their biennial review work up with echocardiography, angiography and intravascular ultrasound (IVUS). We enrolled 9 healthy control patients to undergo CMR alone. Echocardiography was used to analyse wall motion abnormalities and systolic function. CMR images were analysed qualitatively for regional wall motion abnormalities and quantitatively for volumetric analysis, strain (S) and strain rate (SR). All results were compared to IVUS and angiography assessments.

Results: Qualitatively, CMR detected regional wall motion abnormalities corresponding to angiographic disease in three patients that were not detected on echocardiography. However, quantitative strain analysis suggested regional wall motion abnormalities in an extra 9 patients. Detection of regional wall motion abnormality using quantitative strain analysis was associated with a higher mean stenosis grade (p=0.04) and reduced graft survival (p=0.04) compared to those with no quantitative wall motion abnormality. Overall,
only longitudinal strain was abnormal in patients compared with controls, but there was no correlation between any of the global indices of strain or strain rate and IVUS measurements.

Conclusion: CMR is more sensitive than echocardiography for the visual detection of significant wall motion abnormalities. Quantitative CMR strain analysis at rest may give additional information to discriminate those at greatest risk.

4.2 INTRODUCTION

Heart transplantation is a life-saving procedure that remains the treatment of choice for patients with end-stage heart disease and poor anticipated survival. In the recent era, one-year survival is approaching 90%. However, coronary allograft vasculopathy (CAV) remains the leading cause of death in children beyond the first year post transplant.

The typical lesion consists of a diffuse intimal and medial thickening that affects epicardial coronaries arteries as well as microvasculature. As a result, it is a concentric disease that often starts in the smaller vessels. Hence, invasive luminal angiography, which is the gold standard for clinical coronary imaging, is relatively insensitive for the diagnosis of CAV. Additionally, as a result of the denervation inherent to the transplantation, patients fail to display classical clinical warning signs of angina. Intravascular ultrasound is a much more sensitive method for the detection of CAV but it is invasive and cannot be used in smaller children.

In current clinical paediatric practice, the detection of CAV is achieved by regular invasive angiography in combination with haemodynamic measurements and left ventricular functional assessment by echocardiography. In our institution, the follow-up protocol consists of annual echocardiography, 24-hour Holter monitoring, 24-hour BP monitoring and
cardiopulmonary exercise test. In addition, they undergo biannual angiography. For those older than 10 years of age, intravascular ultrasound (IVUS) is performed alongside angiography. However, follow-up with catheter angiography places an extensive burden of invasive testing using an insensitive technique and many institutions are moving toward rationalizing the use of invasive testing. Echocardiography remains the most practical method for imaging follow-up in clinic. However, Cardiac Magnetic Resonance (CMR) cine analysis is a rapid and more reproducible method for the assessment of cardiac function. Furthermore, strain analysis has demonstrated the ability to quantitatively detect changes in myocardial function and contractility in different cardiac conditions such as hypertensive or hypertrophic cardiomyopathy as well as Duchenne muscular dystrophy even before changes in left ventricular ejection fraction (EF) are observed. In particular, quantitative strain analysis of conventional CMR cine imaging has been shown to be superior to traditional visual analysis for the detection of significant wall motion abnormalities.

The purpose of this study was therefore to assess the incremental value of routine cine CMR analyses and quantitative strain analysis to detect left ventricular wall motion abnormalities (WMA’s) at rest, in paediatric heart transplant recipients. In this context, WMA’s are likely to be secondary to established CAV. The hypothesis tested was that routine CMR analysis of cardiac function detects resting WMA’s and therefore gives additional insights into the detection of CAV. Demonstrating the utility of this imaging protocol may facilitate future trials aimed at rationalizing invasive testing.

4.3 METHODS

This was a prospectively recruited, imaging biomarker validation trial. The study was performed in one of the two nationally commissioned centres for heart and lung paediatric transplantation in the UK. At our institution, an average of 20 transplants a year are
performed, the majority of which are due to an underlying aetiology of dilated cardiomyopathy.

4.3.1. Study population

Patients that had received heart transplantation in childhood, were included if they were undergoing biennial follow-up with angiography and IVUS as per hospital protocol. All patients older than ten years (old enough to have an IVUS) were invited to participate in the study while attending their routine annual review follow up. Twenty-four patients were invited. Patients were excluded if they had any contra-indication to CMR (e.g. contra-indicated metallic implant or device) or if they had any contra-indication to intravenous gadolinium-based contrast agent (e.g. end-stage renal failure). Twenty-three were included and were prospectively enrolled and twenty-two completed the study (in one patient, a technical problem precluded the completion of the CMR). They underwent CMR in addition to their usual follow up with intravascular ultrasound (IVUS) and coronary angiography on the same day. The immunosuppressant regime was based on Unit policy and consisted of double immunosuppression with tacrolimus and mycophenolate mofetil.

