

**Myocardial fibrosis in repaired tetralogy of Fallot;
Predicting ventricular arrhythmia and sudden cardiac death**

Thesis submitted for the degree of *Doctor of Philosophy*
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*For my parents &
To Mohamed and Sophia*

Statement of Originality

I certify that this thesis, and the research to which it refers, represents my own work except where referenced or acknowledged otherwise in accordance with the standard referencing practices of the discipline.

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Abstract

We are faced with new challenges in the growing population of adult survivors with repaired tetralogy of Fallot (rTOF). The risk of premature death persists and drives eager pursuit for the accurate identification of patients at high-risk of malignant ventricular tachycardia (VT) and sudden cardiac death (SCD).

It is previously known that inducible VT predicts mortality in rTOF patients. We show that the burden of right ventricular (RV) late gadolinium enhancement (LGE) defined fibrosis $> 25\text{cm}^3$ quantified by high-sensitivity 3D LGE can predict inducible VT as a proxy endpoint for mortality. Patients with minimal RV LGE $< 10\text{cm}^3$ were extremely unlikely to have inducible VT suggesting those with minimal RVLGE avoid an invasive study.

In a prospective study of 550 rTOF patients, a high-risk subgroup of patients with a 4.4% annualised risk of death and 3.7% annualised risk of life-threatening VT/SCD were identified. RVLGE was a strong predictor of outcome. We demonstrated how RVLGE can be integrated with other independent predictors into weighted risk scores ready for clinical use.

Diffuse fibrosis defined by RV T1 shows promise as a subtle biomarker of adverse remodelling. An imbalance in the expression of fibrosis biomarkers suggests that a state of high-collagen turnover exists and correlates with adverse remodelling.

In conclusion, myocardial fibrosis plays a central role in predicting death and malignant VT in rTOF. This work identifies biomarkers to help risk stratify and enable more personalised and targeted care in the life-long follow up of adult rTOF patients.

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Good geographical correlation is seen between 3D LGE scarred regions (A) and low-voltage scar seen on bipolar voltage maps taken using CARTO 3 version 7 (**B**) in the RVOT (yellow dotted arrow) and RV septum (yellow arrow). Scar on the bipolar voltage maps is demonstrated by red/yellow/green colours (0.5mv- 1.5mv) and healthy myocardium is represented as purple. Similarly a good match with LGE-defined scar was seen with activation mapping where brown areas showed slow or no conduction (SNO zone) in the RVOT free wall and septal surface (**C**) A re-entry mechanism was induced through an

‘anatomical isthmus’ that is between the pulmonary valve annulus and VSD patch with clockwise activation. Critical activation across the isthmus (red circle) induced a well-tolerated monomorphic VT (TCL 280ms). **D)** VT ablation was carried out. Subsequent mapping showed complete isthmus line block (red circle) causing a change in the direction of activation to travel inferiorly around the tricuspid annulus (TA) and on to the anterior RV with late activation (red) occurring adjacent to the ablation line as a result. Images adapted from Ghonim et al 2020.

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previously published. The RV is divided in to 6 segments (yellow numbers 1-6).Regions of RV LGE were scored according to linear extent (0= no enhancement 1 = up to 2 cm, 2=up to 3 cm, 3= 3 or more cm in length) and number of trabeculations enhanced including the moderator band (0= no enhancement, 1 = 1 trabeculation, 2=2-4. Scoring of LVLGE was performed using the universally accepted 17-segment LV model. Points were attributed to the proportion of LGE present in each myocardial segment, as visually judged: 0= No LGE, 1= up to 25% 2=up to 50%, 3= up to 75% and 4= up to and including 100% of the myocardium enhanced

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Table 3.2: Univariable predictors of inducible VT.

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Table 4.1 Patient characteristics and associations with all-cause mortality and ventricular arrhythmia.

*Repair for tetralogy of Fallot with pulmonary atresia and no systemic-pulmonary collaterals in 44 (8%), double outlet RV variant in 13 (3%), with absent pulmonary valve in 10 (2%). [†]Redo surgical pulmonary valve implantation occurred in 113 at baseline and 118 during follow up. Percutaneous pulmonary valve implantation occurred in 32 during follow up). [‡]12-lead ECG was available in 500 (91%). [§]B-type natriuretic peptide (BNP) was available in 384 (70%). Echocardiography data on restrictive RV physiology was available in 447 (81%), ^{||} Restrictive RV physiology with large RV [top decile RVEDVi ($\geq 150\text{ml/m}^2$) in 16 (14%)] restrictive RV physiology and small RV [lowest quartile RVEDVi ($\leq 115\text{ml/m}^2$) in 55 (47%)]. Right ventricular systolic pressure (RVSP) was available in 446 (81%), tricuspid annular planar excursion (TAPSE) in 473 (86%), LV E/A ratio in 509 (93%), and LV E/E' in 477 (87%).

[#]Peak oxygen uptake (PVO_2) was available in 423 (77%). [^]Holter monitoring was available in 142(26%). Non-sustained VT was recorded in 66 patients during follow up (median 12 beats: [8-18], median total cycle length 350ms: [300-3884ms]). EDVi; end-diastolic volume indexed to body surface, EF; ejection fraction, ESVi; end-systolic volume indexed to body surface area, LGE; late gadolinium enhancement, NYHA; New York heart Association classification, PES; programmed electrophysiological study, RAAi; right atrial area indexed to body surface area, RV;right ventricle, LV;left ventricle, RVOT; right ventricular outflow tract.

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Collagen volume fraction (%) MMP-2: TIMP-1 ratio; ratio of matrix metalloproteinase 1 expression to tissue inhibitor of matrix metalloproteinase 2

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Collagen volume fraction (%) MMP-2: TIMP-1 ratio; ratio of matrix metalloproteinase 2 expression to tissue inhibitor of matrix metalloproteinase 1

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List of Abbreviations

AICD	Automated implantable cardiac defibrillator
ACHD	Adult congenital heart disease
ANP	Atrial natriuretic peptide
AUC	Area under the curve
BNP	B-type natriuretic peptide
BMI	Body mass index
BSA	Body surface area
bSSFP	Balanced steady state free precession
CHD	Congenital heart disease
CI	Confidence interval
CMR	Cardiovascular magnetic resonance
CPEX	Cardiopulmonary exercise
CVF	Collagen volume fraction
ECM	Extra-cellular matrix
ECV	Extra-cellular volume
EF	Ejection fraction
FoV	Field of View
FLASH	Fast low angle single shot
GBCA	Gadolinium based contrast agent
LAAi	Left atrial area indexed to body surface area
HR	Hazard ratio
HCT	Haematocrit

IQR	Interquartile range
LAAi	Left atrium area indexed to body surface area
LGE	Late gadolinium enhancement
LV	Left ventricle
LVEDP	Left ventricular end-diastolic pressure
LVEDVi	Left ventricular end-diastolic volume indexed to body surface area
LVESVi	Left ventricular end-systolic volume indexed to body surface area
LVOT	Left ventricular outflow tract
MOLLI	Modified Look-Locker inversion recovery
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
NSVT	Non-sustained VT
NYHA	New York Heart Association
PA	Pulmonary artery
Peak VO₂	Maximum oxygen uptake
PES	Programmed electrical stimulation
PICP	Carboxy-terminal pro-peptide of type I procollagen
PIINP	Amino-terminal pro-peptide of type III procollagen
PR	Pulmonary regurgitation
PS	Pulmonary stenosis
PSIR	Phase sensitive inversion recovery
PV	Pulmonary valve
PVR	Pulmonary valve replacement
RAAi	Right atrial area indexed to body surface area

RER	Respiratory exchange ratio
ROC	Receiver operator curve
ROI	Region of interest
RV	Right ventricle
RVEDVi	Right ventricular end-diastolic volume indexed to body surface area
RVESVi	Right ventricular end-systolic volume indexed to body surface area
RVOT	Right ventricular outflow tract
rTOF	Repaired tetralogy of Fallot
RVSP	Right ventricular systolic pressure
SCD	Sudden cardiac death
TAPSE	Tricuspid annulus plane systolic excursion
TE	Time to echo
TOF	Tetralogy of Fallot
Ti	Inversion time
TIMP	Tissue inhibitor metalloproteinase
TR	Repetition time
VENC	Velocity encoding range
VE/VCO2	Minute ventilation/carbon dioxide production slope
VLA	Ventricular long axis
VSD	Ventricular septal defect
VT	Ventricular tachycardia
2D	Two-dimensional
3D	Three-dimensional

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Preamble

This thesis aims to address a current unmet need in the contemporary management of adult patients with repaired tetralogy of Fallot (rTOF), namely the improvement of risk stratification for life-threatening ventricular tachycardia (VT) and sudden cardiac death (SCD). The influence of myocardial fibrosis on clinical outcomes is explored using late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR), T1 mapping and serum biomarkers of fibrosis.

Chapter 1 reviews the available background literature and the current challenges facing risk stratification in patients with rTOF. Previous risk models and known individual risk factors are explored including the role of focal and diffuse myocardial fibrosis. A basic review of CMR physics, LGE CMR, T1 mapping and serum biomarkers of fibrosis is included. The aims and hypothesis are summarised.

Chapter 2 details the methods and materials used in this thesis.

In **Chapter 3**, the results from a prospective study to determine whether right ventricular (RV) focal myocardial fibrosis quantified by three-dimensional (3D) LGE CMR can predict inducible VT are presented with clinical relevance explained.

In **Chapter 4** the results of a prospective study to determine the prognostic value of LGE CMR in rTOF are presented including presentation of risk scores devised. The relevance of the findings of this study to clinical practice is discussed.

In **Chapter 5**, the incremental role of RV T1 defined diffuse fibrosis as an early indicator of adverse remodelling is explored with results presented from a cross-sectional study.

Furthermore, insights into serum biomarkers of fibrosis and their association with adverse remodelling post pulmonary valve replacement are presented in **Chapter 6**.

In **chapter 7**, the clinical relevance of the findings from all the sub-studies that comprise this thesis are contextualised in an overall general discussion. The direction for future work is discussed.

In **Chapter 8**, the overall conclusions derived from each sub-study of this thesis are reiterated and summarised.

Chapter 1: Introduction

Tetralogy of Fallot- aetiology and epidemiology

Tetralogy of Fallot (TOF) is the most common cyanotic congenital heart condition accounting for 10% of congenital heart disease (CHD) patients and occurring in 1 in 3600 births¹⁻³. It is characterised by the presence of right ventricular outflow tract (RVOT) obstruction, right ventricular hypertrophy (RVH), a large outlet ventricular septal defect (VSD) and an over-riding aorta as described in 1888 by Etienne-Louis Fallot. Abnormal antero-cephalad deviation of the outlet septum during foetal development leads to RVOT obstruction, the dominant lesion in the 'tetrad' of malformations described. The degree of antero-cephalad deviation of the septum determines the severity of cyanosis.

Tetralogy of Fallot (TOF) is a heterogeneous condition that encompasses a spectrum of different anatomical variations with differing clinical severity. These may include the presence of branch pulmonary artery stenosis, double outlet right ventricle (DORV), absent pulmonary valve, pulmonary atresia with VSD and major aorto-pulmonary collateral arteries. It may also be associated with additional congenital lesions: atrial septal defect, atrioventricular septal defect (trisomy 21), right aortic arch, persistent left superior vena cava, ascending aortopathy, aortic root dilatation and anomalous coronary artery origins in 3-5% of cases. A genetic substrate to TOF is recognised, most commonly microdeletion of the q11 region on chromosome 22 that is associated with Di George syndrome or Velocardiofacial syndrome^{4,5}.

Repaired tetralogy of Fallot

Prior to the development of cardiopulmonary bypass, only a palliative shunt such as a Blalock-Thomas-Taussig shunt (BTS) was possible. A BTS involved end-to-end anastomosis of a subclavian artery to the ipsilateral pulmonary artery ideally on the opposite side of the aortic arch. A modified BTS involved a Gore-Tex® (W.L. Gore and Associates, USA) interposition graft connection between the subclavian and pulmonary artery and had the advantage of preserving arterial supply to the upper limb and being easier to connect to small subclavian arteries.

Other palliative procedures performed without the requirement for cardiopulmonary bypass included a central shunt between the ascending aorta and right pulmonary artery (Waterston's shunt) or a Brock procedure which involved relief of RVOT obstruction using a punch biopsy-like instrument through an incision in the right ventricle. This procedure was in order to improve pulmonary blood supply and reduce right to left shunting of blood from the VSD. Palliative shunts were not without their own problems with potential for them to cause pulmonary over-circulation, pulmonary artery distortion and to thrombose⁶.

After cardiopulmonary bypass was established, total TOF repair became possible following a palliative shunt, as a two-stage surgical approach⁷. The first total repair was performed by Lillehei in 1954⁷. Fallot repair involves closure of the VSD usually with a patch and relief of RVOT obstruction infundibular muscle resection and RVOT reconstruction with transannular, RVOT or PA patching, homograft or right ventricle- pulmonary artery (RV-PA) conduit depending on the anatomical variant and coronary anatomy. Surgeries in this earlier era often involved generous transannular patch implantation via large ventriculotomy incisions

that inevitably led to residual pulmonary regurgitation (PR) and large akinetic scarred areas in the RVOT.

Thanks to advances in cardiothoracic surgery, surgical outcomes vastly improved in infancy regardless of patient weight. This enabled patients to undergo a primary total repair under the age of one year rather than a two-stage repair⁶. The main advantage of primary repair is to avoid chronic hypoxia and RV hypertrophy and its suspected sequelae of RV impairment and myocardial fibrosis^{6,8}. Modern surgical approaches such as this also take in to consideration more conservative reconstruction of the RVOT and VSD closure via atriotomy and trans-pulmonary artery incisions, thus avoiding ventricular scar formation and preservation of ventricular myocardium⁹⁻¹¹. Furthermore, there is a shift to preserve the pulmonary valve as much as possible, even if this at the expense of mild residual pulmonary stenosis or RVOT obstruction^{2,6,12}.

Tetralogy of Fallot survival

Survival prospects for patients with repaired tetralogy of Fallot (rTOF) have been revolutionised secondary to advances in diagnosis, cardiothoracic surgery techniques, improved myocardial protection during surgery, and post-operative patient care. There is now a growing adult population of rTOF patients with more adults than children¹³. Survival 20 years post repair is currently greater than 90% with mortality at 40 years after primary surgery estimated to be 14%¹⁴. In a meta-analysis involving 4583 patients (39 studies), sudden cardiac death (SCD) occurred in 1.8% of patients after 8 years of follow-up, equivalent to 0.15% annualised risk of SCD¹³. Although this seems low, it is still higher than that for the general population of healthy adults¹⁵.

Despite modern surgical techniques, TOF repair is not curative. Years of pressure and volume loading from residual haemodynamic pulmonary valve dysfunction leads to adverse mechano-electrical remodelling that can be catastrophic, predisposing to ventricular tachycardia (VT) and SCD¹⁶. Even with contemporary management of rTOF patients, where there is proactive timing of pulmonary valve replacements (PVR) in order to minimise long term complications of PR¹⁷⁻²⁰, the risk of VT and SCD is not aborted and is estimated to be 11.9% and 3.7% -8.6% respectively by 35 years of follow up²¹. Following the third and fourth decade of life, the risk of clinically significant arrhythmia is known to rise exponentially²². This inspires the need to improve risk stratification in rTOF patients to identify those individuals who are at greatest risk of VT and SCD, and are therefore most likely to receive benefit from an automated implantable cardiac defibrillator (AICD)¹ for primary prevention.

Automated implantable cardiac defibrillator (AICD)

Decisions regarding primary prevention AICDs in patients with adult congenital heart disease (ACHD) are challenging and are not taken lightly. This is because of the significant and well reported physical and psychological morbidity associated with these devices²³⁻²⁵. Patients are at risk from procedural complications, infection secondary to multiple generator box changes throughout their lifetime, inappropriate shocks and AICD shock related depression and anxiety²³⁻²⁵. In a multicentre study that followed 121 rTOF patients with primary prevention and secondary prevention AICDs, a high complication rate (30%) and annual risk of inappropriate shock of 6% was reported²³. Finding the right balance between the potential risks of morbidity and complication from an AICD with the risk of

missing a potentially catastrophic event such as ventricular arrhythmia or SCD, is a significant challenge that arises frequently in daily clinical practice.

Primary prevention AICD guidelines in rTOF patients

Currently, guidelines to aid primary prevention ICD decision making in rTOF patients are somewhat elusive^{1, 26} in contrast to the clearer guidelines available for patients with acquired heart diseases (ischaemic or dilated cardiomyopathy)²⁷. Unlike patients with acquired heart disease, randomised controlled trial data are lacking in CHD due to the large number of patients required to power these studies, the heterogeneity of the conditions and that earlier conclusions may not be relevant to a more contemporary cohort of patients¹⁵. Recent European Society of Cardiology (ESC) guidelines in 2020¹ and PACES/HRS guidelines²⁸ in 2014 recommend that AICD should be considered in patients with multiple risk factors for SCD namely: LV dysfunction, non-sustained VT, symptomatic VT, QRS duration $\geq 180\text{ms}$, extensive RV scarring on CMR and inducible VT on electrophysiology testing (class IIa recommendation)¹. The precise details on which or how many of these risk factors should be present in a patient, remain unspecified²⁹ and are left to the discretion of the clinical team.

Risk stratification for ventricular arrhythmia and sudden cardiac death

For more than two decades, efforts have been made to identify risk factors for VT and SCD in rTOF patients however, no single marker on its own has been accurate enough in predicting this outcome. The challenge lies with predicting a relatively rare event, SCD with incidence 0.15% per year, from a large population^{15, 23}. At least 10 years ago, Bricker and colleagues had suggested that a study of approximately 1700 rTOF patients followed up for

10 years will be required to enable full multivariable statistical analysis due to the low event rate³⁰.

Previously proposed stratification scores

Following a multicentre prospective study in 2009 by Khairy et al²³, a risk stratification score was proposed and although not in clinical guidelines, is often used to guide management in clinical practice. An important disadvantage is that application of the full score requires data that is invasive to acquire namely: results from programmed ventricular stimulation and LV end diastolic pressure (EDP)²³. These invasive tests are not frequently indicated and are only for selected patients in clinical practice, therefore rendering the score limited in its day-to-day clinical applicability. A study that applied this risk score to its cohort of 174 rTOF patients found that it was only possible to provide a complete risk calculation in 10% of patients with the majority of the invasive data missing³¹. The absence of invasive data that are seemingly important and are weighted heavily against other parameters in this scoring tool make its application and use difficult to interpret. Secondly, this scoring tool was derived from a study of rTOF patients with primary prevention AICD's already *insitu* and was used to predict freedom from appropriate AICD shock²³. Therefore, the risk score was inherently biased towards predicting appropriate AICD shock in an already high-risk patient group.