Institution Review Board approval was obtained (09/H0713/53) and informed consent was taken from patients and/or their parents/guardians as appropriate. Longitudinal survival and graft survival (i.e. if listed for re-transplantation) were recorded. Whereas all children required general anaesthesia (GA) for angiography and IVUS, the CMR studies were performed without GA or sedation.

Nine healthy age-matched controls were also recruited. These controls were undergoing clinical CMR for exclusion of cardiovascular disease (e.g. exclusion of anomalous coronary origin, exclusion of right ventricular cardiomyopathy or exclusion of aortic root abnormality) and were found to have a normal cardiovascular system.
4.3.2. CMR

Patients underwent CMR at a 1.5 T clinical magnetic resonance scanner (either Achieva/Ingenia, Philips Healthcare, Best, Netherlands or Avanto, Siemens Medical Solutions, Erlangen, Germany, using a 32-element cardiac coil). CMR data were acquired in 4 chamber and short axis orientation using a standard balanced steady state free precession (SSFP) retrospectively VCG gated sequence for cine imaging.

Volumetric and functional ventricular assessment was performed using a multiple breath-hold, standard multi-slice 2D cine SSFP sequence. The temporal resolution of this sequence was between 20 and 30ms. The end-diastole and end-systole were defined as the mid-ventricular temporal frame, in which the image showed the largest and smallest left ventricle (LV) cavity area. Papillary muscles were excluded from the analysis of the ventricular volume. Visual detection of regional wall motion abnormality (RWMA) was subjectively assessed on cine images independently by two blinded observers, each with more than 5 years’ experience in CMR. Disagreement between observers occurred only in one patient and consensus was reached after re-discussion.

4.3.3. Echocardiography

Echocardiography studies were performed using either a GE Vivid 7 or a GE E9 echocardiography machine (General Electric Medical Systems, Wisconsin, USA). Measurements (EF, left-ventricular end-diastolic diameter and left-ventricular end-systolic diameter) were taken from the stored digital data by a single investigator with more than ten years’ experience. The observer was blinded to other investigations. They also commented on the presence of wall motion abnormality. All measurements were made using standard views and according to current guidelines. All images were reviewed by a second blinded observer and no disagreements were noted. Echocardiography was performed on the same day as the CMR.
4.3.4. Strain analysis

Strain analysis was performed using Feature Tracking (FT) Diogenes software (TomTec® Germany). The subendocardial contours were drawn manually at the end-diastolic frame. Automatic contour tracking was employed by the software to track myocardial displacement through the rest of the cardiac cycle. The contouring was then checked visually to ensure accurate tracking. If inaccuracy was noted, the end-diastolic frame was manually corrected and contour detection was repeated. Left ventricular deformation was assessed in terms of longitudinal, radial and circumferential components: the short axis was used to calculate radial and circumferential strain (S) and strain rate (SR) at 3 different level of the left ventricle (LV) (basal, mid-ventricular and apical), covering the 16-segment LV model. The 4 chamber cine was used to calculate longitudinal S and SR. The same operator performed all measures following a standard protocol. Normal values were obtained from nine healthy controls children with similar age range to those included in the study.

Wall motion abnormalities and their corresponding coronary artery territory were evaluated using “bull’s-eye” plots of the strain and strain rate values by two independent observers. Observers were blinded to all information, including the qualitative reading. In order to qualify as a RWMA, the colour-coded bull’s eye plot had to show relative reduction in strain in more than two contiguous AHA segments that could potentially reflect a major coronary distribution. In case of discrepancy between observers, which only occurred in one patient, the results were discussed and agreement reached.

4.3.5. IVUS and Coronary Angiography

Conventional coronary angiography and IVUS were undertaken, usually on the day after the CMR scan, as part of clinical routine follow up. Catheterization is normally performed under general anaesthesia for children and adolescents, according to institutional protocol but for older adolescents, having the procedure under local anaesthesia is encouraged. IVUS was
performed in the left anterior descending coronary artery with automated pullback at 0.5 mm/s (temporal resolution of 30 frames per second). A suitable length of vessel was imaged to allow analysis of at least 30 cross-sectional images evenly spaced (at ≈1.5-mm intervals in mid-diastole) over the same segment of the left anterior descending coronary artery that was analysed at the previous annual review (identified by branch points). Mean percent area stenosis, maximal intimal thickness (MIT) and Stanford grade were recorded

Mean percent area stenosis is the ratio of the mean intimal area to the sum of the mean intimal and luminal areas. In addition, a semiautomatic interactive edge-detection software (QIVUS Clinical Edition, Medis Medical Imaging Systems) was used to improve reproducibility of measurements

Coronary angiography images were analysed after catheterization by the operator; any abnormalities were subsequently confirmed by a second experienced independent observer. Both observers were blinded to the strain analyses.

4.4 Statistical Analysis

SPSS 16.0 (SPSS Inc. Chicago, IL) was used for data analysis. P<0.05 was considered to indicate a statistically significant difference. Continuous variables were reported as means and standard deviations. Existence of WMA was expressed as dichotomous data. Independent 2-tailed T-tests were used to compare continuous variables between groups and a Pearson correlation coefficients were used to compare IVUS values with global strain data.

Additionally, a Kaplan-Meier survival curve was constructed using a temporal variable of survival time since CMR scan and censure points of death or listing for re-transplantation. Survival curves were compared using a log-rank test.
ANOVA was used to compare the continuous strain values between the Stanford grading CAV severity groups. Intra-observer strain agreement in analysis was tested in the transplant cohort using Bland-Altman analysis and interclass correlation coefficient. Post-hoc analysis of statistical power of the trial to detect a change in circumferential strain was also performed.

4.5. RESULTS

Twenty-two patients (11 males) between 10 and 17 years old (median age 14 years) were enrolled in the study. Median age at transplant of these patients was 8 years (range 0.5 to 15 and a half years), (Table 7).

Table 7: Patient characteristics are expressed as mean ± standard deviation where appropriate.

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14±2.1</td>
</tr>
<tr>
<td>Male</td>
<td>11(50%)</td>
</tr>
<tr>
<td>Age at transplant (years)</td>
<td>8.2±5.1</td>
</tr>
<tr>
<td>Time since transplant (years)</td>
<td>5.7±5</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>26±15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>11(50%)</td>
</tr>
<tr>
<td>Other cardiomyopathies</td>
<td>5(23%)</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>4(18%)</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>2(9%)</td>
</tr>
</tbody>
</table>
### Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus only</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Tacrolimus and MMF</td>
<td>19 (86%)</td>
</tr>
<tr>
<td>Additional steroids</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Statins</td>
<td>22 (100%)</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>17 (77%)</td>
</tr>
</tbody>
</table>

At the point of CMR, median time post-transplant was 5.75 years (range 1 to 12.5 years).

Four patients did not undergo an IVUS as 2 of them were too small for IVUS and, in the other 2, the disease was angiographically so advanced that the attending physician felt the IVUS was not necessary. Mean length of clinical follow up after CMR was 2 years (range 0.4 to 4.5 years). No patient had previously documented angiographic evidence of CAV prior to enrolment in the study (All were CAV grade 0 according to ISHLT guidelines for nomenclature)\(^{15}\).

Regarding major adverse events, there were three deaths and one re-listed for transplantation during follow-up. All major adverse events were due to CAV (diagnosed in three cases on angiography and in one case, only on post-mortem).

Regarding angiographic disease, at the time of their CMR study, 3 patients had angiographic disease (two died and one re-transplanted during the follow-up period, as above). Regarding IVUS, one patient had Stanford grade 1 disease, one patient had Stanford grade 2, twelve patients had Stanford grade 3 and 6 patients had Stanford grade 4 coronary disease. In our cohort, IVUS maximum intimal thickening for the whole group was 0.63 (SD 0.27) and mean IVUS stenosis 20.13 (SD 5.07). Two of our patients, with severe CAV on angiography, did not undergo IVUS due to the clear evidence of advanced disease on angiography alone.
4.5.1. Qualitative analysis

Echocardiography did not detect qualitative wall motion abnormalities in any patients. Ejection fraction measured by echocardiography was also normal for all cases. However, CMR cine image analysis detected qualitative wall motion abnormalities in three patients. All 3 patients had angiographic disease. Furthermore, these 3 were the only patients with angiographic disease.

4.5.2. Quantitative strain analysis

Echocardiography showed normal EF and dimensions in all cases.

CMR similarly showed normal left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV). However, despite normal volumes, CMR revealed that three patients had borderline or mildly reduced EF because of a lower centile value for end-systolic dimension compared to end-diastolic dimension (Table 8). Two of these had angiographic disease. The other patient with reduced EF died suddenly 18 months after the angiogram and CMR, despite not showing evidence of angiographic disease. The post-mortem report in that patient revealed CAV as the cause of death. Quantitative analysis was performed using CMR. Intra-observer variability was tested for the transplant cohort. Bland Altman Analysis of Intra-observer differences in circumferential strain show excellent reproducibility. 95% Confidence intervals are between 3.3% and -1.8%
and the intra-class correlation coefficient was 0.88. There was no significant mean bias.

Figure 25: Bland Altman Analysis of Intra-observer differences in circumferential strain show excellent reproducibility. 95% Confidence intervals are between 3.3% and -1.8%. Intra-class correlation coefficient is 0.88.

Strain analysis confirmed visually detected RWMA and revealed subtle wall motion abnormalities corresponding to coronary territories in a further 9 patients that were not apparent visually (Table8).