Since 2009, a modification of the original score proposed by Khairy *et al* was published in 2017 by Bokma and colleagues³². This modified score, replaced invasive data (LVEDP and VT inducibility) with non-invasive cardiovascular magnetic resonance imaging (CMR) parameters found to be independent predictors of adverse outcome: RV ejection fraction

(EF) <30% and LV ejection fraction (EF) <45%³². Although it was a large study involving 575 rTOF patients, it did not investigate all known risk factors for SCD and malignant ventricular arrhythmia in rTOF. As recent as 2020, Waldmann and colleagues studied appropriate AICD shock from a national registry of 167 rTOF patients with both primary and secondary prevention AICDs *insitu*³³. Patients were scored according to how many features of the ESC guidelines were present (LV impairment, QRS duration > 180ms, non-sustained VT and positive ventricular stimulation). QRS fragmentation when combined with the guideline based scores was found to be a significant predictor of appropriate AICD shock³³. The main limitation of this study was its inherent bias towards including already clinically high-risk patients and its use of appropriate ICD shock as a proxy endpoint for survival. Furthermore, non-sustained VT was included in the score despite several reports of it being benign^{21,56}. Also the study did not include comprehensive CMR evaluation with LGE or other non-invasive tests.

A comprehensive risk scheme that can be applied to all unselected patients and one that integrates all non-invasive risk factors for life-threatening VT/SCD is still awaited and motivates this research.

Risk factors for VT and SCD

Multiple risk factors for VT and SCD have been identified. These include patient characteristics, structural, haemodynamic and electrophysiological features.

Patient and surgical characteristics

Late surgical repair was significantly associated with SCD in a large multicentre study of 793 patients as well as older age²¹. Prior palliative shunt and ventriculotomy have also been reported as important predictors^{21,23}. Concerning symptoms such as syncope, fast palpitations and symptomatic non-sustained VT were found to be predictors of appropriate AICD shock³⁴. Obesity was also shown to be an independent predictor of clinically significant ventricular arrhythmia³⁵.

Structural and haemodynamic features

Residual pulmonary regurgitation (PR) that was at least moderate was the predominant haemodynamic lesion that predicted SCD²¹. Other structural predictors of adverse outcome reported from a large multi-centre registry (INDICATOR) included cardiovascular magnetic resonance imaging (CMR) derived RV EF < 45%, and RV mass/volume ratio of >0.3g/ml/m²¹⁷. An RV EF < 30% and LVEF < 45% were found to be independent predictors of outcome in 2017³².

The akinetic region in the RVOT can be aneurysmal and is related to previous transannular patch or large ventriculotomy incisions. A large akinetic region in the RVOT was found to predict first onset ventricular arrhythmia³⁶ and is associated with increased RV volumes and impaired RVEF³⁷. Furthermore, a large akinetic RVOT segment is mechanistically associated with delayed electrical conduction through the right ventricle, causing further ventricular desynchrony³⁸. Other structural features that predicted both atrial and ventricular arrhythmia included the finding of right atrial area indexed to body surface area (RAAi) $\geq 16\text{cm}^2/\text{m}^2$ ³⁶ as measured by CMR and raised right ventricular systolic pressure (RVSP) measured by echocardiography¹⁷.

The prognostic value of markers of LV systolic dysfunction in rTOF patients has also been studied³⁹. An LVEF <35% measured by CMR is a predictor of VT and SCD¹⁷. A reduced mitral annulus plane systolic excursion (MAPSE) as well as global longitudinal LV strain on two-dimensional (2D) echocardiography have been found to predict VT and SCD and have been proposed as potential early markers of LV dysfunction⁴⁰. With regards to LV diastolic dysfunction, invasively measured LV EDP was also shown to be prognostic²³, although this was not true for echocardiographic markers of LV diastolic dysfunction⁴⁰.

The involvement of the LV in rTOF, a predominantly 'right-heart disease', has been speculated to be related to LV myocardial ischaemia and hypoxia suffered before the time of surgical repair and peri-operatively⁴¹. Secondly, myocardial fibres are shared between the RV and LV and there is sharing of the pericardial space, therefore ventricular-to-ventricular interdependence and septal shift is also a mechanism by which LV dysfunction also occurs⁴²⁻⁴⁶.

Neurohormonal markers and exercise measures

Elevated Neurohormonal markers, atrial natriuretic peptide (ANP)⁴⁷, b-type natriuretic peptide (BNP)⁴⁸ and reduced peak oxygen consumption⁴⁹ also have been reported as predictors of ventricular arrhythmia and SCD.

Electrophysiological factors

A QRS duration of ≥ 180 milliseconds or an increase by ≥ 3 millisecond per year on the surface ECG⁵⁰ and fragmented QRS complexes were all predictive of SCD^{33, 51}. Other groups have also shown that an increased JT dispersion and late potentials seen on signal-averaged

ECG⁵² also to be risk factors for SCD. These ECG features are thought to be related to underlying progressive RV dilatation and fibrosis in the ventricular septum. Ventricular arrhythmia is not the only electrophysiological consequence in rTOF; atrial arrhythmia also occurs frequently and is a risk factors in itself for SCD^{17, 21, 53, 54}.

Non-sustained VT has been reported as a predictor of ventricular arrhythmia and sudden death²³ and often it is included⁵⁵ within the composite endpoints of studies predicting ventricular arrhythmia and mortality. On the other hand, there are also conflicting reports that non-sustained VT could be more benign,^{21, 56} bringing to question its use as a surrogate endpoint for malignant ventricular arrhythmia and mortality in studies and its place in AICD guidelines^{1, 26}. Similarly, a higher burden of ventricular ectopy has been associated with an increased risk of SCD⁵⁷. However, again there is conflicting data against this²¹ with ventricular ectopy found to be common in rTOF patients (found in 83% on 24 hour Holter monitoring⁵⁵) and a non-specific finding⁵⁸.

Programmed ventricular stimulation

In one of very few prospective studies with a survival endpoint carried out in patients with ACHD, inducible VT during invasive programmed ventricular stimulation was predictive of mortality by almost five-folds⁵⁹. The high negative predictive value of 91% for invasive programmed ventricular stimulation versus its lower predictive value of 77% makes its potential use as a 'rule out' test clinically attractive. Inducible VT in a low-risk group is non-specific and is unlikely to warrant aggressive prophylaxis with an AICD. On the other hand, patients who are deemed high-risk due to their non-invasive risk profile could avoid this invasive test with there being enough concern to implant an AICD.

The benefit from ventricular stimulation may be best demonstrated therefore, in patients clinically judged to be of intermediate risk and in whom there is no clear consensus on primary prevention. Recently published ESC guidelines in 2020 recommend as a class IIa recommendation programmed ventricular stimulation in rTOF patients with LV/RV dysfunction, non-sustained VT, symptomatic VT, QRS> 180ms, and extensive RV scarring on CMR¹. These less helpfully, also happen to be the exact indications for AICD implantation as well¹.

Ventricular arrhythmia in rTOF

Myocardial scar substrate for ventricular tachycardia (VT).

Dense myocardial scar (replacement fibrosis) has been shown histologically to be present at surgical incision sites in patients with rTOF such as the RVOT reconstruction site, VSD patch site and LV apical vent site, as well as in RV trabeculations remote to surgery^{47, 60}. These regions of scarring or patch material together with valve annuli form areas of 'electrically dead' non-conducting tissue. Between these non-conducting tissues are healthy myocardium (anatomical isthmuses) from which monomorphic re-entrant tachycardias can arise⁶¹⁻⁶³.

The majority of scar related ventricular arrhythmia in rTOF patients with preserved ventricular function is a fast monomorphic VT with a an average total cycle length of ~ 250ms that can lead to haemodynamic instability and degeneration to ventricular fibrillation (VF)²³. Patients with impaired ventricular function are also at risk of developing polymorphic VT in addition.

Anatomical isthmuses in rTOF

The slowing of electrical impulses through anatomical isthmuses (AI) have also been recently shown in electrophysiology studies to be an underlying cause for VT⁶³. Four AI's in rTOF have been identified on post-mortem specimens⁶² and electro anatomical mapping⁶¹: AI-1, between the RVOT scar and tricuspid valve annulus, AI-2, between the RVOT scar and pulmonary valve annulus, AI-3, between the VSD patch site scar and pulmonary valve annulus, and AI-4, between the VSD patch site and tricuspid valve annulus.

Electrophysiology studies have demonstrated that the narrower the width of the AI, the slower the electrical conduction through the isthmus and therefore, greater likelihood for re-entry tachycardia in the presence of an ill-timed ectopic beat⁶³.

Myocardial fibrosis

Pathophysiology

Myocardial fibrosis is the final common pathophysiological process that links a wide spectrum of both congenital and acquired heart conditions. It has been shown to have implications on ventricular function and arrhythmia⁶⁴⁻⁶⁸. It is the process by which there is remodelling of the extracellular matrix (ECM) secondary to an insult, which can lead to abnormalities in the composition and quality of the ECM, which in turn can impair myocardial performance⁶⁹.

Fibrosis or scarring is characterised by an increase in myofibroblasts and their deposition of excessive amounts of type I and III collagen into the extracellular matrix (ECM). The ECM which connects neighboring cardiac myocytes and blood vessels is the 'scaffolding' of the

heart that maintains its structural integrity and function. When its composition is altered and disrupted due to the overproduction of collagen, a reduction in compliance of myocardial tissue and diastolic impairment can ensue and eventually systolic impairment can follow⁴¹.

Mechanisms for fibrosis

The precise mechanism by which fibrosis occurs is complex. Triggers such as injury or increased mechanical stress on the myocardial ECM from high pressure or volume loading, causes a release of inflammatory cytokines which in turn cause migration and proliferation of cardiac fibroblasts⁷⁰. These cardiac fibroblasts are transformed into their active forms, myofibroblasts by transforming growth factor-Beta (TGF- β)^{71, 72}. Active myofibroblasts produce pro-collagen type I and III which are cleaved into collagen type I and III and are subsequently deposited in the ECM. Pro-collagen type I peptide (PICP) and pro-collagen type III peptide (PIIICP) are byproducts of the production of collagen and are therefore circulating plasma markers of collagen biosynthesis. Excess proliferation of myofibroblasts and their production of collagen leads to fibrosis, myocardial stiffness and diastolic dysfunction^{73, 74}.

Regulation of the fibrosis process

Under normal physiological conditions, myofibroblasts also have a role in regulating collagen deposition and collagen degradation that occurs in the ECM. They do this by producing matrix metalloproteinases (MMPs) enzymes that digest collagen and also produce their antagonist, tissue metalloproteinase inhibitors (TIMPs) which block the break-down of collagen. The balance of MMPs and TIMPs maintains ECM homeostasis. The relative levels of circulating plasma MMPs and TIMPs reflect the *status quo* in the cardiac remodelling process. In patients with hypertensive heart disease and hypertrophic cardiomyopathy,

higher levels of TIMPs were found in comparison to lower levels of MMPs in keeping with expansion of the ECM due to collagen production and LV hypertrophy⁷⁵⁻⁷⁸. In patients with pulmonary hypertension, raised TIMP-4 relative to MMP-2 was significantly associated with severe RV hypertrophy and worsening markers of disease severity and the poorest survival⁷⁹⁻⁸¹. On the other hand, patients with dilated cardiomyopathy were found to have higher levels of MMPs relative to lower amounts of TIMPs present⁸²⁻⁸⁵ which suggest that a process of increased degradation of the ECM dominated in these patients. These circulating serum markers of collagen turnover may add further insight into the myocardial health and composition in adult rTOF patients. This has not been previously studied and is explored further in this research.

Replacement fibrosis

Broadly there are two types of myocardial fibrosis that can occur: replacement fibrosis (scarring) and diffuse (interstitial) fibrosis⁸⁶. Replacement fibrosis occurs following direct damage or apoptosis of myocytes such as from ischaemia or surgical incision. Myocytes are replaced with type I collagen. This type of fibrosis is considered irreversible and corresponds with scarred myocardial areas seen macroscopically^{87, 88}.

Diffuse fibrosis

Diffuse fibrosis is secondary to increased collagen deposition within the ECM as a response to abnormal loading conditions on the myocardium for example, secondary to residual pulmonary regurgitation in rTOF⁴¹. It is considered as a precursor for established replacement fibrosis⁸⁹ and is on the same continuum as focal fibrosis, despite traditional binary views on the two types of myocardial fibrosis. Importantly, diffuse fibrosis has been demonstrated to be potentially reversible^{90, 91}. Mineralocorticoid antagonists⁹², Angiotensin

converting enzyme inhibitors⁹³ and angiotensin II receptor inhibitors⁹⁴ are demonstrated to have anti-fibrosis properties, causing regression of collagen deposition and the promotion of reverse cardiac remodelling.

There is increased enthusiasm in non-invasively detecting the various forms of fibrosis and understanding their prognostic significance for a more targeted treatment approach. Non-invasive biomarkers, if proven reliable, could become helpful in monitoring disease activity and guiding heart failure therapy.

Restrictive RV physiology

The hallmark of restrictive RV physiology is laminar antegrade flow in the pulmonary artery occurring in late diastole and present throughout the respiratory cycle. This is detected by the 'a' wave on Doppler echocardiography of the pulmonary artery. This phenomenon is caused by an impairment of RV compliance and filling in end-diastole that causes the RV to act as a rigid conduit which transmits atrial contraction directly to the pulmonary artery^{95, 96}.

Mechanisms for RV myocardial fibrosis have been proposed such as long term hypoxia and peri-operative ischaemia^{47, 97}. RV restrictive physiology has been demonstrated in patients with severe degrees of myocardial fibrosis detected by late gadolinium enhancement (LGE) CMR, in keeping with myocardial fibrosis being responsible for this phenomenon⁹⁸.

Restrictive RV physiology has been reported at different stages of follow up of patients with rTOF. It is prevalent, affecting a significant proportion of patients (28-68%) at different

stages, or in approximately 50% by 23 years of age⁹⁸⁻¹⁰⁰. In infancy, RV restrictive physiology is associated with a prolonged and stormy postoperative recovery^{99, 101}. Paradoxically, older children and young adult patients with rTOF and RV restrictive physiology have the advantage of superior exercise capacity¹⁰², reduced pulmonary regurgitation, narrower QRS complexes⁵⁰ and less risk of ventricular arrhythmia⁵⁰ in comparison to patients with non-restrictive, larger RVs. Later on in the patient's adult life, it has been reported that the patients with RV restriction have reduced exercise tolerance and quality of life,^{103, 104} whereas some patients retain the protective benefit of this phenomenon.

A possible explanation for the differences in clinical behaviour reported in adult rTOF is that there are two distinct RV restrictive physiology phenotypes as described by Lee and colleagues in 2013^{105, 106}. The first type is referred to as 'primary restriction' which occurs as a result of myocardial disease causing stiffness but with patients retaining normal ventricular volumes. The second type ('secondary restriction') is caused by an initially compliant RV that dilates to its maximum stretch capacity and then undergoes a fibrotic process of remodelling that renders it to become restrictive^{105, 107}. It has been demonstrated that patients with restrictive RV physiology and larger RV volumes (indexed RV end diastolic volume $\geq 170\text{ml/m}^2$) had significantly impaired exercise capacity when compared to patients who had RV restriction and smaller RVs¹⁰⁵. Furthermore, patients with restrictive RV physiology have tended to have larger right atrial dimensions, tricuspid regurgitation and late Fallot repair which are in themselves predictors of atrial arrhythmia³⁶.

Cardiovascular magnetic resonance (CMR) in adult congenital heart disease

Cardiovascular magnetic resonance (CMR) has become an important diagnostic tool in the management of patients with CHD enabling their life-long follow up without radiation exposure¹⁰⁸⁻¹¹⁰. It is the gold standard modality for assessing ventricular volumes and function¹¹¹. Particularly in conditions where the RV is of interest such as in TOF, CMR can accurately assess the uniquely shaped RV without making geometrical assumptions and without the challenge of restricted acoustic windows such as with echocardiography¹¹². The accuracy and reproducibility of CMR in quantitative RV assessment has been demonstrated to be superior to other imaging modalities¹¹²⁻¹¹⁶.

Advances in CMR have enabled the non-invasive detection of replacement fibrosis using late gadolinium enhancement (LGE) techniques and diffuse fibrosis with T1 mapping. The detection of fibrosis was previously only possible using invasive methods such as with tissue biopsy or on post-mortem.

Common CMR challenges in patients with CHD are problems with ECG gating due to arrhythmia, breath-holding difficulties as well as metallic artifacts from sternal wires and trans-catheter devices.

Cardiovascular magnetic resonance (CMR) – basic Physics

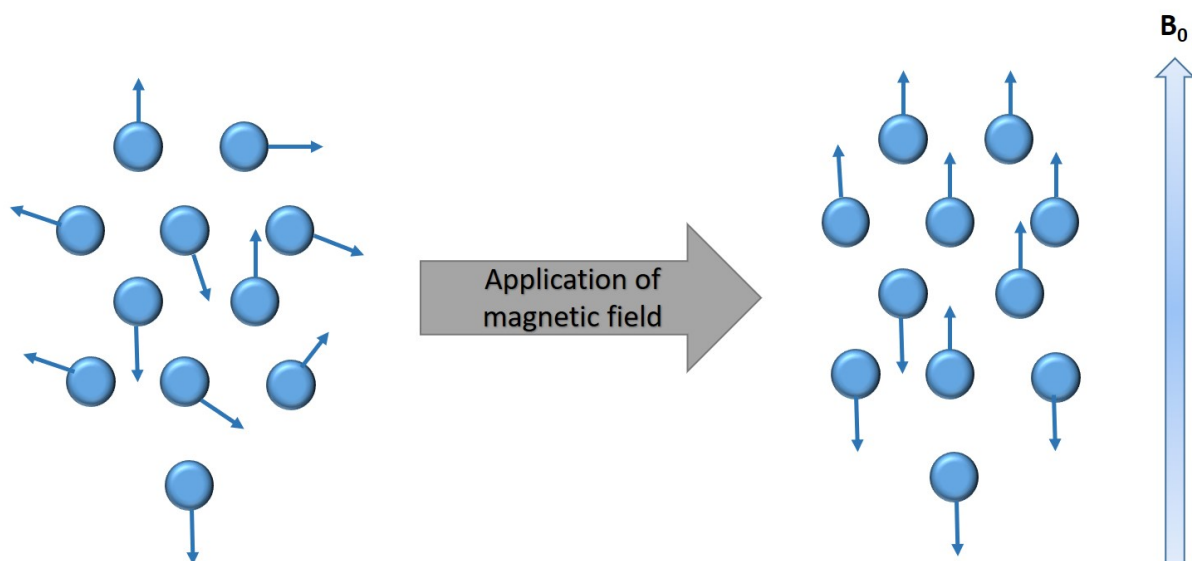
The hydrogen atom is the most abundant atom in the human body. Each atom is comprised of a nucleus that contains a positively charged proton and has a surrounding shell of negatively charged electrons. Each charged proton spins (precesses). This moving charge

creates an electrical current which in turn creates a magnetic field. So that in effect, each hydrogen proton in the human body is effectively acting like a small magnetic bar¹¹⁷.

A) The patient is placed in the scanner

When the patient is placed inside the magnetic core of the CMR scanner, each proton acting as an individual magnet immediately aligns to the magnetic field of the scanner so that overall the patient's magnetic field is longitudinal and parallel to the magnetic field of the scanner (**see Figure 1.1**). The protons continue to spin at a speed that is proportional to the strength of the magnet¹¹⁷.

Figure 1.1:



Protons outside of the magnetic field are aligned in a random fashion. When a strong magnetic field is applied, the protons switch their alignment from random orientation to the excess majority being parallel to the magnetic Field B_0 .

B) A radiofrequency wave is applied

A radiofrequency wave that matches the frequency of the spin of the protons is then applied to the patient whilst inside the scanner. This causes the transfer of energy to protons which makes them turn away from their relaxed longitudinal and parallel state to anti-parallel. Longitudinal magnetisation is then decreased. The extra energy transferred to these protons also causes them to spin in phase with each other. This creates a second magnetic vector, transverse magnetization¹¹⁷.