Table 8: Summary of patients' findings in echocardiography, angiography and CMR.: Details of the coronary vessel affected in patients with angiographic disease as well as coronary territory corresponding to WMA detected on CMR quantitative analysis. In all patients, echocardiographic findings were normal and only 2 patients had impaired LV systolic function by CMR EF (1 additional patient had an EF in lower limit of normality).
<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Vessels</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No/75</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>No/51</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>No/78</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>No/82</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>No/67</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>No/70</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>No/57</td>
<td>3 VESSELS DISEASE</td>
<td>LAD</td>
</tr>
<tr>
<td>8</td>
<td>No/73</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>No/72</td>
<td>LAD + RCA</td>
<td>RCA</td>
</tr>
<tr>
<td>10</td>
<td>No/81</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>No/79</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>No/67</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>No/65</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>No/67</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>No/65</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>16</td>
<td>No/48</td>
<td>RCA + LAD</td>
<td>LAD + RCA</td>
</tr>
<tr>
<td>17</td>
<td>No/81</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>18</td>
<td>No/68</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>19</td>
<td>No/74</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>No/76</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>21</td>
<td>No/67</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>22</td>
<td>No/69</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Overall, those patients with quantitatively demonstrated wall motion abnormalities showed a higher mean stenosis (23%) compared to those with no quantitative wall motion abnormality (17%) p=0.04. Furthermore, those that demonstrated wall motion abnormalities on quantitative analysis, demonstrated reduced graft survival over the follow-up period (p=0.04)
by log-rank testing.

Figure 26: Kaplan-Meier survival curve showing difference of survival between transplanted patients showing WMA and those with normal LV function and contractility: patients with WMA demonstrated worse outcome, with 70% survival at one year and just over 40% survival at 4 years.

Number at risk n=12.

Longitudinal strain values were lower than in normal controls. However, other global strain and strain rate values were similar between controls and patients. Looking at Stanford grade, only the early diastolic strain rate falls as severity increases but this is not significant on ANOVA testing (p=0.17).
Table 9: Correlations between IVUS mean stenosis and S and SR values in 20 patients that underwent IVUS.

<table>
<thead>
<tr>
<th></th>
<th>Rad S</th>
<th>Circ S</th>
<th>Circ SR</th>
<th>Rad SR</th>
<th>EDSR</th>
<th>Long S</th>
<th>Long SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson correlation</td>
<td>-0.06</td>
<td>0.130</td>
<td>0.044</td>
<td>-0.030</td>
<td>-0.152</td>
<td>0.260</td>
<td>-0.031</td>
</tr>
<tr>
<td>mean stenosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig 2 -tailed</td>
<td>0.820</td>
<td>0.617</td>
<td>0.861</td>
<td>0.905</td>
<td>0.546</td>
<td>0.314</td>
<td>0.907</td>
</tr>
<tr>
<td>mean stenosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>-0.331</td>
<td>0.405</td>
<td>0.325</td>
<td>-0.184</td>
<td>-0.414</td>
<td>0.414</td>
<td>0.118</td>
</tr>
<tr>
<td>Maximal intimal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig 2 tailed</td>
<td>0.179</td>
<td>0.096</td>
<td>0.188</td>
<td>0.465</td>
<td>0.088</td>
<td>0.099</td>
<td>0.652</td>
</tr>
<tr>
<td>Maximal intimal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean for Healthy</td>
<td>25.56</td>
<td>-25.73</td>
<td>-1.83</td>
<td>1.55</td>
<td>2.23</td>
<td>-19.72</td>
<td>-1.06</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD for healthy</td>
<td>9.17</td>
<td>5.30</td>
<td>0.44</td>
<td>0.64</td>
<td>0.83</td>
<td>2.96</td>
<td>0.29</td>
</tr>
<tr>
<td>controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean for Patients</td>
<td>20.71</td>
<td>-24.73</td>
<td>-1.79</td>
<td>1.55</td>
<td>2.41</td>
<td>-15.28</td>
<td>-0.93</td>
</tr>
<tr>
<td>SD for patients</td>
<td>6.76</td>
<td>5.43</td>
<td>0.54</td>
<td>0.41</td>
<td>0.58</td>
<td>7.0</td>
<td>0.92</td>
</tr>
<tr>
<td>p-value for</td>
<td>0.177</td>
<td>0.643</td>
<td>0.839</td>
<td>0.343</td>
<td>0.574</td>
<td>0.021</td>
<td>0.590</td>
</tr>
<tr>
<td>comparison of control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to patients</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

4.6. DISCUSSION

The main finding was that compared to echocardiography, qualitative and quantitative RWMA by CMR detects more abnormalities. Those detected by CMR did indeed have a higher mean grade of stenosis. Furthermore, in this cohort, CMR RWMA was able to detect all the cases that had angiographic disease. It is fair to say however, that qualitative CMR WMA (visual assessment) was not as sensitive as quantitative CMR WMA.

Absolute systolic values of S and SR alone were not helpful to discriminate between patients or identify patients with disease. We also found a poor correlation with IVUS results suggesting that the absolute values may have a limited role in clinical practice. However, when examining deformation in accordance with the 16 segments model, using a comparative ‘bulls-eye’ plot rather than absolute values, we were also able to identify abnormal regions of systolic myocardial contraction corresponding to typical coronary artery territory patterns in 12 patients. These 12 patients showed a higher mean stenosis on IVUS and showed reduced graft survival on follow-up.

Strain imaging has previously been shown to improve the sensitivity and specificity of stress imaging techniques with the ability to quantify regions of wall motion abnormality. A reduction in peak systolic strain percentage will be seen in LV segments associated with inducible ischemia and accurate measurements of time to peak strain may also give information on regional wall motion abnormalities (RWMA). It should be noted that longitudinal and radial strain seem to entail more limitations regarding reproducibility compared to circumferential strain. FT strain analysis in our cohort potentially enables us to discriminate between patients and identify those in our cohort who display signs of coronary involvement. Discriminating between patients may allow us to select the subgroup that displays signs of disease and requires further diagnostic work up. On the
other hand, patients with no wall motion abnormalities may potentially continue with non-invasive follow up and could avoid going through IVUS and angiography. Aside from saving the patients from unnecessary invasive diagnostic techniques, this may allow a more efficient use of resources in the future.

S and SR values were largely abnormal within our population, when compared to previous normal values published in the literature but the magnitude of this difference is difficult to interpret in the absence of other data available in the transplanted population. S and SR values were largely abnormal within our population, when compared to previous normal values published in the literature but the magnitude of this difference is difficult to interpret in the absence of other data available in the transplanted population.

Several publications have correlated abnormal strain values with fibrosis and further investigation of this is worthwhile specifically in the heart transplant population. More studies are required to evaluate fully the usefulness of FT in the follow up of patients after heart transplantation but our experience shows that it is certainly a promising screening tool that could reduce the burden of invasive techniques, especially if associated with T1 mapping and ECV calculation, which unfortunately was not included in the research protocol of this study.

An increasing number of transplant units are using less invasive techniques, especially in the adult population where there has been an important increase in the use of computed tomography in the detection of CAV. A recent meta-analysis showed very good results in this respect. The latter concludes that it is a reliable non-invasive option. Coronary computed tomography angiography is beginning to be used in other paediatric centres.

4.7. LIMITATIONS

One major limitation of the study was not performing echocardiographic strain analysis in order to compare strain and strain rate in both modalities: CMR and echocardiography. This could have been a helpful resource that could eventually been added to a proposed
diagnostic protocol. Echocardiography and CMRI were done on the same day but unfortunately on two different sites due to the co-location of the research project. Echocardiography was performed as part of the routine clinical protocol by a physiologist. None of them were involved in the research project and unfortunately due to the workload of the transplant clinic, time did not allow for the acquisition of additional images that would have guaranteed the possibility of post processing.

Our cohort appears to present a high degree of CAV using Stanford Grading, given that 16 patients had SG 3 or 4. This seems higher than in most studies, but if we look at angiography disease, CAV was only found in three patients. Many institutions probably will report a lower rate of paediatric CAV as they do not routinely perform IVUS. Even in centres that do perform IVUS, protocols vary and the quantity of image sampling may vary. Additionally, it could be noted that donor age, (with only 7 paediatric donors) is higher than many other reported cohorts. Perhaps, a slightly higher rate of CAV has been helpful, specifically with respect to study purposes, in this cohort to allow for longitudinal follow-up of outcomes.

In addition, the control group consisted on patients with normal findings at the time of MRI who were screened for arrhythmogenic right ventricular cardiomyopathy or vasculopathy and some of these patients may develop abnormalities in the future. However, obtaining perfectly normal populations is very difficult in paediatric research, even with non-invasive MRI studies.

The study findings are limited by the lack of fibrosis detection as well as the lack of other CMR signs of CAV such as myocardial or coronary late enhancement detection. However, the protocol described is advantageous for children in terms of tolerability and simplicity with off-line strain analysis for a more detailed assessment. It is also limited by the sample size. For example, although our data suggests 100% sensitivity for the detection of angiographic disease using CMR, the sample size makes this value unreliable (95% confidence interval
for this is wide from 31% to 100%). Furthermore, post hoc testing of statistical power showed that the study had 80% power to detect a change in circumferential strain of 6%. It is possible, therefore, that the study was underpowered to show subtler strain changes between controls and transplant patients. However, global strain and strain rate values also showed no correlation with IVUS measurements (Table 8). Hence, we recognize that additional studies are needed to confirm these findings and establish CMR for the routine follow-up of children.