C) The radiofrequency wave is switched off

When the radiofrequency wave is switched off, longitudinal magnetisation relaxes back to its full state. This is described by a time constant T1. The newly formed transverse vector relaxes back to its starting position zero. This transverse direction of magnetisation is described by a time constant T2¹¹⁷.

D) The patient emits a signal

Different types and sequences of radiofrequency pulses can be delivered, for example a 90° pulse (saturation pulse), or a 180° pulse (inversion pulse) followed by a 90° pulse. These radiofrequency pulses tilt the longitudinal and transverse magnetic fields to different degrees. The net magnetic field (sum of longitudinal and transverse vectors) is the signal measured when the radiofrequency wave is switched off¹¹⁷.

E) The signal is used to reconstruct an image

The signals transmitted are detected and sorted by a complex process called Fourier's transformation¹¹⁸. This enables images to be formed.

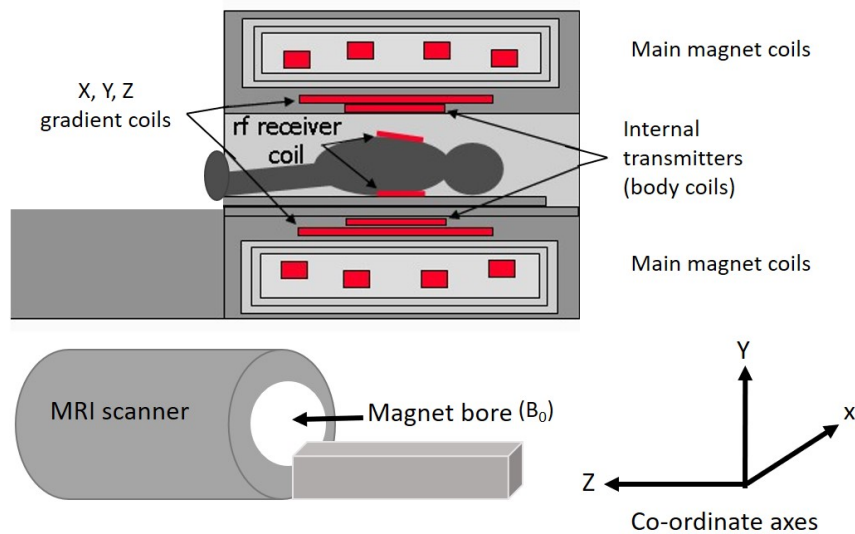
Components of the CMR scanner

A typical CMR scanner for clinical work has a magnetic strength of 1.5 Tesla (or 15000 Gauss). This is approximately 30,000 times the magnetic strength of earth. The magnet is surrounded in cryogen fluid cooled to -269 degrees. The cooling effect surrounding the magnet, removes resistance for the electrical current that is applied through it so that a magnetic field is generated and is constantly present. The magnet is therefore effectively always turned on.

The patient is surrounded by coils inside the machine and covering the chest wall which transmit radiofrequency pulses and receive signals. There are also gradient coils which are responsible for selecting the slice thickness of the image and creating spatial resolution.

There are also shimming coils which reduce any inhomogeneity that may be present in the magnetic field **(see Figure 1.2)** ¹¹⁷.

Figure 1.2:

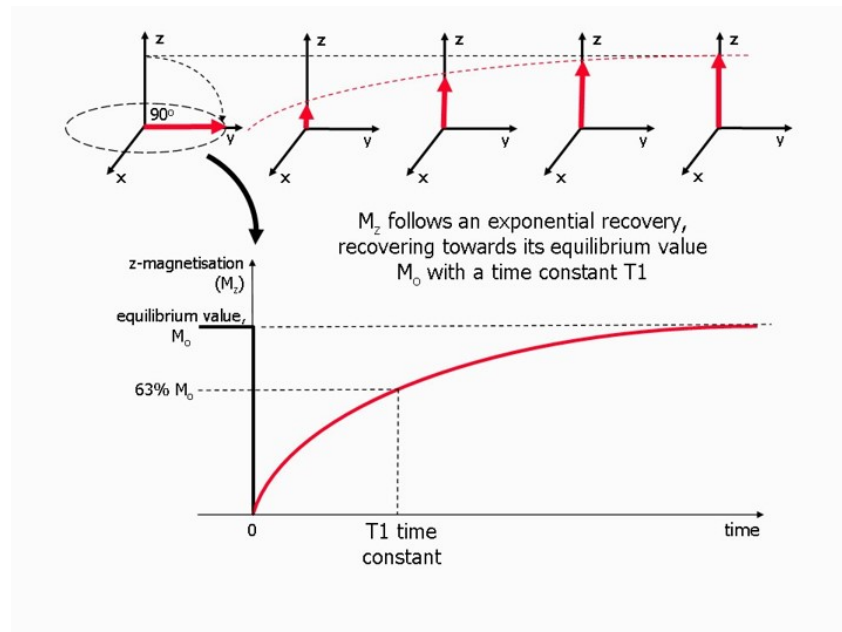


The components of the MRI scanner are shown (top image). The magnet coils and gradient coils surround the patient. The gradient coils are in three-dimensions (X,Y,Z) and enable slice selection and spatial resolution. Body coils surrounding the patient transmit radiofrequency (RF) pulses, and a surface coil attached to the chest of the patient receives RF transmissions. Reference co-ordinates with relation to the magnetic field are shown (lower image). When a patient is placed inside the scanner, a new magnetic vector is induced (Z) and is in parallel to the static magnetic field B_0 . Image reproduced from Ridgeway et al¹¹⁷.

T1 weighted images

Following excitation of the magnetic field with a radiofrequency pulse, the magnetic field relaxes back to its equilibrium state in the longitudinal direction. The rate of which it returns to its fully relaxed state follows an exponential curve (recovery curve). The rate at which the magnetic field relaxes back to 63% of its original state is known as T1 (see Figure 3). T1 relaxation is dependent on each proton's ability to exchange energy with its surroundings. Tissues that have high water content, have protons that exchange energy with their surroundings less easily and therefore take longer for the magnetic field to return to its equilibrium state. These tissues have a longer T1 time constant compare to other tissues with less water content.

Figure 1.3:



T1 relaxation diagram. Following the delivery of a 90-degree radiofrequency pulse, the net longitudinal magnetisation (Z) is abolished and then recovers back to its original state. As it is difficult to determine exactly the point in which longitudinal relaxation has fully completed. T1 is defined as the time taken for 63% of the original longitudinal magnetisation to recover. Reproduced by Ridgeway et al¹¹⁷.

Gadolinium based contrast agent

The differences between healthy and fibrosed myocardium may be subtle on CMR; however, following administration of intravenous gadolinium-based contrast agent (GBCA), areas of replacement fibrosis in the myocardium are emphasised. Gadolinium, when combined with a chelating agent is rendered non-toxic and has a high molecular weight. Its large molecular weight confines GBCA distribution to only the extracellular compartment⁴¹.

The extracellular compartment comprises ~ 15% the myocardium in a healthy heart. In disease states, this extracellular compartment expands due to increased collagen deposition.

Therefore, when GBCA agent is administered, its distribution remains within a larger extracellular volume⁴¹.

Gadolinium based contrast medium has paramagnetic properties that allow it to interact with spins promoting more rapid exchange of energy, thus where the gadolinium contrast is concentrated have shorter T1 constants. This results in a high signal (bright) when imaged¹¹⁸.

Replacement fibrosis identified by two-dimensional (2D) LGE CMR

Late gadolinium enhancement (LGE) CMR can detect replacement fibrosis using an inversion recovery fast gradient echo sequence. Following the application of a non-selective 180° inversion recovery pulse, the longitudinal component of magnetisation returns to its relaxed state. The rate at which it returns to its relaxed state follows an exponential curve with a time constant (T1).

Approximately 10-30 minutes following contrast injection, there is a higher concentration of GBCA in areas of replacement fibrosis than in normal myocardium. Therefore, there is a difference in T1 values and different magnetisation recovery curves. The time along the magnetisation recovery curve where the normal myocardium passes through 0 (has no signal) is known as the inversion time (Ti). This Ti varies with GBCA dose, time after administration, and with patient-specific factors such as kidney function and disease state. In order to maximise the signal from fibrosed myocardium against a background of healthy neighboring myocardium, the optimum Ti should be when the myocardium is nulled (appears black).

Pitfalls of 2D LGE CMR

The wash-out kinetics of GBCA can vary between patients. Depressed myocardial contractility and kidney function may slow down GBCA washout, whereas an intra-cardiac shunt may cause rapid washout of GBCA¹¹⁹. In practice this can make finding the optimum Ti for nulled myocardium challenging. This technique therefore requires careful visual inspection of each acquired image to ensure correct tissue contrasting¹²⁰.

Arrhythmia may be another cause for image artefact. Data acquisition on every second R-R interval allows time for full relaxation of longitudinal magnetisation. This in turn improves the signal-to-noise ratio and helps reduce sensitivity from variable R-R intervals¹²¹⁻¹²³.

Imaging the RV is challenging due to its unique shape, thin wall, close proximity to pericardial/mediastinal fat and metallic artefact from sternal wires common in CHD. The spatial resolution of currently available 2D LGE CMR sequences¹²²⁻¹²⁴ (7mm slice thickness) is not adequate to image the typically ~ 3 mm wall of the right ventricle. The inadequate spatial resolution for the thin walled RV makes it susceptible to partial volume effects. Partial volumes effects are seen when a single voxel in the area of interest is shared by tissue from an adjacent structure, for example: RV myocardium and pericardial fat. This can give rise to a false positive LGE scan and can be minimised with high spatial resolution imaging.

Three-dimensional (3D) LGE CMR

Unlike 2D LGE CMR, three-dimensional (3D) respiratory-navigated LGE CMR can image the whole heart at a higher spatial resolution^{125, 126}. The spatial resolution of 3D LGE CMR is 1.5x1.5 x4mm and when reconstructed in any plane has an image voxel size of

0.7x0.7x2mm. The enhanced spatial resolution of 3D LGE CMR makes it particularly appealing for studying the thin walled RV. Without the use of a respiratory-navigator, coverage of the whole heart with contiguous slices is virtually impossible because patients would not be able to sustain the long breath-hold required. In this sequence, a continuous level adaptive windowing strategy (CLAWS) defines the window for the diaphragm position that is tracked by the respiratory navigator¹²⁷. This in-turn allows for patients to breath freely.

LGE CMR in rTOF

Patients with rTOF have shown myocardial enhancement at the surgical sites such as: RVOT patch, VSD patch site, and apical LV vent site on LGE CMR as well as in areas remote to cardiac surgery such as in RV trabeculation and moderator band⁴⁷. This has been validated against histological findings⁴⁷. The extent of myocardial enhancement has correlated with clinical arrhythmia, exercise capacity and neuro-hormonal dysfunction such as elevated B-type natriuretic peptide (BNP) levels in a cross-sectional study⁴⁷.

The prognostic and incremental value of LGE CMR remain unknown. It is also unknown how it should be weighted clinically against other known risk factors in patients with rTOF when considering its use within a risk stratification algorithm. This is explored in this research.

Concern regarding Gadolinium deposition in the brain

Gadolinium deposits were found in the globus pallidus and dentate nucleus region of the brain on post-mortem examinations^{128, 129}. There is a concern that multiple doses of GBCA over many years could lead to this deposition. The clinical implication of the cerebral deposits remains unknown^{130, 131} and therefore, it is important that contrast administration is evidence based.

LGE quantification

It is expected that almost all patients with rTOF will have at least some degree of scarring (myocardial fibrosis) detected by LGE CMR at sites of their previous surgery. Quantification of LGE can therefore be important in determining its prognostic value and differentiating those patients who are most at risk of sudden death.

When quantifying LGE in the LV a range of different methods can be used. The most sensitive and reproducible of these are LGE visual presence scores¹³²⁻¹³⁴, semi-automated full width half maximum technique^{135, 136} and semi-automated quantification of LGE by identifying signal intensity greater than two standard deviations more than remote healthy myocardium¹³⁶.

Quantification of LGE in the RV in contrast to the LV is challenging due its thin wall and complex shape. LGE visual presence scores would not be helpful in rTOF because LGE is almost always expected. The endocardial and epicardial contouring required for the full width half maximum technique is technically difficult for the thin RV and is susceptible to partial volume effect and falsely elevated LGE quantity.

A visual segmental LGE scoring system for the RV in patients with rTOF reported by Babu-Narayan and colleagues in 2006^{47, 137} had an advantage that it accounted for the unique anatomy of the RV post TOF repair. This was shown to have high inter-observer and intra-observer reproducibility. One of the major appeals of 3D LGE CMR is that it can offer volumetric quantification of LGE in the whole heart at high spatial resolution which makes it particularly suitable for quantifying LGE in the RV.

T1 mapping

An important limitation of LGE CMR is that it is dependent on the differences in signal intensity between healthy and diseased myocardium to detect fibrosis and therefore it cannot detect interstitial (microscopic) fibrosis, which diffusely affects the myocardium^{41, 138}.

T1 mapping is a non-invasive technique that indirectly estimates the amount of diffuse fibrosis in the myocardium using CMR^{139, 140}. It has been validated against ex-vivo whole heart histology in dilated and ischaemic cardiomyopathy^{141, 142} and from endomyocardial biopsies taken from LV in patients with aortic stenosis, amyloidosis and hypertrophic cardiomyopathies¹⁴³⁻¹⁴⁶.

Tissues have different T1 constants primarily due to their differing concentration of water and GBCA shortens T1. With T1 mapping, an estimate is calculated from a series of different T1-weighted images during the magnetisation recovery process. A myocardial T1 map (parametric map) of the image is reconstructed where each pixel's signal intensity corresponds to its T1 relaxation time. This involves a series of assumptions about alignment of the pixels in all images of the supporting series (typically subject to errors such as inter-cycle differences, breath-hold imperfection, and corruption by blood signal artifacts)¹⁴⁷⁻¹⁴⁹.

T1 mapping general concepts

There are three main steps that must occur for a T1 map to be generated:

A) Excitation of the magnetic field with a radiofrequency pulse

The magnetic field must be excited in the longitudinal direction by either an inversion recovery 180° radiofrequency pulse or a 90° saturation radiofrequency pulse. The most common T1 mapping sequences used in research studies and clinical practice are Modified Look-Looker Inversion recovery sequence (MOLLI)¹⁵⁰ where typically 3 ECG-triggered 180° inversion recovery radiofrequency pulses are delivered¹⁵⁰.

The second most common T1 mapping sequence used is a Saturation-recovery single-shot acquisition (SASHA) sequence which uses 90° saturation pulses¹⁵¹. SASHA is more accurate at estimating T1 when compared to a MOLLI sequence however, is less precise mainly because of the use of a 90° saturation pulse to reduce the contrast-noise ratio, allowing for more noise on the T1 map⁴¹. Although there is some debate on whether MOLLI or SASHA is the most optimum T1 sequence, a trade-off with preference towards maximising the sensitivity of sequence to measure disease versus accuracy is debated^{41, 152}.

B) Sampling at different time points of the magnetisation recovery curve

As the longitudinal magnetisation relaxes back to its resting state following the delivery of the radiofrequency pulse, sampling occurs along the exponential recovery curve at different time points (inversion times, T_i). This occurs during mid-diastole for when the heart is most still in order to minimise error from cardiac motion (**see Figure 1.4**).

C) Fitting of the sampled curve in-order to extract a T1 value.

In a MOLLI sequence, sampling of T1 occurs at different inversion times (T_i) along the IR curve are then taken and fitted based on each pixels T1 signal (**see Figure 1.4**). T1 is calculated in each image pixel from a 3-parameter curve fit to the corresponding pixels by applying the following equation:

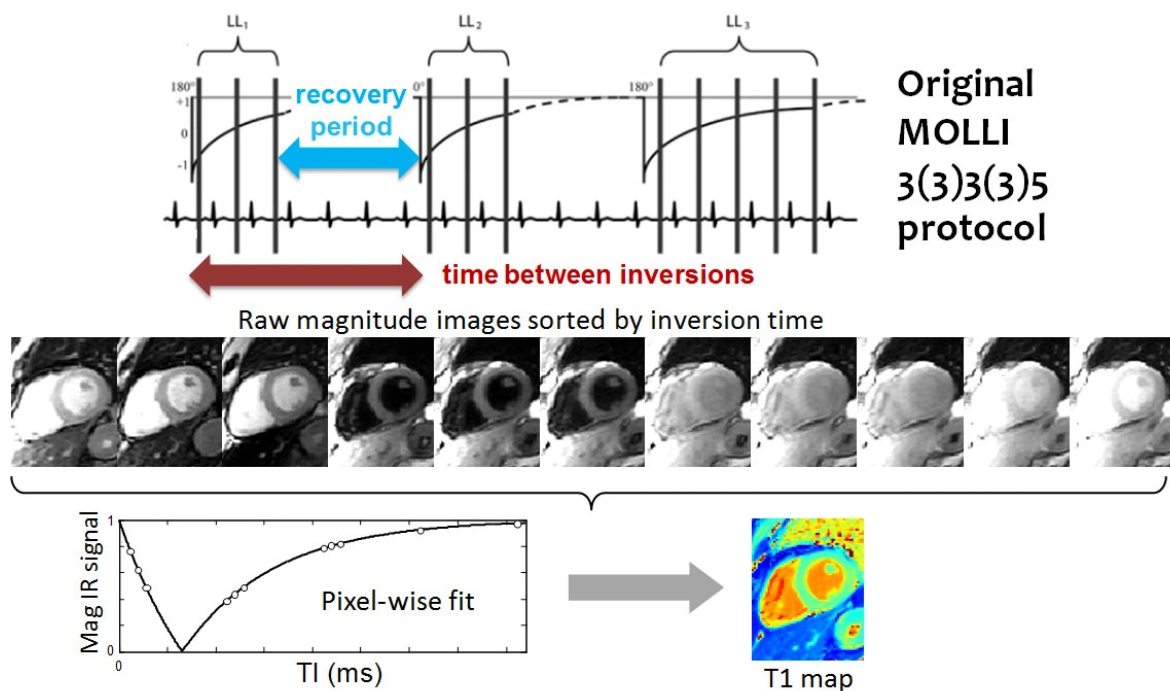
$$S(t) = A - B \exp(-t/T1^*)$$

$$T1 = T1^*((B/A)-1)$$

Here ' t ' is the inversion time, ' $T1^*$ ' represents the T1 relaxation time and ' A ' and ' B ' are fitting parameters related to the equilibrium magnetisation^{149, 152}.

With this method, a T1 map is acquired of a single slice of the heart within one single breath hold and at high resolution (2x2mm)¹⁵³.

Figure 1.4:



Original protocol Modified Look-Locker Inversion Recovery (MOLLI) obtains T1 maps over 17 heart beats. Each black vertical line above represents an inversion recovery (IR) radiofrequency pulse. In this protocol 3, 3, then 5 IR pulses are applied to produce 3, 3 and 5 images. The gaps in between the lines represents the time allowed for the IR curve to recover back to baseline (3 heart beats). This is referred to as a 3(3)3(3)5 MOLLI. Sampling of T1 occurs at different inversion times (T_i) along the IR curve. The samples are then fitted based on pixel T1 signal and is performed using the different measured inversion times. Figure reproduced from Kellman *et al*¹⁵² based on the original protocol diagram by Messroghli¹⁵⁰.

Extracellular volume (ECV) measurements

In the absence of myocardial oedema¹⁵⁴, expansion of the ECM is assumed to be secondary to deposition of collagen (diffuse fibrosis)¹⁴³. The ECM itself cannot be directly measured however, the extracellular volume (ECV) defined as the volume of the myocardium that is not occupied by myocytes, can be estimated as surrogate to the ECM¹⁵⁵. ECV measured by T1 mapping CMR for the left ventricle closely has been robustly histologically validated. Studies have shown that it correlates with diffuse fibrosis quantified histologically (collagen volume fraction)^{142, 143, 156}.

The measurement of ECV requires GBCA administration which is known to have confined distribution to the extracellular compartment. ECV is derived from the following formula¹⁵⁶:

$$ECV = \left(\frac{\frac{1}{T_1} \text{postcontrast myocardium} - \frac{1}{T_1} \text{precontrast myocardium}}{\left(\frac{1}{T_1} \text{post contrast bloodpool} - \frac{1}{T_1} \text{pre contrast blood pool} \right)} \right) * (1 - \text{haematocrit})$$

ECV is derived by measuring the change in myocardial T1-relaxation rate (1/T1) pre and post contrast and comparing this to the change in blood pool T1-relaxation rate pre- and post-contrast administration¹⁵⁰. It requires knowledge of the hematocrit for its calculation¹⁵⁰. The more fibrosis in the extracellular space, the longer the native T1 because from a physics perspective, the average water content of the myocardium is slightly higher with more interstitial fluid (intracellular cytoplasm containing a high concentration of macromolecules)⁴¹. However, on the images post-contrast, there will be more GBCA accumulation in the increased interstitial space of abnormal myocardium, therefore shortening post-gadolinium T1 relative to normal myocardium. The changes in 1/T1 for the myocardium, when used in the formula to calculate ECV, result in a larger ECV value.

In normal subjects, gender-related associations of ECV with age have been demonstrated with females and older subjects having higher ECV's. In healthy subjects, the normal ECV for the left ventricle has reported to be between 18% and 30% at 1.5T^{155, 157, 158}. The normal ECV for the right ventricle has been reported in healthy control subjects to be 29 – 4.2% at 1.5T¹⁷¹.

Native T1 measurements

Unlike ECV measurements, native T1 measurements do not require administration of contrast agent and therefore their application is appealing particularly in CHD patients who are likely to require multiple scans from a young age. In practice, they are faster to apply than ECV measurements and reduce scan time which is a further advantage¹⁵⁹.

Native T1 mapping is a non-contrast technique that detects pathological changes in the myocardium that are associated with a relative increase in the concentration of water (oedema) or increased interstitial space caused by protein deposition (from increased collagen deposition) or other proteins such as amyloid.

Higher native T1 values in the myocardium reflect longer T1 relaxation time secondary to increased water/protein content in the ECM. The increase in native T1 is not specific to diffuse fibrosis because it occurs also (more strongly) in other disease processes such as myocarditis and infarction¹⁶⁰. Similar to ECV, healthy females have a higher LV native T1 than males. The normal native T1 for the healthy left ventricle is reported to be between 900-1200ms at 1.5T^{157, 158}. In 40 healthy controls, native T1 for the healthy right ventricle was reported as 937ms ± 118ms at 1.5 T¹⁷¹.

T1 mapping predicts outcome in acquired heart disease

Diffuse LV fibrosis as defined by LV T1 mapping has been shown to be an important predictor for SCD, VT, and heart failure in hypertrophic cardiomyopathy and dilated cardiomyopathy^{139, 140, 161}. For Fabry's disease¹⁶² and cardiac amyloidosis¹⁶³, T1 mapping has played a crucial role in diagnosing these diseases.

Challenges with RV T1 mapping

Given the success of T1 mapping application to the LV in order to quantify diffuse fibrosis, there is increasing enthusiasm in also applying T1 mapping to the RV, particularly amongst the ACHD research community. However, RV T1 mapping faces several challenges. The RV is a thin walled structure (~3mm) that is highly mobile, it is in close proximity to pericardial fat, blood pool and often sternal wires. These factors predispose RV T1 mapping to partial volume effects where a single voxel may be contaminated by an unintended structure other than RV myocardium. Due to these challenges that are specific to the RV, the simple application of conventional T1 mapping sequences designed for the usually thicker and conical shaped left ventricle cannot be assumed.

T1 mapping in adult congenital heart disease

Due to the challenges with applying T1 mapping to the RV, most studies in CHD have examined diffuse fibrosis in the systemic LV¹⁶⁴ or the hypertrophied systemic ¹⁶⁵ RV. Studies have been performed in patients following atrial switch patients with transposition of the great arteries (TGA), congenitally corrected TGA¹⁶⁶, Eisenmenger syndrome and pulmonary hypertension^{167, 168}.

Left ventricle (LV) T1 mapping in the patients with rTOF

T1 mapping has been applied to the LV in rTOF patients using different sequences. In these mostly cross-sectional studies, increased native T1 and LVECV correlated with longer cardiopulmonary bypass time and aortic-cross clamp time at the time of repair in adults and children, suggesting early onset of diffuse fibrosis secondary to peri-operative ischaemia ¹⁶⁹, ¹⁷⁰. Increased LV ECV is also associated with older age, late repair, increased QRS duration, elevated BNP levels, dilated atria¹⁶⁴ and impaired exercise capacity^{164, 171}. Increased LV

native T1 was also associated with worsening NHYA class and exercise impairment. LV ECV was also shown to relate to atrial, ventricular arrhythmia and SCD¹⁶⁴. Studies so far have been limited by small sample size and low event rate, with ventricular ectopy and non-sustained VT augmenting the primary outcome¹⁷¹⁻¹⁷³.

Right ventricle (RV) T1 mapping in patient with rTOF

Despite the absence of dedicated T1 mapping sequences for the RV, studies have examined RV diffuse fibrosis using standard LV T1 mapping sequences in rTOF patients^{169, 171-174}.

Elevated RV native T1 was found to correlate with dilated RV and a greater degree of PR¹⁷¹.

In a prospective study of 44 patients an increased RVECV predicted major adverse cardiovascular events (MACE) whereas an increased LVECV did not¹⁷⁴. This raises the question as to whether RV T1 mapping has potential as a non-invasive biomarker that can monitor adverse remodelling and help plan the optimal timing for PVR in rTOF. This question is explored in this research.

Of note, in the same prospective study¹⁷⁴ patients with rTOF had higher RV ECV compared to healthy controls (which was only possible to measure in 50% of healthy subjects). The thin RV wall of 1-2 pixels in healthy control subjects makes the application of T1 mapping and the determination of a normal reference range for RV T1 even more challenging.

In this thesis, a bespoke method for performing T1 mapping in the RV is explored.

Relationship between RV and LV diffuse fibrosis in rTOF

Studies that looked at biventricular diffuse fibrosis in rTOF patients have found that T1 markers of diffuse fibrosis for the LV and RV closely correlate with each other^{171, 173}. The

phenomena of ventricular-to-ventricular interdependence is well recognised and in part secondary to sharing of common fibers, septum and pericardial space^{41, 42, 175, 176}. However, also on a cell signaling level, paradoxical movement of the septum due to an impaired or dilated RV can downregulate angiogenic factors in the LV wall which in turn can cause LV impairment¹⁷⁷.

Incremental role of diffuse and replacement fibrosis in rTOF

To date, the incremental role of diffuse and focal fibrosis in rTOF patients remains undetermined. In a study where both biventricular T1 mapping and LGE was acquired, no correlation between the extent of diffuse fibrosis and replacement fibrosis was found¹⁷¹ yet they both were associated with ventricular arrhythmia¹⁷¹. This suggests that diffuse and replacement fibrosis may be occurring due to different reasons in rTOF (e.g. secondary to abnormal hemodynamic and surgical incisions respectively) conflicting to previous reports that describe them as a continuum. There is speculation that diffuse fibrosis may be occurring in the 'gray' zone perimeter that surrounds replacement fibrosis and could lead to slowing of conduction in this area required for a macro-re-entry tachycardia¹⁷¹. The potential incremental value in measuring diffuse fibrosis with T1 mapping compared to focal fibrosis defined by LGE will be also studied in this thesis.

Increasing the sensitivity of the MOLLI sequence to myocardial fibrosis

T1 mapping sequences have variable degrees of accuracy and precision where there is often a trade-off between the two. Accuracy of T1 mapping describes of how far the T1 estimate is from the true T1 signal and is related to systematic biases, whereas precision refers to its reproducibility¹⁵².

Robson and colleagues speculated in 2013 that it is more clinically beneficial to have a MOLLI sequence that allows the extraction of a T1 signal that sensitively reflects myocardial fibrosis, rather than a technically accurate 'perfect T1' signal¹⁷⁸.

The role of magnetisation transfer (MT) in improving the sensitivity of MOLLI sequence to detect disease has been explored^{140, 152, 178-180}.

MT is an MRI property that has been demonstrated in *ex vivo* myocardial tissue^{181, 182}. It is the process whereby protons belonging to water bound to intracellular macromolecules exchanges its spin polarisation with the protons in the surrounding free pool of water following the application of a radiofrequency pulse¹⁸³. This results in a reduction in the signal in the free pool. The MT will vary according to the differing concentrations of water and large macromolecules in the intracellular and extracellular space and therefore can be exploited as an endogenous contrast used in MRI for tissue characterisation.

Magnetisation transfer (MT) is a confounding bias that reduces T1 accuracy by shortening and underestimating T1 derived by MOLLI imaging¹⁵². However, an increase in MT may improve sensitivity to diffuse fibrosis and improves precision in effect of the imaging response to diffuse fibrosis and at the expense of less accuracy^{140, 184}. At the same time, a weak increase of native T2 by increased interstitial space in diffuse fibrosis has been shown also to improve sensitivity¹⁷⁸. The MOLLI sequence settings can also be changed to increase T2 sensitivity, by increasing the time between bSSFP RF pulses; unfortunately, this increases its delicacy regarding off-resonance problems, especially if combined with the increased flip-angle required for MT sensitisation¹⁵².

An increase in MT effect can be created by increasing the flip angle¹⁵². The higher flip angle drives the magnetisation of the intracellular bound protons more strongly towards zero during each MOLLI single shot. When this occurs, there is stronger magnetisation transfer effect between free water protons and macromolecules. The increased MT effect is more pronounced on native T1 mapping post contrast T1 (where the T1 is already shortened by the contrast), making such a modified MOLLI sequence potentially slightly more sensitive to abnormalities in myocardial composition such as diffuse fibrosis. However, it must be noted that the MT effect is very weak.

This research uses a specifically optimized MOLLI sequence developed to be especially sensitised to the effects of MT. Its correlation with clinical outcomes is presented in Chapter 5. The imaging parameters used in this sequence are detailed in Chapter 2: Methods (the CMR protocol).

Specific Thesis Aims

Overall aim of thesis

To examine the role that myocardial fibrosis has on predicting VT and SCD in adult rTOF patients including its non-invasive assessment with CMR and other biomarker candidates.

Thesis aim 1

To determine whether non-invasive 3D LGE CMR can be used to predict inducible VT as a surrogate endpoint for mortality.

Thesis aim 2

To examine the prognostic value of LGE CMR and to identify the high-risk subgroup of patients for developing VT and SCD who may benefit from targeted therapy.

Thesis aim 3

To examine the role of RV T1 mapping in the non-invasive assessment of RV diffuse fibrosis and to determine its potential as a biomarker of adverse remodelling.

Thesis aim 4

To gain mechanistic insight into the fibrotic process on an ultrastructure level and its effects on adverse remodelling post pulmonary valve replacement, using histology and blood biomarkers of fibrosis.

Chapter 2: Methods

The general methodology for this research is described in this chapter. More specific methods are detailed in each of the results chapters.

Patients identification and inclusion criteria

All adult patients (≥ 16 years) with repaired rTOF that did not have contraindication to CMR were invited to participate in this research. This included patients with TOF variants of pulmonary atresia and VSD without significant major aortopulmonary collateral arteries (MAPCAs), absent pulmonary valve and Fallot-type double outlet right ventricle (DORV). Patients were identified from the ACHD department at the Royal Brompton Hospital. The majority had been referred for a clinically required CMR scan.

Specific ethics approvals were obtained prior to patient recruitment for the study of myocardial fibrosis with gadolinium contrast, the physics development of CMR sequences and for the gifting of myocardial tissue samples for histological assessment of fibrosis.

Patients gave their informed and written consent prior to recruitment.

Exclusion criteria

Patients with complex pulmonary atresia and VSD with MAPCAs or those who had undergone previous unifocalisation of MAPCAs were excluded from the study. Patients who had contraindication to MRI or gadolinium contrast administration were not recruited. This included patients who had $\text{eGFR} < 30$, severe claustrophobia, metal eye fragments, $\text{eGFR} < 30 \text{ ml/min/1.73m}^2$, and those patients who had abandoned pacing leads. At the start of this body of research in 2017, patients who had a pacemaker device implanted before 2010 had contraindication to CMR according to departmental policy (as assumed non-conditional).

However, as the research progressed, this practice changed with patients with non-conditional pacemakers and AICD's now able to have CMR scans after the clinical indication for CMR was agreed in an electrophysiology multidisciplinary team meeting for each patient¹⁸⁵. Patients with devices who required contrast administration for clinical purposes were recruited for research and additional research sequences were kept to a minimum in order to minimise radiofrequency exposure.

Patient preparation for CMR

In preparation for the scan, patients completed an MRI and gadolinium contrast safety questionnaire. A peripheral cannula was inserted and less than 1ml of blood was removed to check that they had an eGFR of $>30\text{ml/min/1.73m}^2$ at the point of care testing machine. Patients who have pacemaker devices or implantable loop recorders had their devices interrogated prior to entering the magnet to prevent loss of data. Physiologists adjusted the pacemaker settings to asynchronous pacing modes e.g. AOO or VOO. These were re-adjusted back to the patient's normal settings following their scan.

The CMR protocol

During this research, all CMR images were acquired on a 1.5T MR scanner (Avanto, Siemens Healthcare, Erlangen, Germany) with an anterior phased array 6-channel surface coil and 6-9 channels of a posterior array spine coil. Data was acquired in a single scan. The protocol took between 75-90 minutes to acquire.

MRI compatible ECG electrodes were attached to the patient with particular care taken to ensure good quality ECG capture. Retrospective ECG gating was used for all patients with sinus rhythm and if they had rarely occurring ectopic beats. Patients with frequent ectopy,

bigeminy, trigeminy or atrial fibrillation were scanned with prospective gating to improve image quality, accepting that ventricular volumes may not be accurate as a trade-off.

Patients entered the MRI bore in the supine, head-first position (or prone, feet-first position to combat mild claustrophobia) **see Figure 2.1**. They were carefully positioned so that the heart was in the magnet's centre (isocentre). This was important to reduce MRI errors that can occur when the heart is not in the centre of the magnet.



Figure 2.1: 1.5T CMR scanner (Avanto, Siemens) with 70cm diameter bore used to carry-out this research at the Royal Brompton Hospital. The patient is in the supine head-foot position and is positioned optimally so that the heart is at the isocentre (middle) of the magnet.

Pilot scouts (localisers)

First, the position of the patient's heart at the isocentre of the scanner was confirmed using free breathing multi-slice single shot acquisitions taken in the 3 different orthogonal planes: coronal, sagittal and transaxial. The patient's position in the scanner was optimised at this stage if required. The necessary coils were selected.

Multi-slice imaging

For anatomical delineation, 'black-blood' multi-slice imaging in transverse, sagittal and coronal planes were acquired using half-Fourier acquisition single-shot turbo spin-echo (HASTE) sequence during free breathing. These planes were also repeated with 'white-blood' multi-slice images in the same planes using fast-imaging steady-state free-precession sequence (TruFISP) but with breath held in expiration (**see Figure 2.2**).

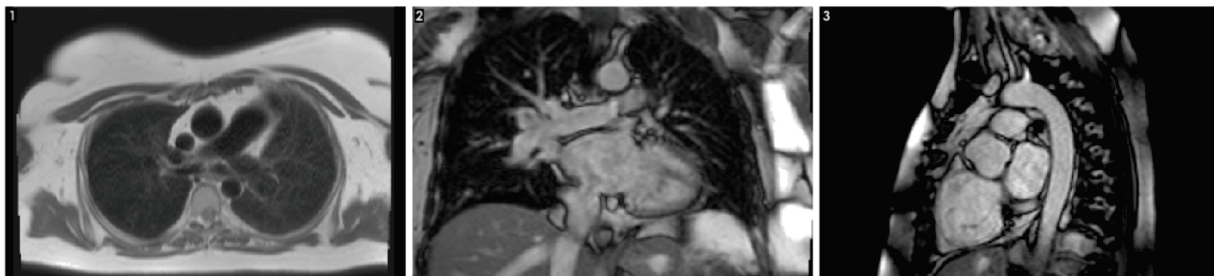


Figure 2.2: Example of a transaxial half-Fourier acquisition single-shot turbo spin-echo (HASTE) multislice (1) and TruFISP multi-slices in the coronal (2) and sagittal plane (3).

Imaging parameters: Typical imaging parameters for the HASTE multi-slice acquisitions involved 35 contiguous slices with no gap from just above the aortic arch to include the bottom of the heart. The resolution was 1.4mm x 1.4mm x 6mm slice thickness. The field of view (FoV) read 360 mm x field of phase (FoV) phase 80% optimised to avoid wrap around artefact. There was continuous retrospective ECG gating. The time between radiofrequency pulses (TR) was 454ms, TE(1.17ms), with 888Hz/pixel bandwidth. The Flip angle was 79°.

TruFisp. Multi-slice images were acquired in the same planes as the HASTE images with retrospective ECG gating , 25 contiguous slices taken over 2 breath-holds, resolution 1.6mm x 1.6mm x 6mm slice thickness, with FoV read and phase optimised to minimise wrap around artefact and breath-hold time, TR 454ms, TE 1.17ms, Flip angle 79°.

Cine imaging

Long axis and short axis cines were acquired using breath hold Steady State Free Precession (SSFP) sequence. Initially a ventricular long axis (VLA) and a basal short axis scout are taken and are used to plan the LVOT view which is cross-cut. The VLA, basal short axis and LVOT views were then used to plan the four-chamber view.

For the right heart, the RVOT sagittal view was planned using the multi-slice transaxial and coronal images and is cross-cut. An RV in-and-out view and an RV oblique view that is similar to the subcostal para-oblique 'Fallot view' on paediatric echo images, are obtained by reviewing the RVOT, LVOT and four-chamber images. An RV long axis view is acquired (two-chamber view of the RV, tricuspid valve and the right atrium). Right and left pulmonary artery cines are acquired in the coronal plane and are cross-cut further to produce a cine of the pulmonary artery bifurcation.