4.8. CONCLUSION

Conventional CMR cine analysis of cardiac function is a simple and effective means for follow-up of patients after heart transplantation. It offers incremental diagnostic value over echocardiography alone. Retrospective quantitative analysis of strain from these routine cine images may increase the sensitivity for the detection of clinically significant wall motion abnormalities and this may have prognostic implications.
CHAPTER 5. OVERALL DISCUSSION

5.1 INTRODUCTION

This thesis has documented some novel and exciting findings. We have shown that ECV correlates well with changes on IVUS thus linking fibrosis to coronary disease. Late gadolinium enhancement angiography was not only associated with disease on IVUS but was also localised to the same anatomical areas and like IVUS it could detect abnormalities when angiography was falsely reassuring. CMRI strain analysis for wall motion abnormalities detection also successfully identified coronary disease on angiography and corresponded to typical coronary artery territory patterns. Children with abnormal CMRI strain had decreased graft survival, despite normal angiography.

We feel confident that this work has demonstrated the importance of CMR in the management of children after heart transplantation. The transplant community have been slow to take up CMR but this work and the associated papers may help CMR to become more main-stream in heart transplant management. Of course, for children the replacing of an invasive test is always welcome but there are special reasons why we feel CMR should be adopted in paediatric heart transplantation.

The psychological burden of post paediatric heart transplant is substantial and reducing invasive testing has potential to improve the quality of life for the children. Many children have post-traumatic stress as do their parents and an annual general anaesthetic for coronary angiography is often a huge obstacle. In fact, many children need intensive psychological help just to arrive in the anaesthetic room.
Transplanted children also have an increased risk of renal failure because of the use of calcineurin inhibitors. The contrast agent used for conventional coronary angiography is nephrotoxic and each administration is deleterious for the kidneys. In addition, in these post-transplant children, in whom immunosuppression already increases their risk of malignancy, each coronary angiogram adds to their life time risk of cancer. Coronary angiography also entails a significant risk of complications, leading frequently to haematoma, clots and potential bleeding if heparin is given.

The current gold standard, IVUS, is not a technique without risk either and dissection of the coronary from the guidewire has been described. Moreover, each invasive diagnostic test induces a substantial financial cost to the NHS with equipment for IVUS, staffing and day bed occupancy. The lack of need for general anaesthesia also means quicker procedures and the possibility of increasing the number of patients seen in each list, optimising the resources and a greater cost-benefit ratio.

Therefore, if CMR was adopted instead of invasive techniques we believe it would lead to: a reduction in the risk of cancer, a reduction in the risk of renal failure, and the prevention of physical damage to the femoral or radial arteries as well as the coronary arteries themselves. It will also greatly improve the quality of life in a psychologically vulnerable population and potentially make significant financial savings to the NHS.

This thesis studied a paediatric population but the findings could translate into adult practise and we have collaborated with Papworth to facilitate this.
5.2. DISCUSSION OF KEY FINDINGS

Chapter 2, demonstrated that the combination of particular CMR sequences with the appropriate contrast agent led to better coronary arteries characterisation and visualisation. In particular, the combination of gadobenate dimeglumine with an inversion recovery pre-pulse SSFP sequence and a new imaged based navigator resulted in significantly improved image quality as well as reduced scan time. This proved to be particularly useful in visualising coronary arteries on paediatric patients, even the youngest children with high heart rates. This was a key finding for this thesis as it demonstrated a technique that could be easily applied to the paediatric heart transplant population. For the reasons discussed above (5.1) we firmly believe that non-invasive assessment of coronary arteries is desirable in children post heart transplant.

In chapter 3, we established a correlation between IVUS data and ECV. We believe this is an important finding in transplant cardiology as it establishes a link between CAV and fibrosis. This would support the paradigm that the severity of large vessel disease (IVUS) is linked to small vessel coronary disease and thus fibrosis. We recognise there are differences of opinion regarding the link between epicardial and microvascular disease. Several groups advocate a correlation between them\(^{43,51,60,271}\), whereas for other authors their course is independent\(^{55,111,249}\). However, based on our experience, in the absence of an inflammatory process myocardial fibrosis is likely to be caused by microvasculopathy. ECV seems to be a reliable biomarker of fibrosis and we have shown a link to epicardial disease on IVUS. This is important in transplant cardiology, as late graft failure typically presents with restrictive cardiomyopathy physiology with a scarred and stiff ventricle and biatrial enlargement. Detecting early fibrosis and preventing its progression appears to be fundamental to improving outcomes post-transplant. New drugs such as everolimus appear to limit fibrosis and CMR could be key to assessing the benefit of this and other new treatments.
Additionally, qualitative analysis of late gadolinium enhancement angiography appeared to be robust and showed a significant association between the degree of enhancement found in CMRA and IVUS maximum intimal thickness, alongside the previously published work from our group, by Hussain *et al* it underscores the potential of CMRA as a new paradigm potentially replacing IVUS. Furthermore, the CMRA not only demonstrated vessel wall enhancement in those patients who presented with angiographic disease but it was also concordant in severity and localisation. Moreover, CMRA was able to identify patients with coronary artery involvement that had a normal angiography but where IVUS demonstrated Stanford grade greater than three. All except one of those patients also had high ECV, reinforcing the diagnostic value of ECV as a detection tool.

One disappointing aspect of the research was that our results for myocardial enhancement have not been robust and we are uncertain of their additive value to the protocol. Our series, presented a much lower incidence than previously published data. The latter could identify atypical or typical scar in the majority of patients. These groups reported the following percentages for the incidence of infarct typical scar: 37% for Steen *et al.*, 11% for Butler *et al.* and 9% for Miller *et al*; as well as infarct atypical scar in 51% for Steen *et al.*, 40% for Butler *et al.* and 49% for Miller *et al*.

As discussed in chapter 3, those studies included adults and the discrepancy could be due to the younger donor age of our cohort with a presumably healthier heart. Additionally, this sequence requires 11 breath-holds and can be challenging for younger children, therefore, we decided to not included scar imaging in the following proposed protocol.

Finally, chapter 4 showed how using CMRI strain analysis for wall motion abnormalities detection we were able to identify all the patients with coronary disease on angiography. Furthermore, on assessing deformation in accordance with the 16 segments model we could identify patients with abnormal regions of systolic myocardial contraction, which corresponded to typical coronary artery territory patterns. These patients had higher mean
stenosis on IVUS and decreased graft survival on follow up, despite normal angiography. We recognise that the addition of strain analysis to the routine echocardiography protocol would have been beneficial. Unfortunately, that was not undertaken in the study performed for this thesis, but we recognise it should be considered in future work and has been added to our proposed protocol for further assessment of paediatric transplant patients.

These interesting results allowed us to formulate a hypothesis that answers the research question raised at the beginning of this thesis. We can indeed, using a multi-parametric CMRI approach, detect accurately, safely and non-invasively those post-transplant children who have signs of CAV. Thus identifying those that would need further diagnostic work up and differentiating them from those who could safely continue with non-invasive follow up.

Obviously, these results need to be validated in additional studies which include more patients. Due to the nature of the population and its general scarcity the ideal way to validate our findings would be through a multicentre study that would allow a sufficient number of patients to be able to draw a conclusion.

5.3. PROPOSAL ON HOW THESE KEY FINDINGS CAN INFLUENCE CLINICAL MANAGEMENT OF CHILDREN POST HEART TRANSPLANTATION

Based on that hypothesis our work supports a new paradigm in paediatric transplantation; only patients determined to be at risk of CAV from non-invasive testing should undergo invasive testing.

In keeping with widely published data, this thesis has demonstrated the poor sensitivity of angiography in detecting CAV. We therefore propose a new diagnosis protocol for CAV detection and management.
At the moment patients undergo IVUS and angiography every two years; in addition, on a yearly basis, concomitantly with their clinical review they have 24-hour blood pressure and ECG monitoring, an exercise test (for those old enough), blood testing and a routine echocardiography.

We believe that the following algorithm would offer a better alternative to the current one:

- Clinical review and blood test
- 24h holter and 24h BP + CPET
- Echocardiography with TDI and strain analysis
- MRI with ECV, LGE angiography and vessel wall abnormalities detection using CMR strain analysis

If normal:
- Continue with yearly non-invasive F/U

If abnormal:
- IVUS and MDCT
- Consider change in immunosuppression regime

Figure 27: New diagnosis algorithm proposal for CAV.

As mentioned earlier in the thesis MDCT is increasingly used in the transplanted population and it was demonstrated in a relatively recent meta-analysis\textsuperscript{268} that it is a reliable non-invasive alternative to coronary angiography with an excellent sensitivity, specificity and negative predictive value for CAV detection.
5.4. FUTURE DIRECTIONS

5.4.1. PROTOCOL OPTIMIZATION USING IMPROVED NAVIGATOR AND NEW CONTRAST AGENT

The above protocol (figure 27) seems to offer a viable alternative to current invasive assessment and we believe that it can be further optimised by integrating some of the new advances in cardiac magnetic resonance imaging.

The whole heart image acquisition does require patient cooperation as it relies on adequate diaphragm tracking and therefore can be lengthy and time consuming, in addition the quality of the images can also be suboptimal. The application of a cardiac tracking navigator, as published by Henningsson et al \textsuperscript{220,221,226,272} as previously described in this thesis, would lead to a much shorter scanning time. If the latter technique were combined with a new intravascular agent, such as Vasovist \textsuperscript{®} (gadofosveset trisodium) or even better MultiHence\textsuperscript{®} (gadobenate dimeglumine) it should result in higher quality and improved accuracy of coronary imaging. A new study could be designed to investigate these new advances in imaging.