A short axis stack is planned from the VLA and four-chamber view in the final end-diastolic frame. Care is taken to ensure that the second slice of the stack is aligned parallel to atrioventricular groove and that the stack includes enough slices to enable complete biventricular coverage from base to apex. Slices were acquired every 10mm (7mm slice thickness with 3mm gap). **See Figure 2.3.**

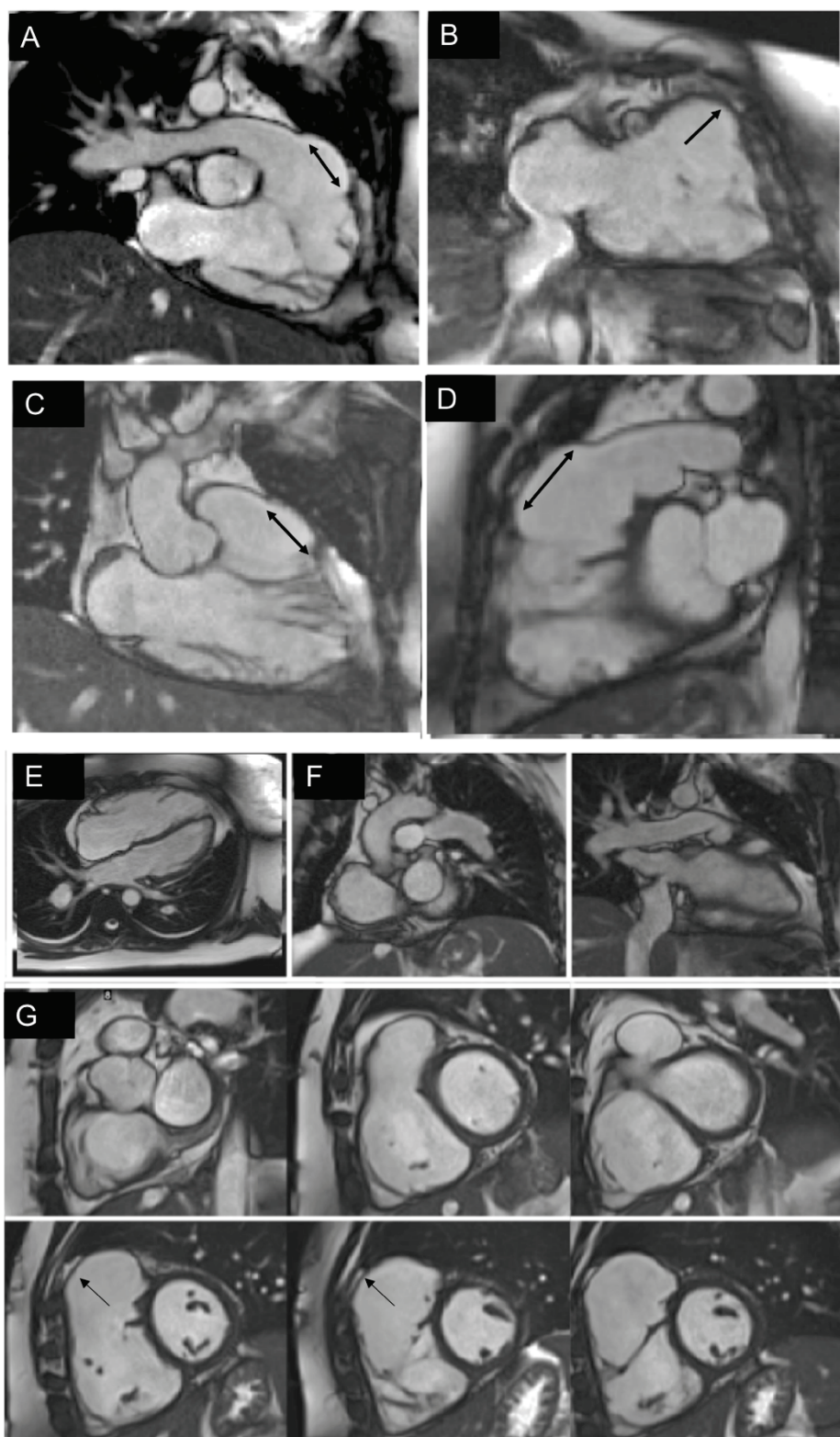


Figure 2.3: Cine images acquired with a bSSFP sequence. RV Long axis images shown **A-E**. RV in and out view (**A**) RV ventricular long axis (**B**), RV oblique view (**C**), Sagittal RVOT (**D**), four-chamber view (**E**), coronal branch pulmonary artery views (**F**), Short axis stack (**G**). There is a

thin walled, akinetic and aneurysmal region in the RVOT seen in long and basal short axis images (black arrows). The RV is dilated in all views.

Imaging parameters: Typical bSSFP cine images were acquired with breath-held in expiration and retrospective ECG triggering. The resolution was 1.9 mmx 1.9mmx 7mm slice thickness, FoV read 360 mm x FoV phase 69%, TR 40 ms, TE 1.13ms, 15 raw data lines for each frame within the cardiac cycle. Each cardiac cycle was reconstructed to include 25 frames.

Flow imaging

In-plane flow cines of the RVOT in the sagittal plane were acquired using a breath-hold velocity map. This helped to provide qualitative assessment of the degree of pulmonary stenosis or regurgitation present. Through plane velocity maps were then acquired at the level of the sinuses of the pulmonary artery to enable a pulmonary regurgitant fraction to be derived (**see Figure 2.4**). A through plane velocity map at the pulmonary sub-valvar level was also performed to assist with qualitative estimation of pulmonary regurgitation.

For calculation of cardiac output and aortic regurgitant fraction, a through-plane velocity map was acquired at the level of the aortic sino-tubular junction. In-plane and through-plane velocity maps were performed for the branch pulmonary arteries to determine peak velocity and differential blood flow to the right and left lung only if stenosis was noted on cine images. Care was taken to ensure that an appropriate velocity encoding range, VENC (upper threshold of velocity beyond which aliasing occurs) was set to avoid artefact and

inaccuracies in measurements. Patients often required multiple flow acquisitions to titrate the VENC accordingly to avoid excessive velocity aliasing.

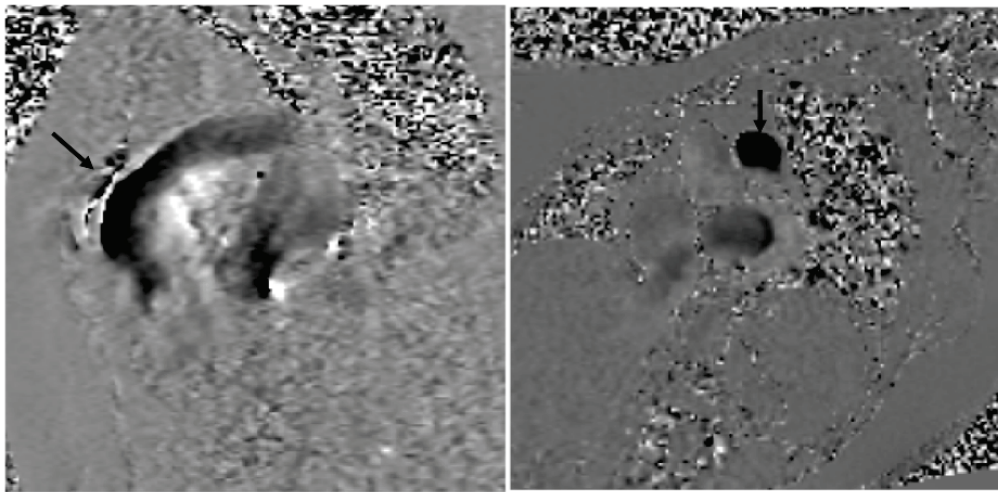


Figure 2.4: Velocity mapping images acquired in-plane (left) and through-plane (right) showing severe pulmonary regurgitation as the black jet in diastole.

Imaging parameters: Velocity mapping sequences were acquired with breath-held in expiration and with continuous retrospective ECG triggering. The typical resolution for a through plane flow acquisitions was 0.8mm x 0.8 mm x 8mm slice thickness and 0.7 mmx 0.7mmx 6mm slice thickness for in plane velocity mapping. The FoV read and FoV phase were adapted accordingly to avoid wraparound artefact and to minimise breath-hold duration. Care was taken not to use phase oversampling. 5 raw data lines were acquired per frame within the cardiac cycle. Each cardiac cycle was made up of 20 frames. The TR was 56ms, TE 3.24ms, flip angle was 25°, bandwidth 391Hz per pixel.

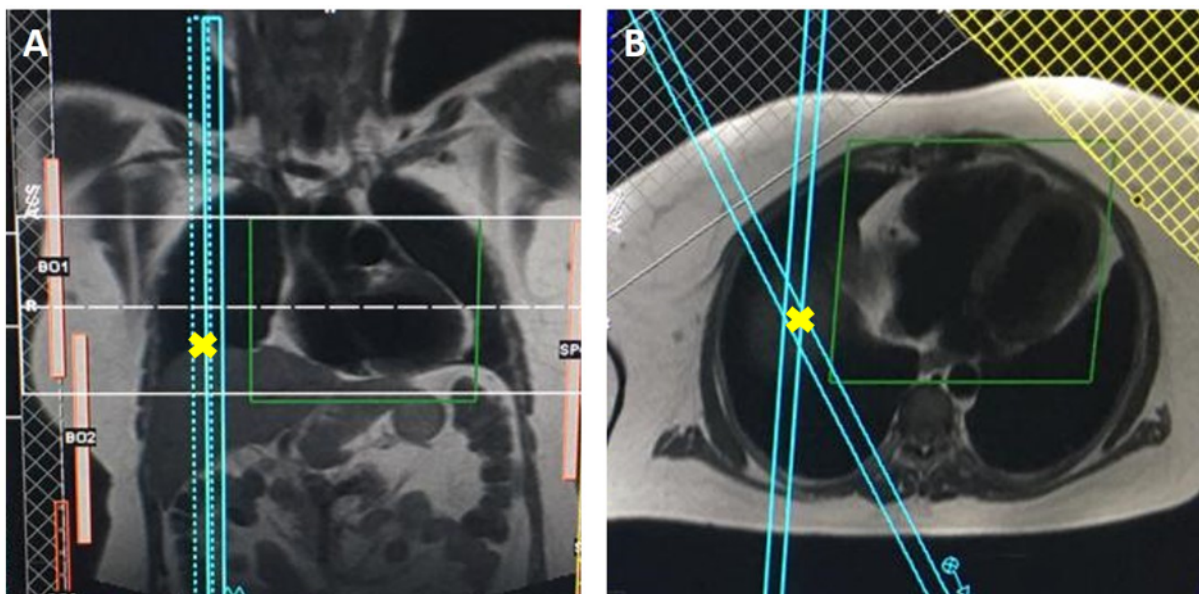
Three-dimensional (3D) balance Steady State Free Precession (bSSFP)

A free breathing respiratory-navigated and ECG-triggered 3D balanced steady state free precession (bSSFP) 'roadmap' of the heart was acquired in the coronal plane. This enabled high-resolution contiguous slice coverage of the whole heart (reconstructed to resolution

0.7x0.7x2mm). Images were acquired in the diastolic still time measured manually from typically a four-chamber cine image to minimise blurring artefact from cardiac motion. This sequence used a continuously adaptive windowing strategy (CLAWS) to produce the fastest possible acquisition regardless of the respiratory pattern¹²⁶.

Imaging parameters: The 3D bSSFP was acquired with resolution 0.9mm x 0.9mm x 1.8mm slice thickness. Typically, 144 slices per slab to cover the chest were used but this was adapted according to the size of the patient. The FoV read was 400mm, FoV phase 100%, TR 288ms, TE 0.9ms, flip angle 70°, bandwidth 970Hz per pixel.

Figure 2.5:



The three-dimensional (3D) balance Steady State Free Precession (bSSFP) with continuously adaptive windowing strategy (CLAWS) were planned from the coronal (A) and transaxial images (B). The respiratory navigator (Blue lines) was placed at the dome of the right hemidiaphragm (except in a patient with dextrocardia, dextroposition, right sided pleural effusion or diaphragmatic palsy). A shim box was applied to improve magnetic field homogeneity (green box) and saturation bands (yellow and white grids) were applied to reduce artefact from soft tissue. The navigator and shim box position from the 3D bSSFP were then copied to produce 3D LGE images acquired in the transaxial plane.

Early gadolinium enhancement

After completion of the anatomical and functional assessment aspects of the CMR protocol as detailed above, gadolinium contrast agent (Gadovist, Bayer Schering, Berlin, Germany) was injected at dose 0.12mmol/kg followed by a 10ml flush of 0.9% normal saline. A slightly larger dose of Gadovist contrast was used compared to the standard dose of 0.1mmol/Kg, usually required for clinical CMR protocols that do not involve 3D LGE and T1 mapping research sequences. The extra 0.02mmol/kg of contrast agent administered was used to ensure that it would last for the length of the scan (75-90mins) without being prematurely washed out.

Early gadolinium enhancement images of the RV and LV long axis were taken within the first 3 minutes following contrast administration, using a breath-hold inversion ECG triggered inversion recovery turbo flash sequence with a Ti of 440ms¹⁸⁶

Two-dimensional (2D) late gadolinium enhancement (LGE)

Ten minutes following administration of gadolinium, 2D LGE images of the LV, RV long axis and short axis stack were taken. This was performed using a breath-hold, ECG-triggered, segmented fast, low-angle shot inversion-recovery sequence. Phase sensitive inversion recovery (PSIR)¹⁸⁷ was used in the image reconstruction and had the advantage of being less sensitive to the choice of Ti.

At the start of acquisition of 2D LGE images, a Ti of 260ms was often selected. As the scan progressed, the Ti was manually increased to keep up with the gadolinium washout. The Ti was chosen by meticulous visual inspection of the myocardium to ensure that it was optimally nulled⁴⁷. Optimum nulling was defined as sharp contrast definition between

healthy myocardium (jet black) and scarred areas (white) and blood pool (grey). Subtle areas of LGE were confirmed by cross-cuts and phase-swaps of phase-encoding directions (head-foot and right-left). These were used together with cine imaging to look for corresponding regional wall motion abnormalities thereby further adding confidence to the LGE findings.

Imaging parameters: The 2D LGE PSIR sequence was performed with resolution 0.7mm x 0.7mm x 8mm slice thickness. The FoV read and FoV phase were adjusted to the patient. An inversion recovery radiofrequency pulse was delivered on every alternative heartbeat (trigger 2). For tachycardic patients, an inversion recovery pulse was delivered on every 3rd heartbeat (trigger 3) and on every single heartbeat for bradycardic patients (trigger 1). 23 raw data lines were acquired per cardiac cycle, TR 700ms, TE 3.34ms, flip angle 25°.

Three-dimensional (3D) late gadolinium enhancement

Approximately 20 minutes after administration of contrast, Ti scouting was undertaken to identify the optimum inversion time for complete nulling of the myocardium. This optimum Ti time was then used for 3D LGE image acquisition.

Free breathing, respiratory-navigated and ECG-triggered 3D LGE images were acquired in the trans-axial plane to cover the entire heart (**see Figure 2.6**). This sequence also used a CLAWS algorithm to improve respiratory efficiency^{126, 127}.



Figure 2.6: 3D LGE images of a patient with rTOF reconstructed in to 3 orthogonal planes, coronal, sagittal and transaxial from left to right. LGE is seen in the RVOT patch site (white arrows) and VSD patch site (red asterisks).

Image reconstruction: The 3D LGE sequence typically acquired 64 slices at $1.5 \times 1.5 \times 4 \text{ mm}^3$

voxel resolution which were post processed to $0.7 \times 0.7 \times 2 \text{ mm}^3$ voxel resolution. The FoV read was 380mm and FoV phase 100%, TR 450ms, TE 1.49ms, flip angle 20° , 37 raw data lines were collected per cardiac cycle. The trigger delay (time required for the inversion recovery pulse to be delivered) was adjusted so that the image acquisition occurred in the diastolic still time in order to minimise artefact from cardiac motion.

Native T1 mapping

For this research, specific physics development of a T1 mapping modified Look-locker inversion recovery (MOLLI) sequence tailored for the RV was undertaken with aim to enable T1 estimation at a higher spatial resolution and with greater sensitivity to diffuse fibrosis. The theory and background supporting this sequence is discussed in Chapter 1: introduction (increasing the sensitivity of the MOLLI sequence to myocardial fibrosis).

Obtaining haematocrit

At the time of peripheral venous cannulation, patients were consented to have 2ml of blood withdrawn and tested in the laboratory for a haematocrit level. This haematocrit was used for the calculation of ECV by the equation described in Chapter 1: T1 mapping, extracellular volume measurements.

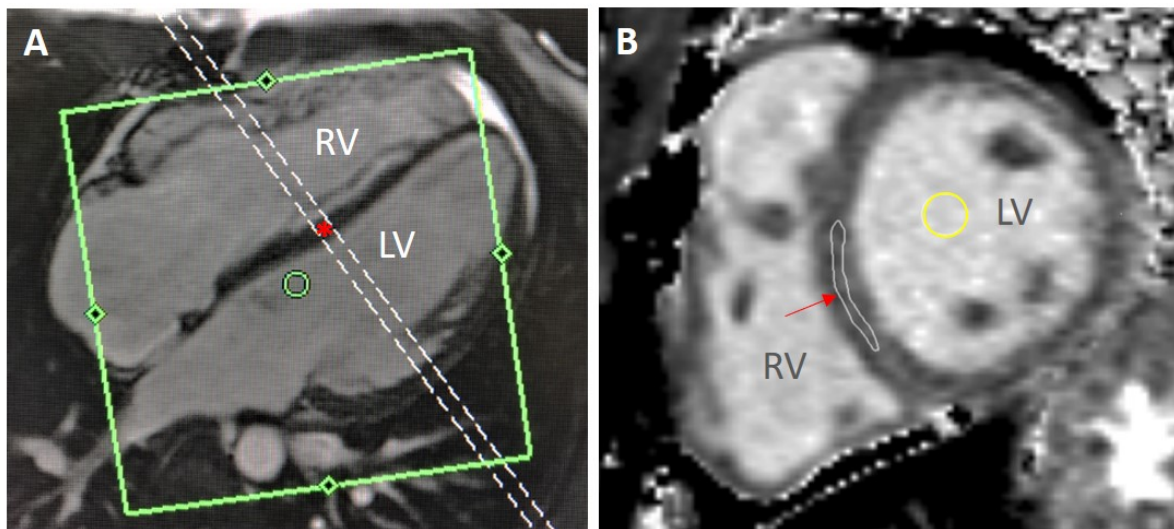
Standard 11 heartbeat MOLLI sequence

T1 mapping was performed with breath-held in expiration, ECG-triggered, MOLLI sequence over 11 heart beats [5(3)3]^{150, 153, 188}. T1 mapping was acquired in the diastolic still time calculated to avoid artefact from cardiac motion. The trigger delay (time between the initiation of the 180° pulse and the first ECG R wave) was calculated using the following formula: mid-diastolic still time minus 210ms.

Acquisition of LV T1 maps

For the LV, T1 maps were acquired at the mid-ventricular level in mid-diastole with care to ensure that the slice was directly perpendicular to the interventricular septum to minimise partial volume effect artefact. The shim box was adjusted to ensure coverage of the LV to remove inhomogeneity in the magnetic field that can affect the T1. This image was repeated to improve accuracy.

Figure 2.7:



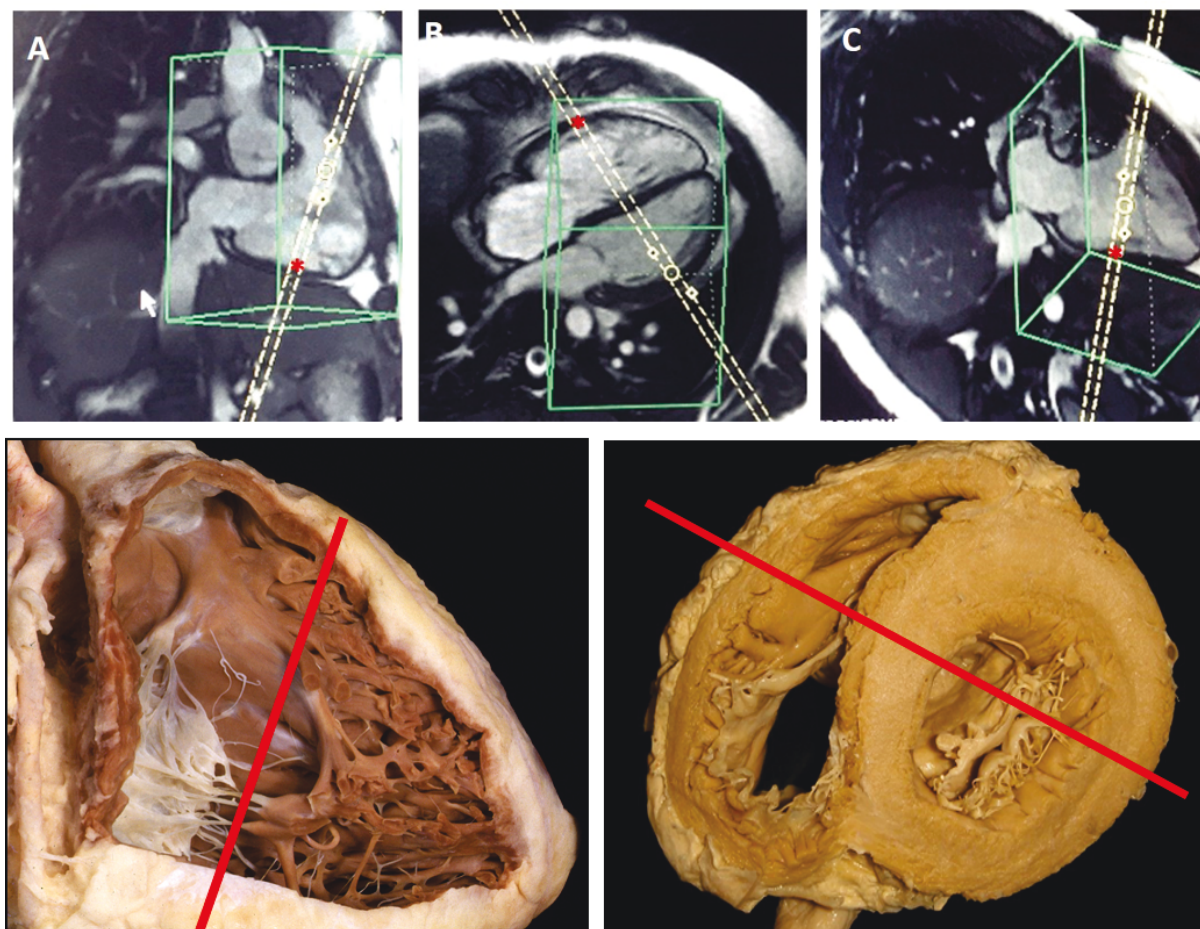
Planning of the LV T1 map is shown (A). Care is taken to ensure that the mid-ventricular slice is acquired perpendicular to the septum and that the shim box (green) covers this area. Images are acquired in mid-diastole. The resulting LV T1 map produced is shown (B). A region of interest (ROI) is drawn (red arrow) in the inner third of the septum to avoid contamination with blood pool. An ROI is drawn in the LV blood pool away from papillary muscles and is used to calculate LV extracellular volume (ECV).

Acquisition of RV T1 maps

For the RV, a short axis slice was acquired in mid-diastole with care taken to ensure that it was taken perpendicular to the thickest part of the RV. Typically, the thickest part of the RV was the inferior wall or the anterior wall, as long as distant from sternal wire artefact. This was also repeated to improve accuracy.

Imaging parameters: The image resolution of the 11 heartbeat MOLLI sequence used was 1.4 x 1.4 x 6mm slice thickness. The sequence involved breath-held in expiration with retrospective ECG triggering. The FoV read was 360mm x FoV phase 85%, TR 280ms, TE 1.13ms, flip angle 35° and bandwidth 1085Hz per pixel.

Figure 2.8:

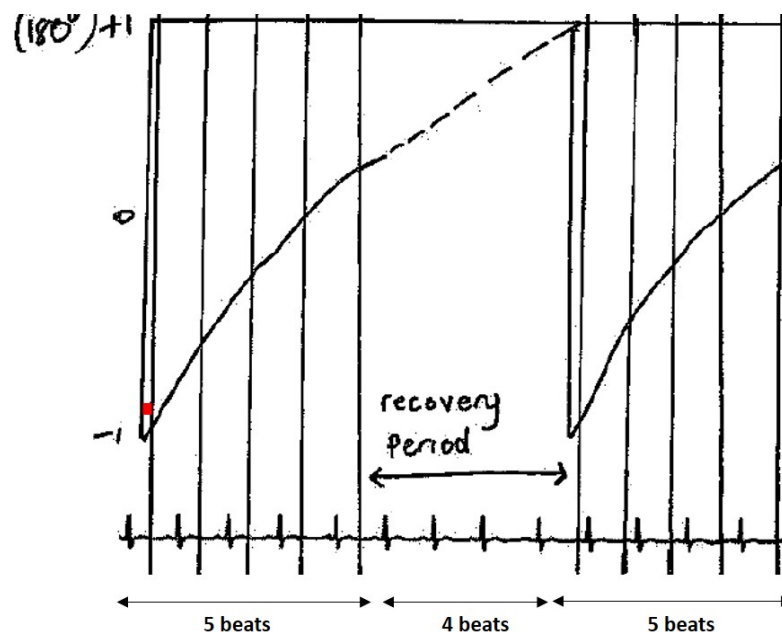


Planning of RV T1 maps. The RV oblique (A), four chamber (B) and two chamber views (C) are used to ensure that an RV slice is acquired perpendicular to the thickest part of the RV, typically the RV inferior wall (red asterisk) in mid-diastole. Due to the unique geometry of the RV, it was not always possible for the slice to be perpendicular to the RV inferior wall in all 3 planes despite best efforts. In most cases, it was also not possible to acquire a single T1 map on which both LV and RV T1 can be analysed. This is because it is challenging to align both perpendicular to the septum and to the RV inferior wall in a single slice, as illustrated on ex-vivo heart slices in the RV oblique and short-axis view (lower panel). Therefore, dedicated T1 maps were acquired for the RV and LV separately. Images courtesy of the Royal Brompton Hospital Morphology department.

The modified 14 heartbeat MOLLI native T1 sequence

A second MOLLI sequence [14 heartbeat, 5(4)5] was applied. This sequence was developed prior to the start of this research with the aim of improving sensitivity to diffuse fibrosis and also at higher spatial resolution to make it particularly suited to the thin RV wall¹⁴⁰. In comparison to the standard MOLLI 5(3)3, it utilises a higher flip angle of 55° to boost the magnetisation transfer effects^{140, 152} with the aim of improving sensitivity to the presence of myocardial fibrosis. It also uses a thinner acquisition slice of 6mm to improve spatial resolution.

Figure 2.9:



Modified 14 heartbeat MOLLI protocol: Five 180° inversion recovery pulses are applied 100 milliseconds before the start of the diastolic still time to acquire 5 images. The inversion recovery pulse recovers towards its original state over 4 heartbeats. This is followed by 5 other inversions.

Imaging parameters: The image resolution of the modified 14 heartbeat MOLLI sequence was $1.4 \times 1.4 \times 6$ mm slice thickness. The sequence involved breath-held in expiration with retrospective ECG triggering. The FoV read was 360mm, TR 300ms and TE 1.15ms. The main

difference between the 11 heartbeat and 14 heartbeat MOLLI protocol was a change in the flip angle from 35° to 55° .

Post contrast T1 mapping

Fourteen-minutes after the administration of contrast, T1 maps of the same slice were repeated using a standard post-contrast 11 heart beat MOLLI [4(1)3(1)2]¹⁸⁸. The trigger delay was adjusted for T1 shortening effects of gadolinium and was calculated as follows: mid-diastolic still time minus 290ms. Care was again taken to ensure that images were acquired in mid-diastole and that they were directly perpendicular to the LV septum and RV inferior or anterior wall.

Analysis

Volumes and functional analysis

Volumes and functional analysis were carried out using a semi-automated software CMRtools (Cardiovascular Imaging Solutions, London, UK). Firstly, the end-diastolic and end-systolic frames where the cavities were smallest were identified visually. For the RV and LV these sometimes differed. The epicardial and endocardial contours were manually delineated on all frames throughout the cardiac cycle during end-diastole. This was repeated in systole. The position of the mitral, tricuspid, aortic and pulmonary valve annulus were tracked in systole and diastole. Finally, volumetric models of the LV and RV were generated.

The papillary muscle and interventricular septum were included as part of the LV mass by semi-automated signal intensity thresholding. RV trabeculations and the moderator band were included within the RV mass as opposed to RV blood pool using the same approach. End-diastolic volume (EDV) and end-systolic volumes (ESV) were calculated as a sum of contoured cavity volumes from each short axis slice from base to apex. LV and RV ejection fraction were calculated using the following method:

$$\text{LV ejection fraction (\%)} = \frac{\text{LVEDV} - \text{LVESV}}{\text{LVEDV}} \times 100$$

$$\text{RV ejection fraction (\%)} = \frac{\text{RVEDV} - \text{RVESV}}{\text{RVEDV}} \times 100$$

LV and RV mass were calculated by the sum of the epicardial volume minus the endocardial volume on all short axis slices from base to apex.

Volumes and mass were indexed to body surface area (BSA m²) calculated by the Mosteller formula:

$$\text{BSA (m}^2\text{)} = \sqrt{\frac{\text{Height (cm)} \times \text{Weight (Kg)}}{3600}}$$

Atrial area measurements

Maximum right and left atrial area were measured by manually contouring each atrium on the four-chamber cine in atrial end-diastole (the frame immediately before the opening of the atrioventricular valves). The atrial appendages were excluded from the analysis. Atrial area measurements were indexed to BSA. A right atrial area indexed to BSA $\geq 15\text{cm}^2$ and an indexed left atrial area $\geq 14\text{cm}^2$ were considered as dilated.

RVOT akinetic length measurements

The akinetic region of the RVOT was identified on cine imaging as the non-contractile, thin walled and sometimes aneurysmal region in the RVOT. The maximum akinetic length of the RVOT was measured longitudinally on the sagittal RVOT cine, RV in-and-out and the RV oblique view. The largest measurement out of all three cine images was recorded.

LGE analysis

Two-dimensional LGE images were scored using a semi-quantitative segmental scoring system. The LV LGE was scored using the 17-segment AHA model where each segment of the LV carried 5 points¹³⁴. For the RV, a segmental scoring system published in 2006 was used. This divided the RV into 7 segments⁴⁷. LGE was scored if present in 2 or more views and confirmed on cross-cuts or phase swaps if subtle. The extent of LGE in the RVOT was measured as the maximum length of enhancement in the sagittal RVOT, RV in-and-out and RV oblique view. The extent of enhancement in the RV septum was measured on the LVOT, RV oblique view, and basal short axis views. Scores were allocated accordingly (**see Figure 4.1**).

It was common that patients with rTOF had a small focus of LGE in the LV apex from apical vent placement at time of surgery. RV and LV septal insertion point enhancement was also very common and is known to be also present in healthy hearts with no significance. LV apical vent LGE and insertion point LGE were therefore excluded from the analysis.

2D LGE reproducibility

Inter-observer reproducibility was tested by scoring of 20 patients by 2 experienced operators, blinded to clinical outcome. Intra-observer reproducibility was tested by re-scoring 10 scans blinded to the results of the first and patient outcome.

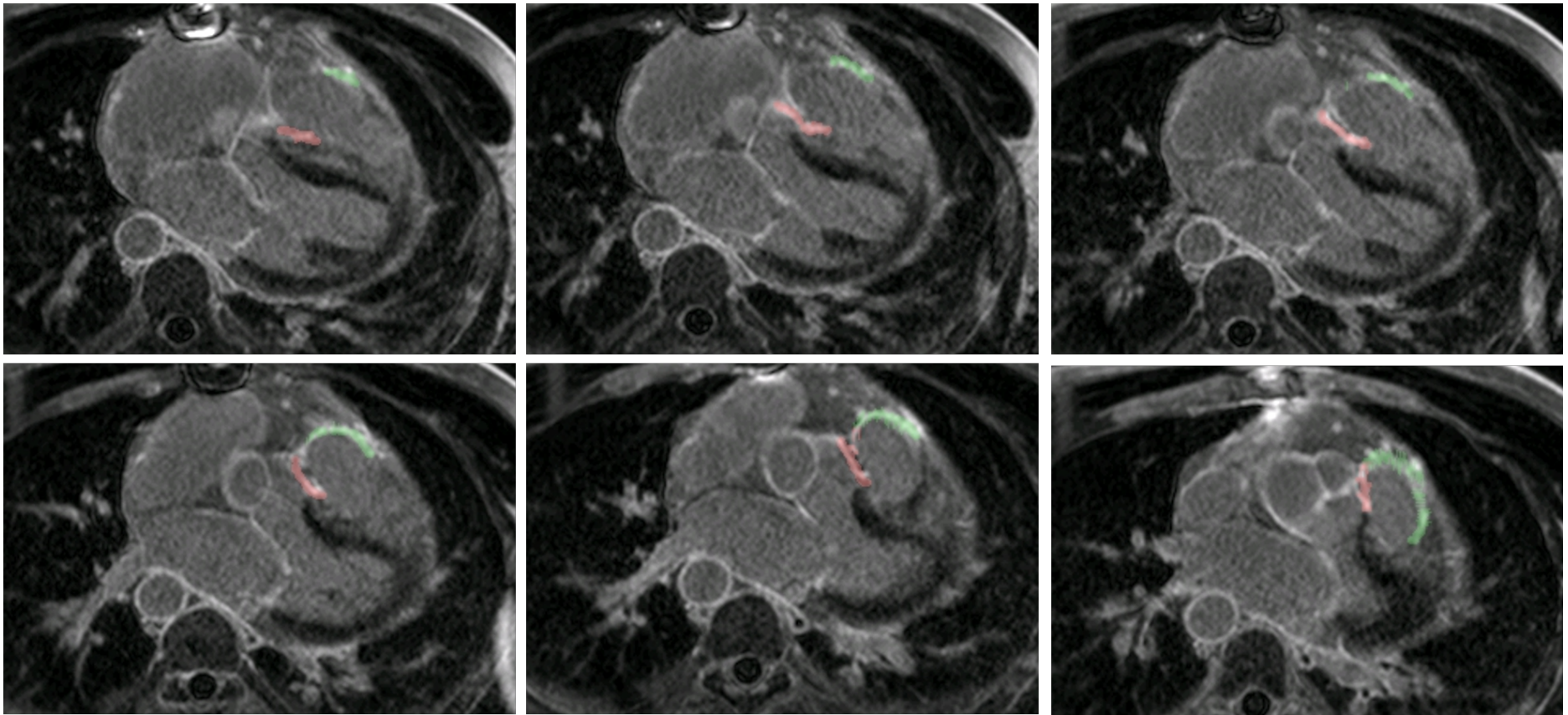
3D LGE volumetric analysis

The volume of LGE in the LV and RV was measured manually using CMR segmentation software Materialise (Mimics, Materialise, Le). Each pixel of LGE was hand selected on each contiguous slice of the ventricle in all 3 orthogonal planes: sagittal, transaxial and coronal from which a volume of LGE (cm³) was rendered by the software.

3D LGE segmentation reproducibility

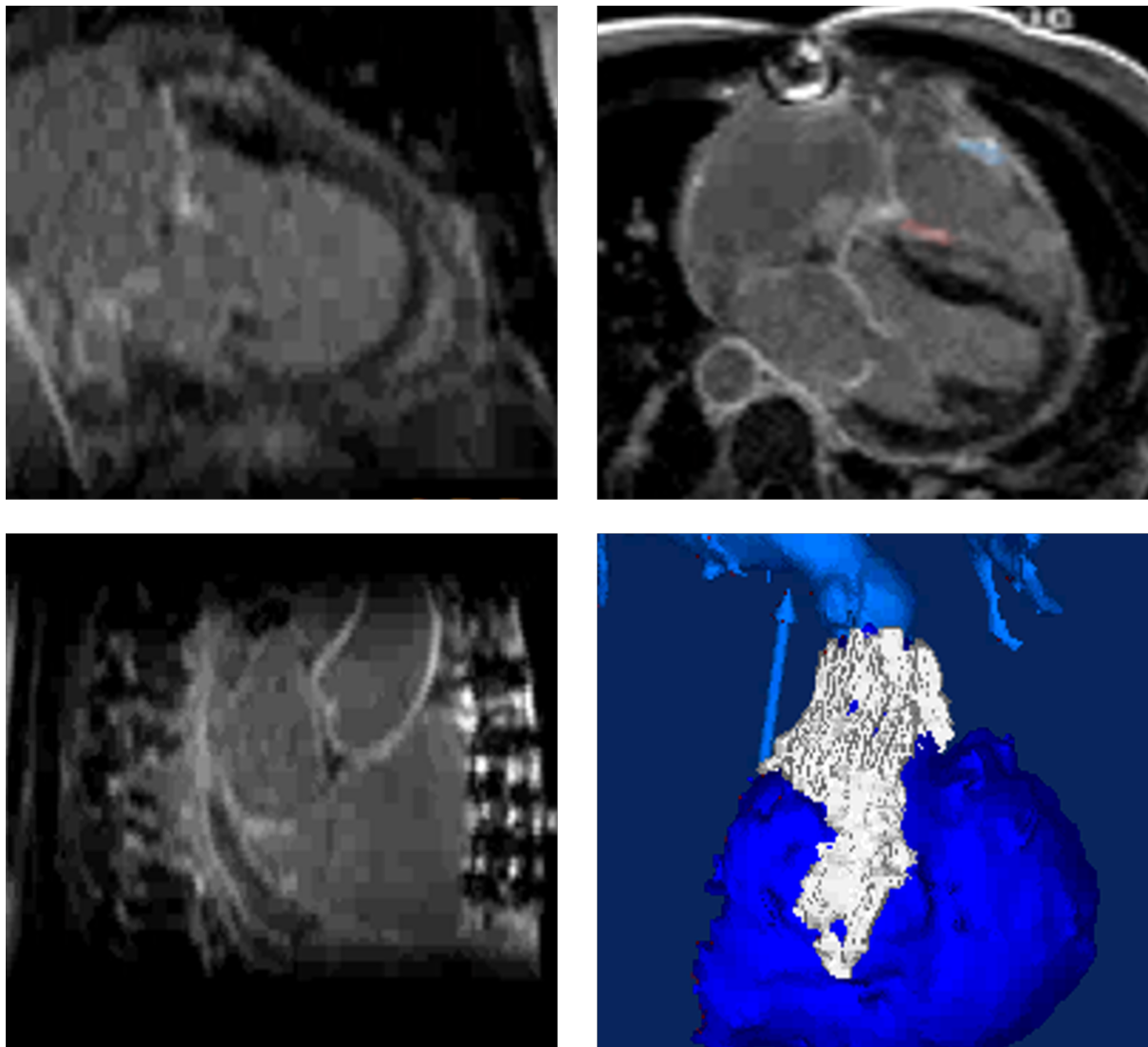
Inter-observer reproducibility was tested by 2 experienced operators segmenting 10 3D LGE acquisitions blinded to the outcome and each other. Intra-observer reproducibility was measured by a single operator re-segmenting the same scan in 10 cases, blinded to the previous segmentation result and patient outcome.