5.4.2. ADENOSINE STRESS PERFUSION MRI

Adenosine stress perfusion CMRI is increasingly used for assessing reversible myocardial perfusion and in the adult population it is emerging as a gold standard \textsuperscript{273}. In children, however it remains slightly more challenging, even if it has been proven feasible and safe \textsuperscript{274,275}. Two recent studies have suggested that stress perfusion CMR is an excellent screening imaging biomarker for paediatric patients with suspected coronary artery disease, with high sensitivity and specificity and a very high NPV of 100% and 88% respectively \textsuperscript{276,277}. Vijarnsorn et al, combined stress perfusion CMR with LGE and wall motion analysis and they were able to predict MACE at 1 year with a PPV of 78% and a NPV of 98%. When
late gadolinium enhancement was associated with a perfusion defect the PPV of adverse cardiovascular events at 1 year increased to 99% demonstrating the robustness of the technique. Myocardial perfusion reserve has been evaluated quantitatively in children. A cohort with Kawasaki Disease were studied by Bratis et al, and they were able to demonstrate functional microvascular abnormalities independently of epicardial disease.  

5.4.3. IMAGING WITHOUT CONTRAST AGENT

In the heart transplant population, chronic renal dysfunction is a very common complication there is an increasing incidence with time after transplantation. This is related to numerous risks factors well described in the literature but amongst them immunosuppressive therapy and particularly the use of calcineurin inhibitors stand out. Chronic renal impairment is associated with increased mortality and morbidity and a relative risk for death of 4.55. Therefore, it is essential in these patients to minimize any possible added kidney toxicity and to preserve kidney function. Contrast agents are known to be nephrotoxic but the heart transplant population need regular coronary imaging. Contrast free imaging is clearly highly desirable in this vulnerable group.

5.4.3.1. Interleaved T2 preparation acquisition

A pilot study published within Kings College has demonstrated the feasibility of an interleaved T2-Preparation acquisition sequence to image the coronary vessel wall without contrast agent. They validated the sequence against the black blood double inversion recovery sequence and used a flow independent free breathing black blood whole heart vessel wall sequence with a interleaved T2prep. Subtraction of the T2prep ON from the T2prep OFF dataset provides a black blood image with high signal intensity from the coronary vessel wall. The whole heart balanced three dimensional, steady-state-gradient-
free-precession (SSFP) echo sequence was acquired in the coronal plane.

A major disadvantage of this technique was the long acquisition time due to the reliability on the diaphragm tracking which recently has been optimised by the addition of a cardiac tracking navigator to the sequence\textsuperscript{281}. This latter publication allows imaging of coronary lumen and coronary vessel wall and reduced scanning time by 1.6 times in comparison to a gated scan. This appears to be an important future direction with huge potential both in the transplant population and in wider cardiovascular research.

5.4.3.2. Dixon water-fat separation sequence

Nezafat \textit{et al}\textsuperscript{282}, applied the Dixon water-fat separation sequence to a cohort of volunteers and compared coronary magnetic resonance angiography, using SPIR (as the currently preferable technique for fat suppression) with Dixon without contrast agent at 3T corroborating results previously shown at 1.5T\textsuperscript{283}. Dixon takes the advantage of the phase shifts due to water fat resonance frequency differences in order to separate water from fat instead of only exciting water and suppressing the fat. Due to the small size of the sample, there was no statically significant difference, however a marked tendency was seen towards higher blood and myocardium SNR and blood/fat CNR. Image quality was better (higher visual score) with Dixon. Finally, there was definitely longer vessel length visualised with the Dixon method. In addition, Dixon also allows steady state cine images acquisition including 4D coronary\textsuperscript{284,285}.

Once again, more studies are needed to confirm this encouraging work; however, the possibility of imaging coronaries without contrast agents make these options very attractive for a cohort of post heart transplant patients.
5.5. CONCLUSION

This thesis has demonstrated the CMR provides a reliable and safe non-invasive, radiation-free tool that accurately identifies those at risk of CAV.

The multiparametric nature of CMR implies that the same protocol using different sequences allows multiple data to be acquired within the same scan. The same diagnostic study provides details of function, possible wall motions abnormalities, perfusion deficits, late gadolinium enhancement (for both the myocardium and coronary arteries), tissue characterization and morphology without any radiation and non-invasively. Potential coronary lesions can be identified and their consequences evaluated. Further studies are required to establish this protocol in common clinical practice. We are hopeful that CMR will become the new paradigm in paediatric heart transplant surveillance because, aside from saving patients from potentially unnecessary invasive diagnostic techniques, it also allows for a more efficient use of resources in an era of increasing financial strain for the NHS. We feel the suggested changes to assessment that have been derived from this thesis offer an important contribution to enhanced care for this group of children,
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