Figure 2.10:



An example of to illustrate how the LGE was segmented from 3D LGE CMR images using software Materialise (Mimics, Materialise, Le). Each pixel of LGE in the wall of the right ventricle (green), right ventricular outflow tract (green) and VSD patch site (red) were hand selected on all contiguous slices in the transaxial view.

Figure 2.11:



A three-dimensional (3D) model of LGE was rendered after the careful hand selection of each LGE pixel in the right ventricle wall, right ventricular outflow tract, VSD patch site including any LGE in areas remote to previous surgery and in the left ventricle. Volumetric analysis of the segmented LGE in each portion of the heart was obtained. The coronal and sagittal planes (left pane) were used to guide the segmentation, in order to ensure that the pulmonary valve annulus (which appears very similar to RVOT LGE in terms of signal intensity) was not included in the RVLGE segmentation.

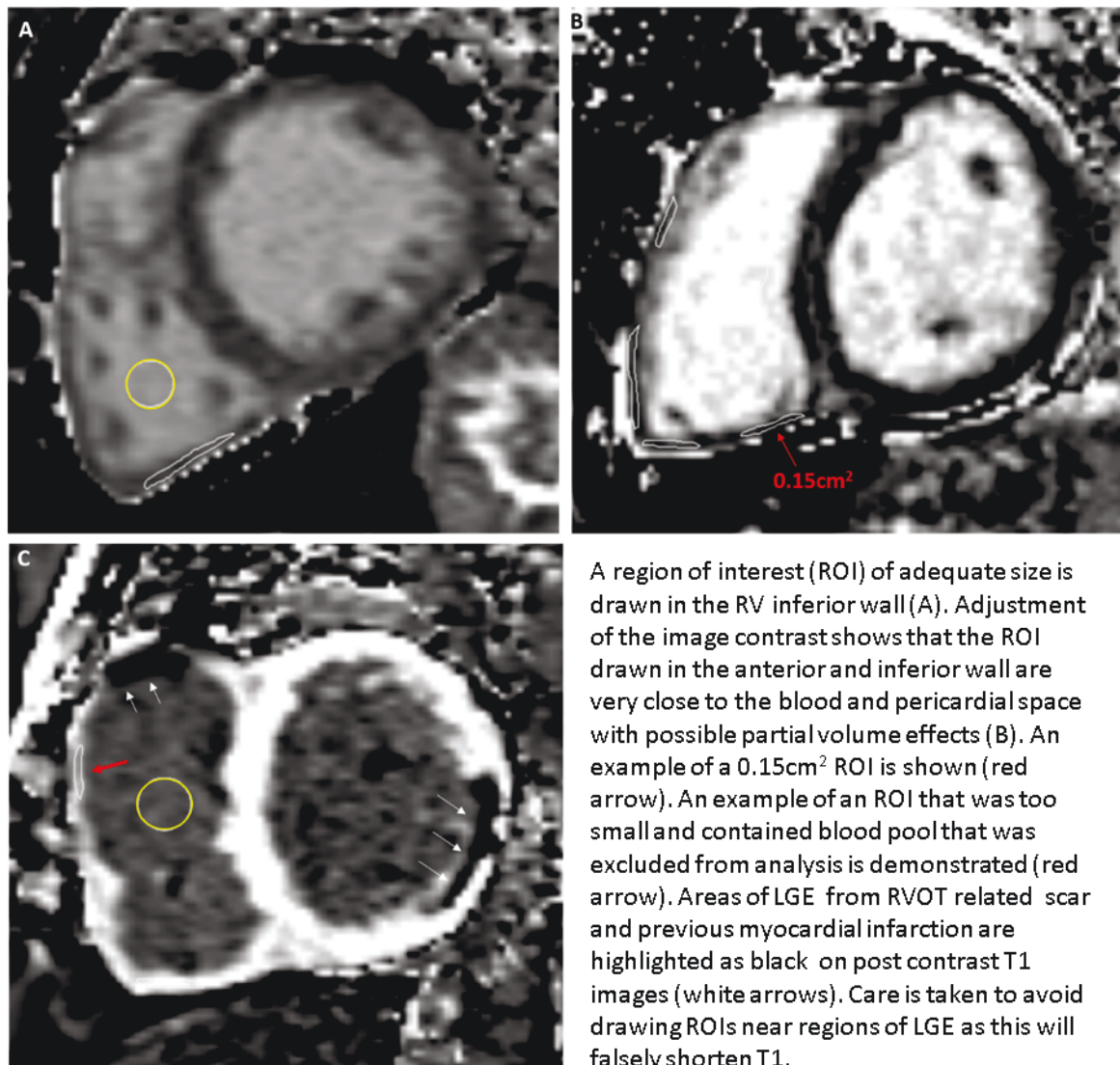
T1 mapping analysis

Native T1 analysis

Analysis was performed using software CMRtools (Cardiovascular Imaging Solutions, London, UK). For measurement of native T1 in the LV, a region of interest (ROI) was drawn on the T1 map in the middle third of the ventricular septum. Care was taken to avoid trabeculations and blood pool which can cause partial volume contamination of the results and a falsely elevated T1 reading. The mean T1 signal within the ROI was derived from the software.

Native T1 was measured in the RV by manually drawing an ROI typically, the RV inferior wall (often the thickest part of the RV). Care was taken again to avoid the blood pool, trabeculations, liver and pericardium. Due to the thin wall of the RV that was at times no thicker than 1 pixel, it was not always possible to draw large enough ROIs. In cases where it was not possible to draw an ROI $\geq 0.15\text{cm}^2$, T1 data was excluded from analysis due to the likelihood of contamination from surrounding structures. Examples of RV T1 mapping analysis and pitfalls secondary to partial volume effects are shown in Figures 2.10 and 2.11 respectively.

Figure 2.12



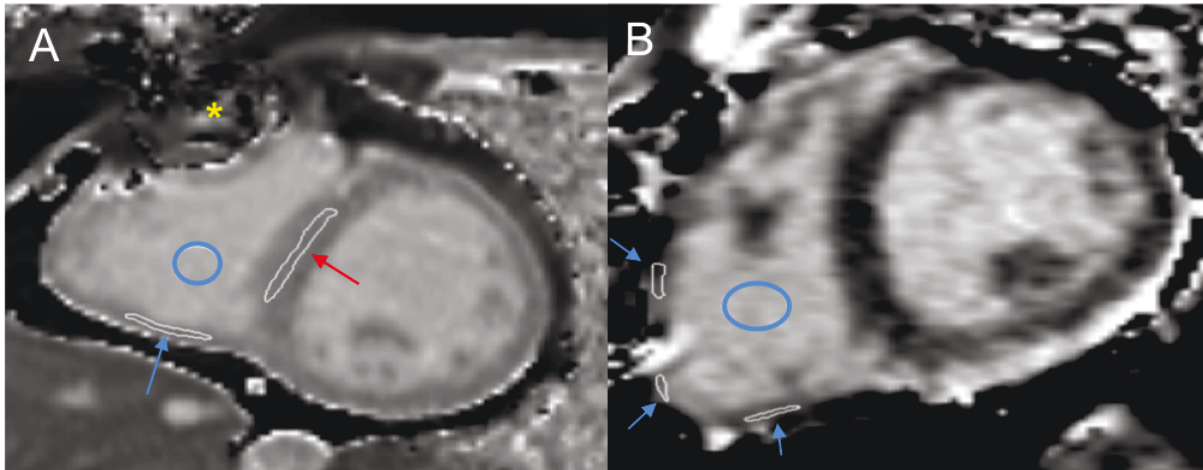


Figure 2.13: Examples of Native T1 maps of a mid-ventricular slice. **A)** For the left ventricle, the mean native T1 signal is measured within the region of interest (ROI) drawn in the LV septum (red arrow) and in the RV inferior wall (blue arrow). Visualisation of the RV anterior wall is significantly impaired by the sternal wire artefact (yellow asterisk) degrading the image quality. **B)** In this dedicated RV mid-ventricular slice acquired perpendicular to the RV inferior wall, it was only possible to draw very small ROIs $< 0.15\text{cm}^2$ (blue arrows) in the RV wall due to proximity of adjacent sternal fat and blood pool. This is an example of T1 map that was excluded from analysis.

Extra cellular volume

For the calculation of ECV (detailed in chapter 1; T1 mapping), T1 was also measured on the post-contrast T1 maps for the LV and RV using the same analysis technique detailed above.

The T1 of the LV and RV blood pool was measured by manually drawing a ROI in the centre of the blood pool of the respective chamber with care taken to avoid trabeculations and papillary muscles. ECV was calculated using the formula described in chapter 1.

Native T1 reproducibility

Inter-observer reproducibility was tested in 10 cases by the measurement of native T1 by 2 experienced operators blinded to outcomes and each other. Intra-observer reproducibility was tested by a single operator repeating the same analysis in 10 patients blinded to the outcome and previous results.

Endomyocardial biopsy and histological analysis

Ethical approval was granted for the collection of wasted myocardial tissue during PVR surgery (such as resected tissue from the RVOT) and for endomyocardial biopsies taken from the body of the RV, to assess for fibrosis. Patients gave their informed and written consent.

When the surgeon judged it to be technically feasible and appropriate to perform, endomyocardial biopsies were resected by scalpel from the body of the RV under direct vision. Biopsies taken under direct vision are considered to be safe to perform¹⁸⁹. The sampled tissues collected were fixed in 10 % neutral buffered formalin and then dehydrated with increasing concentration of ethanol and embedded in paraffin. Blocks of the paraffin were then cut in to 7µm thick slices using a rotary microtome and mounted on to glass slides for staining. Morphological assessment of the histology slides was performed using haematoxylin and eosin (HE) staining¹⁹⁰. Picrosirius red and Masson's trichrome stain was used to stain collagen type I and III^{191, 192}. The slides were analysed using a conventional light microscopy that was linked to an image analyser (Quantimet 500, Leica, UK). Ten fields were randomly selected from the specimen and were magnified x 400. A collagen volume fraction was derived (the proportion of area affected with fibrosis compared to the total field of study)¹⁹³.

Other non-invasive investigations

Data was acquired as part of the patients' routine clinical care. This included height and weight measurements used to calculate body mass index and body surface area. A 12-lead ECG was performed to assess rhythm, rate and measure QRS duration.

Blood samples:

Venous blood samples were sent for BNP level measurements by the clinical biochemistry laboratory. In the sub-study of patients who underwent histological examination of fibrosis at time of PVR, circulating levels of matrix metalloproteinase 2 (MMP-1), tissue inhibitor metalloproteinase 1 (TIMP-1) and carboxy-terminal pro-peptide of type I procollagen in the serum were also measured.

Echocardiogram:

Two-dimensional transthoracic echocardiography was performed according to standard protocol prescribed by the British society of echocardiography. This included cavity dimensions, M-mode, colour, Doppler measurements, diastolic indices, LV ejection fraction using Simpson's biplane and RV fractional area change. RV restrictive physiology was determined by an 'a' wave on pulmonary artery Doppler in end-diastole throughout the respiratory cycle.

Cardiopulmonary exercise testing:

As part of routine clinical follow-up, exercise testing was performed using the modified Bruce protocol. Patients exercised either on a cycle ergometer or treadmill as able. Patients were connected to continuous ECG tracing, blood pressure monitoring and pulse oximetry.

Patients breathed through a mouthpiece that sampled expired concentrations of oxygen and carbon dioxide every 5 seconds whilst exercising.

Patients were encouraged to exercise until they reached a respiratory exchange ratio (RER) ≥ 1 as a marker of high exertion. The duration of exercise, peak oxygen uptake (peak VO_2), ratio of minute ventilation to carbon dioxide (VE/VCO_2) slope and anaerobic threshold (AT) were recorded. The test was stopped when the patient reached limiting symptoms that were typically shortness of breath. If a patient did not reach an $\text{RER} \geq 1$, their exercise data were excluded from analysis as it was considered not to be reflective of their true exercise capacity.

Statistical methods

All statistical analysis was performed with SPSS (IBM Statistics V.22). Data normality was tested using Kolmogorov-Smirnov analysis and normality plots. Normally distributed data was described as means with standard deviations. Medians and interquartile ranges were used to describe data that was not normally distributed. Correlation between continuous data was tested using Pearson's correlation coefficient if normally distributed or Spearman's rank correlation coefficient for non-parametric data. Group comparisons were made with a Mann-Whitney test or paired students t-test. Categorical data were described as frequencies and percentages and were compared using Chi-squared or Fisher's exact test as appropriate. Inter-observer and intra-observer reproducibility were assessed by measuring the coefficient of variation derived by the within-subject standard deviation divided by mean and expressed as a percentage. Intraclass correlation coefficients were calculated using an absolute agreement, two-way mixed model. P values <0.05 were considered

statistically significant. Statistical methods specific to each sub-study are described in each of the results chapters.

Chapter 3: 3D late gadolinium enhancement CMR predicts inducible VT in repaired tetralogy of Fallot

For the work presented in this chapter, a detailed background and literature review were presented in Chapter 1 (introduction) and the conclusions are reiterated in the overall conclusions of this thesis (Chapter 8).

Prospective study recruitment had taken place between 2008 and 2018. My exact role in this sub-study was to complete recruitment between 2016 and 2018. I participated in the CMR data acquisition, reporting, LGE and cavity 3D segmentation. I subsequently analysed the data and wrote the manuscript which in 2020 was published in *Circulation: Arrhythmia and Electrophysiology*¹⁹⁴.

Introduction and aims:

In one of few prospective studies of ACHD patients, inducible VT was an independent predictor of mortality⁵⁹. Hence, invasive programmed electrical stimulation (PES) is selectively recommended in guidelines to further risk stratify patients clinically judged to be of intermediate risk^{1, 28}. In this study, the use of non-invasive 3D LGE CMR in predicting inducible VT as a proxy endpoint of mortality was determined.

Methods:

Patients and study design

Adult rTOF patients were prospectively recruited and consented (>16 years old) for 3D LGE CMR preceding clinically scheduled invasive programmed electrical stimulation from 2008 to 2018. Patients with MRI and or gadolinium contrast contraindications were

excluded. The study was conducted according to the Declaration of Helsinki and was locally registered and approved. Inducible sustained VT was defined as VT >30 seconds or causing haemodynamic compromise requiring cardioversion.

CMR acquisition, analysis and reconstruction

CMR was acquired using a standardised protocol as previously described in Chapter 2: The CMR protocol^{16, 17}. For ventricular volume analysis, RV trabeculations and LV papillary muscles were excluded from the blood pool and included in the mass. Following routine pilot imaging, gadolinium contrast agent was administered at 0.12mmol/kg. A free-breathing whole-heart 3D balanced Steady State Free Precession (bSSFP) 'roadmap' was then acquired to visualise the cardiac chambers and pulmonary veins. Approximately 20 minutes after contrast agent administration, when the myocardial wash-in and wash-out reached a steady state, a free breathing inversion prepared gradient echo 3D LGE acquisition was performed, the inversion time being set to null normal myocardium by meticulous visual inspection of long and short axis 2D LGE images^{47, 120}. Sequence sensitivity to heart rate variations during the acquisition were minimised by making real-time changes to the inversion time as the acquisition proceeded¹²⁶. The 3D LGE images covered the entire heart, typically requiring 64 slices at 1.5x1.5x4mm³ voxel resolution which were post processed to 0.75x0.75x2mm³ voxel resolution¹²⁶. For both roadmap and 3D LGE acquisitions, the continuously adaptive windowing strategy (CLAWS) was used for efficient respiratory gating¹²⁷.

Manual 3D segmentation and fusion of cardiac chambers, vessels and ventricular LGE was carried out using dedicated segmentation software (Mimics, Materialise, Le). LGE volume

(cm³) in each ventricle was recorded. LGE segmentation was performed by myself and another experienced operator co-reviewing for consensus and blinded to the PES. To test intra-observer and inter-observer reproducibility 20 cases were re-segmented by myself and the same operator blinded to our previous segmentations as well as to each other.

Routinely acquired clinical data

Patients routinely underwent 12-lead electrocardiogram (ECG), blood sampling for b-type natriuretic peptide (BNP), echocardiography, cardiopulmonary exercise testing (CPEX), and CMR as part of their standardised ACHD care^{1, 26}. Ambulatory ECG Holter monitoring was performed when clinically indicated by symptoms volunteered or for risk stratification. Non-sustained VT defined as ≥ 3 consecutive ventricular beats ≥ 100 bpm for ≤ 30 seconds duration and sustained atrial arrhythmia defined as ≥ 30 seconds that occurred prior to index CMR were recorded. RV restrictive physiology was assessed on echocardiography using diastolic forward flow through the pulmonary artery, PA ('a wave') throughout the respiratory cycle. LV restriction as a non-invasive surrogate for a raised LVEDP was assessed using standard echocardiographic criteria¹⁹⁵ by a single operator. CPEX was assumed valid within 1 year from the CMR study.

Programmed electrical stimulation (PES) was performed using a standardised protocol under assisted sedation²³. No patients were taking amiodarone prior to the procedure. Electroanatomical mapping (EAM) was performed with CARTO (Biosense Webster Inc., CA, CARTO XP RMT, CARTO 3 RMT up to version 7). PES was achieved at 2 RV sites (RV apex and outflow tract) at twice the diastolic threshold with ≥ 2 eight-beat drive trains (cycle lengths between 440 - 510ms) and with up to 3 extra-stimuli with coupling intervals ≥ 180 ms. If VT was non-inducible, this protocol was repeated with isoprenaline

(200mcg in 500ml normal saline via a peripheral line) until heart rate increased by 20%-50%. RV mapping was performed both in sinus rhythm using an endocardial bipolar voltage map or atrial pacing for intrinsic ventricular activation mapping. Mapping points were typically collected using either a manual mapping catheter (CARTO Navistar Thermocool, Biosense Webster) or a magnetic remote controlled one (CARTO RMT Thermocool, Biosense Webster).

LGE correlation with electro-anatomical mapping (EAM) sub-study

In a sub-study of 22 rTOF patients in whom mapping points were available, correspondence of LGE CMR with EAM was investigated. Registration of 3D LGE CMR segmentation with CARTO bipolar voltage maps for each patient was undertaken using MeshLab software. A two-stage registration process was applied to minimise misalignment: first with landmark registration of the ascending aorta and second with surface registration of the iterative closest point. The surface registration error, defined as the distance between points matched on the 3D LGE segmentations and EAM was measured. EAM regions with bipolar signal amplitude $<1.5\text{mV}$ were identified as scar tissue, as previously reported in several histological validation studies.

To quantify the correspondence between LGE CMR and low-voltage bipolar signal, we defined LGE CMR sensitivity as the proportion of low voltage mapping points within 5mm of LGE-defined scar against total mapping points, where 5mm was the allowance made for LGE to mapping point registration error. Similarly, we defined LGE CMR specificity as the proportion of normal voltage points $\geq 25\text{mm}$ away from LGE-defined scar against total mapping points.

Statistical methods

Continuous data are summarised as mean ($\pm$ SD) or median (interquartile range) as appropriate. Comparisons between groups were made using a Mann-Whitney test, chi-squared test or Fisher's exact test as appropriate. Correlation between continuous data was assessed using Spearman's test (ρ). Receiver-operating characteristics (ROC) analysis was used to determine the discriminative power of RV LGE and the optimum cutoff value with the highest sensitivity and specificity in predicting inducible VT. In addition, RV LGE volume cut-off values achieving 100% sensitivity and 100% specificity were measured. The RV LGE threshold that predicted inducible VT with the most optimum sensitivity and specificity was calculated using the Youden index. The association between RV LGE and other variables with inducible VT was tested using logistic regression analysis.

Given the relatively small number of patients with inducible VT, it was not appropriate to perform full multi-variable analysis. Instead, following univariable analysis, RV LGE volume (cm^3) was combined with other predictive variables in a series of bivariable analyses. All tests were 2-sided and a P value of <0.05 was considered significant. Intra-observer and inter-observer variability was expressed as the coefficient of variation (percent), derived from the within-subject SD divided by the mean, multiplied by 100. Intra-class correlation coefficients were calculated using absolute agreement, 2-way mixed model. Statistical analysis was performed with SPSS (IBM Statistics V.22).

Results

i. Study patients

A total of 69 adult rTOF patients (mean age 40 ± 15 years; male 43, 62%) underwent 3D LGE CMR prior to invasive programmed VT-stimulation. Baseline patient demographics and patient characteristics are summarised in **Table 3.1**.

Of the 69 patients, 22 (31.8%) had inducible VT and 18 (81%) underwent a VT ablation for sustained monomorphic VT (mean total cycle length 259 ± 29 ms). In most patients 14(78%), a linear ablation connecting the pulmonary valve towards the tricuspid annulus (**Figure 3.4**) was performed towards the RV free wall reflecting the operator's preferred approach to avoid ablating close to the conal septum in order to both minimise complications with the conduction system and make VT non-inducible. In 4 patients (22%) however, an ablation very towards the VSD patch was required. All 18 patients that underwent VT ablation were also offered an AICD following this of which 2 have received an appropriate VT shock and 2 an inappropriate shock for atrial tachyarrhythmia at median 6.7 years follow-up.

In 4 patients (18%), VT induction was associated with severe haemodynamic instability due to monomorphic VT in 2 and polymorphic VT in the remaining 2. In comparison, to the 18 patients who had undergone a VT ablation, these 4 patients had significantly worse LV ejection fraction [median 51% (42-56) versus 63% (48-67); $p=0.03$]. There were 4 patients (22%) who declined AICD, of which one died suddenly (presumed arrhythmic).

ii. LGE-defined scar and clinical correlates

All patients had RV LGE at the surgical sites (both RVOT patch site and VSD patch site in 69, 100%). RV LGE was observed in areas apparently remote to surgery: RV trabeculations in 31 (45%) and moderator band in 14 (20%). Patients with this remote type of LGE were older (46 years $\pm$ 14 versus 35 years $\pm$ 14; $p < 0.001$) and had later repairs [repair at 8 years (4-11.4) versus 3.4 years (0.5-6.9); $p < 0.001$]. RV/LV insertion point LGE (typically subtle) was observed in all cases ($n=69$, 100%). Given that we know this is a non-specific finding in normal hearts and that it is routinely observed in our practice, as previously it was excluded from quantification⁴⁷.

Apical LV LGE corresponding with site of previous surgical vent insertion was found in 36 (52%) patients and was not considered relevant for further analyses. Non-apical vent LV LGE occurred in 20 (29%) cases. This was inferolateral and transmural in 3 (15%) and in 13 (65%) discrete foci of LGE occurred in other locations. Four patients (20%) had only papillary muscle LV LGE (see Figure 3.1).

As shown in Table 3.1, supramedian RV LGE volume ($\geq 20 \text{ cm}^3$) was associated with older age ($p < 0.001$), late repair ($p = 0.002$), earlier surgical era, ($p = 0.04$) (see Figure 3.2), increased RVESVi ($p = 0.04$), reduced RV EF ($p = 0.004$), increased indexed right atrial area, RAAi ($p = 0.005$), increased RVOT akinetic length ($p = 0.003$), QRS duration ($p = 0.01$) and restrictive LV filling pressure ($p = 0.02$).

iii. LGE-defined scar, histological and electroanatomical correlates

In the sub-study of 22 rTOF patients (mean age 44 ± 15 years, male 12,55%) in which LGE-defined scar was compared with clinically available bipolar voltage mapping points ($<1.5\text{mV}$), the mean surface registration error was 5mm. In these patients a total of 369 low voltage points ($<1.5\text{mV}$) occurred within a 5mm vicinity of the LGE tissue perimeter versus 151 normal voltage points found in this area. Conversely, there were 35 low voltage points that were more than 25mm away from the LGE tissue perimeter and 141 normal voltage points in that area. LGE CMR was therefore, 71% sensitive and 80% specific for its correspondence with low-voltage areas ($<1.5\text{mV}$) on EAM (**see Figure 3.3 and 3.4**).

In one patient we were able to correlate 3D LGE findings with subsequent surgical excision of the same region (**see Figure 3.5**) and the LGE-defined RV scar was confirmed as fibrosis histologically.

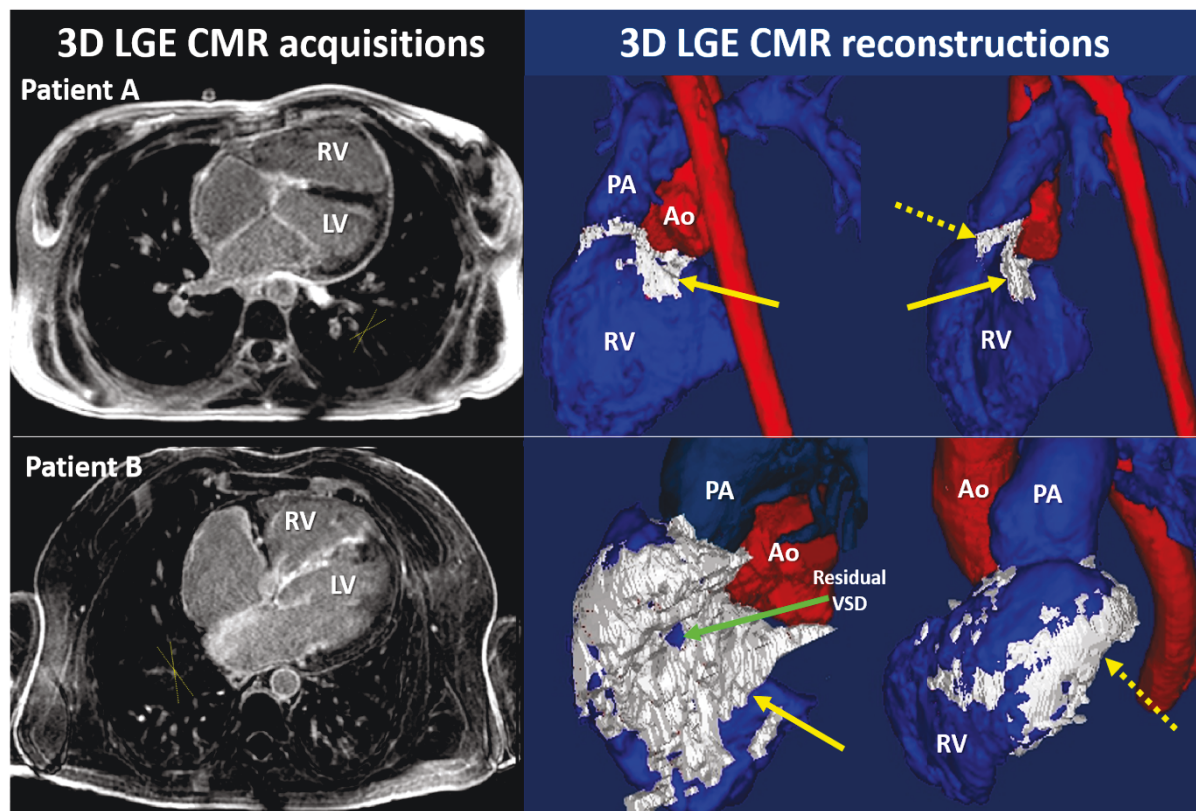
iv. Predictors of VT inducibility

Significant univariable predictors of VT inducibility were RV LGE volume cm^3 (HR 1.15, CI 1.06-1.25; $p=0.001$), QRS duration ≥ 180 ms (HR 3.5, CI 1.2-10.7; $p=0.02$), age (HR 1.6 per decile; CI 1.1-2.3; $p=0.01$), previous non-sustained VT (HR 3.5, CI 1.2-10.1; $p=0.01$) and clinical sustained VT (HR 12.8, CI 2.4-67; $p=0.003$). Non-apical vent LV LGE was also predictive of VT inducibility (HR 1.09, CI 1.02-1.17; $p=0.008$) and remained predictive even when the 4 patients with only LV papillary muscle LGE were excluded (HR 1.1, CI 1.03-1.17; $P=0.007$, **see Table 3.2**). In bivariable analysis, RV LGE volume (HR 1.14, CI 1.04-1.24; $p=0.003$) and clinical sustained VT (HR 8.02, CI 1.26-51.2; $p=0.02$) were the independent predictors of inducible VT

(Table 2), whereas QRS duration, age, non-sustained VT and non-apical vent LV LGE volume did not stay in the model with RV LGE (**see Table 3.3**).

On ROC analysis, RV LGE had a good discriminative power in predicting inducible VT (area under the curve, AUC, 0.81, 95% CI: 0.7-0.9; $p < 0.001$, **see Figure 3.6**). An RV LGE volume of 24.7cm³ had a 72% sensitivity and 81% specificity in predicting inducible VT (Youden index). A 100% sensitivity was achieved at a RV LGE volume $\geq 10.2\text{cm}^3$ and 100% specificity was reached at a RV LGE volume $\geq 36.0\text{cm}^3$ (**see Figure 3.7**). RV LGE volume had a negative predictive value of 100% and positive predictive value of 37% (at 10.2cm³ cut-off volume for RV LGE volume) with 46% accuracy (**see Figure 3.6 and 3.7**).

Figure 3.1:



Two rTOF patients with 3D LGE CMR and corresponding segmented scar and chambers: 21 year-old patient **A** repaired aged 4 years with a RVOT patch, had overall mild LGE burden (4.9cm^3) and was non-inducible for VT. Mild extent 3D LGE reconstructed scar (grey) is seen in the ventricular septum (yellow arrow), and RVOT (dotted yellow arrow). 60 year old patient **B** repaired aged 17 years with transannular patch, had overall extensive LGE burden (53.9cm^3) and was inducible for VT. Severe burden of 3D LGE scar (grey) is seen in the RVOT with extension on to the RV free wall (dotted yellow arrow) and is also seen in the ventricular septum (yellow arrow).

Variable	All patients n=69	Inframedian RV LGE <20cm ³ n=35	Supramedian RV LGE ≥20cm ³ n=34	P-value	LGE correlation, r (p)
Age at scan, y	40 ±15	32 ± 13	48±13	<0.001	0.57 (P=<0.001)
Age at surgery, y	5.8 (1.4-9.3)	2.7 (0.7-5.9)	7.5 (5.6-13)	0.002	0.5 (P=<0.001)
Male sex, n	43	23	20	0.6	
Palliative shunt*, n	29	14	15	0.8	
Transannular patch, n	23	9	14	0.3	
RVOT patch, n	17	9	8	0.7	
RV-PA conduit, n	11	7	4	0.4	
Surgical era ¶	68			0.04	
Redo-PVR†, n	41	18	23	0.5	
NYHA class ≥ II, n	26	11	15	0.3	
Syncope, n	11	4	7	0.5	
Sustained atrial arrhythmia‡, n	23	9	14	0.2	
Non-sustained VT‡, n	24	12	12	0.5	
Sustained VT, n	10	3	7	0.2	
BNP§, ng/L	59 (28-106)	51 (17-100)	69 (8-130)	0.3	0.3 (P=0.02)
QRS duration , ms	162 (145-178)	162 (151-177)	180(161-185)	0.01	0.29 (P=0.01)
QRS duration ≥ 180 ms	19	7	12	0.1	
Peak V02, # mL/kg/min	24.7 (20.6-30)	25.1 (20-31.6)	22.1 (20-30)	0.5	-0.2 (P=0.3)
% predicted V02	71 (45 -85)	80.3 (61-95)	75 (57-85)	0.3	-0.2 (P=0.3)
VE/VCO2 slope	30 (26-34)	28 (24-31.4)	32 (28.5-36)	0.1	0.47 (P=0.02)
RV LGE volume, cm ³	20 (12.6-27)				
LV LGE volume ,non-apical vent,cm ³	3.7 (0.5-9.7)				
Akinetic length of RVOT, mm	38 (28-50)	30(22-42)	43 (35-57)	0.003	0.4 (P=0.001)
RVEDVi, mL/m ²	125 (99-147)	113 (97-147)	133(105-155)	0.2	0.1 (P=0.35)
RVESVi, mL/m ²	65 (47-89)	62 (44-75)	76 (53-92)	0.04	0.29 (P=0.01)
RVEF, %	47 (42-55)	52 (44-59)	43(40-51)	0.004	-0.46 (P=<0.001)
RV:LV volume ratio	1.4 (1.1-1.8)	1.4 (1.1-1.9)	1.4 (1.2-1.9)	0.3	0.05 (P=0.7)

RV mass/volume, g/m2	0.4(0.32-0.47)	0.39 (0.31-0.47)	0.42(0.32-0.49)	0.4	0.07 (P=0.56)
RAAi, cm2/m2	15 (12-17)	13 (12-15)	17 (13-19)	0.005	0.3 (P=0.008)
LVEDVi, mL/m2	85 (73-101)	84 (75-99)	84 (69-110)	0.9	0.04 (P=0.8)
LVESVi, mL/m2	33 (28-43)	33(27-43)	34 (28-48)	0.6	0.1 (P=0.4)
LVEF, %	60 (50-66)	58 (54-67)	55 (49-65)	0.3	-0.2 (P=0.08)
LAAi, cm2/m2	10 (9-12)	10 (9-11)	11 (9-13)	0.7	0.07 (P=0.5)
Pulmonary regurgitation, %	14 (2-32)	21 (4-48)	13 (1-45)	0.3	-0.2 (P=0.2)
Restrictive LV filling**, n	12	2	10	0.01	
Restrictive RV physiology**, n	11	7	4	0.7	

Table 3.1: Repair for tetralogy of Fallot with pulmonary atresia and no systemic-pulmonary collaterals in 5 (7%) for absent pulmonary valve in 3 (4%)* A total of 29 palliative shunts were used: Blalock-Taussig shunt n=19, central shunt n=7, Brock procedure n=3 ¶ Information on surgical era was available in 68, the following decades from 1950's through till 1990's were studied (60's n=11, 70's n=24, 80's n=21, 90's n=12) † 5 cases had percutaneous pulmonary valve implantation with Melody valve. ‡ Holter monitors were available in 54 (78%), §BNP was available in 55 (80%). || 12-lead ECG was available in 69 (100%), # CPEX was available in 50 (72%), ** echo data on LV and RV restriction was available in 66 (96%) and 55(80%) respectively Spearman's coefficient of correlation

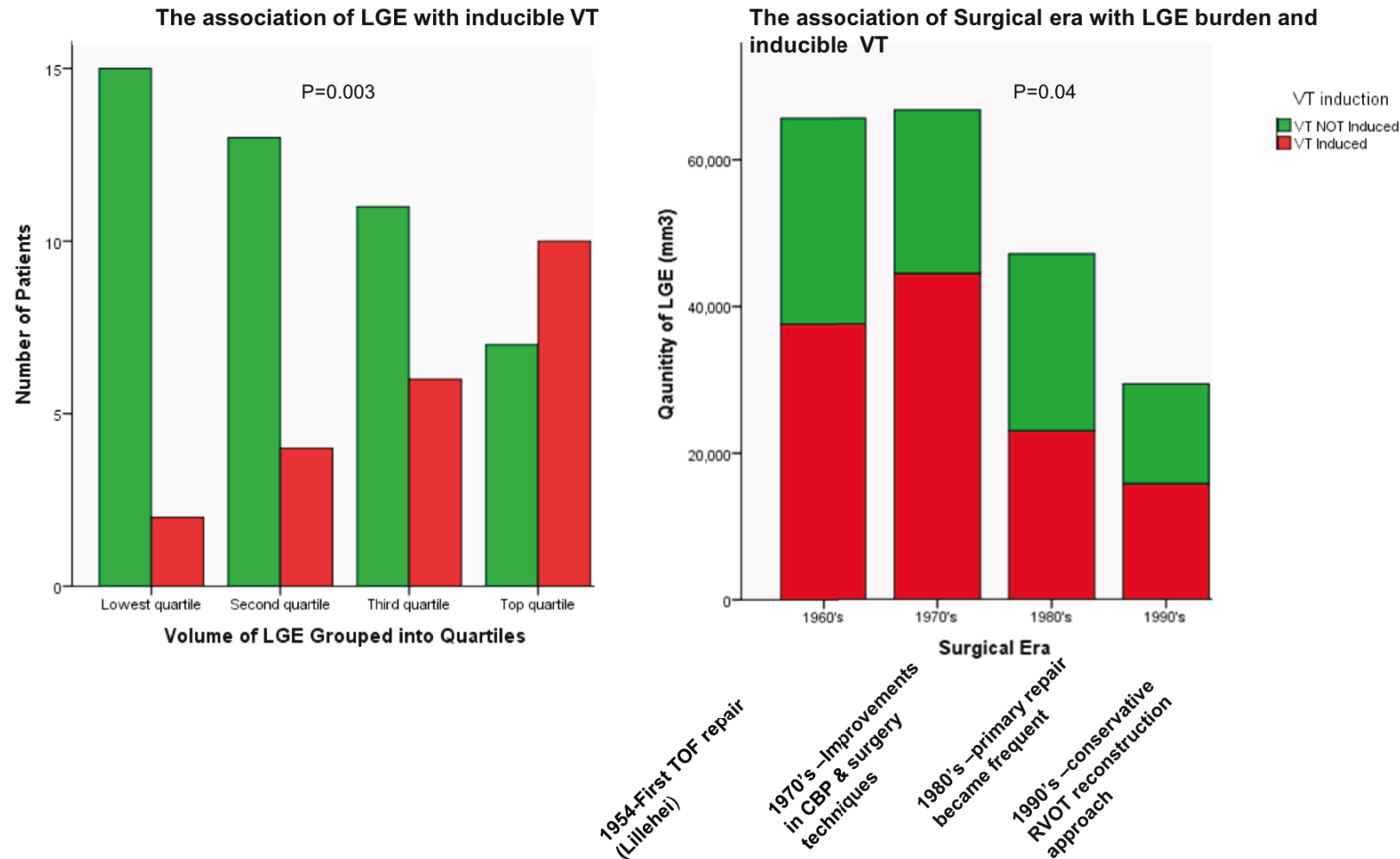


Figure 3.2: Associations of LGE volume with inducible VT and surgical era. Increased LGE volume presented as quartiles is associated with inducible VT (Left). Patients who were operated in the earlier surgical era's (1960's, 1970's) had the worst LGE burden compared to more modern era's (1980's, 1990's) and were more likely to have inducible VT (right).

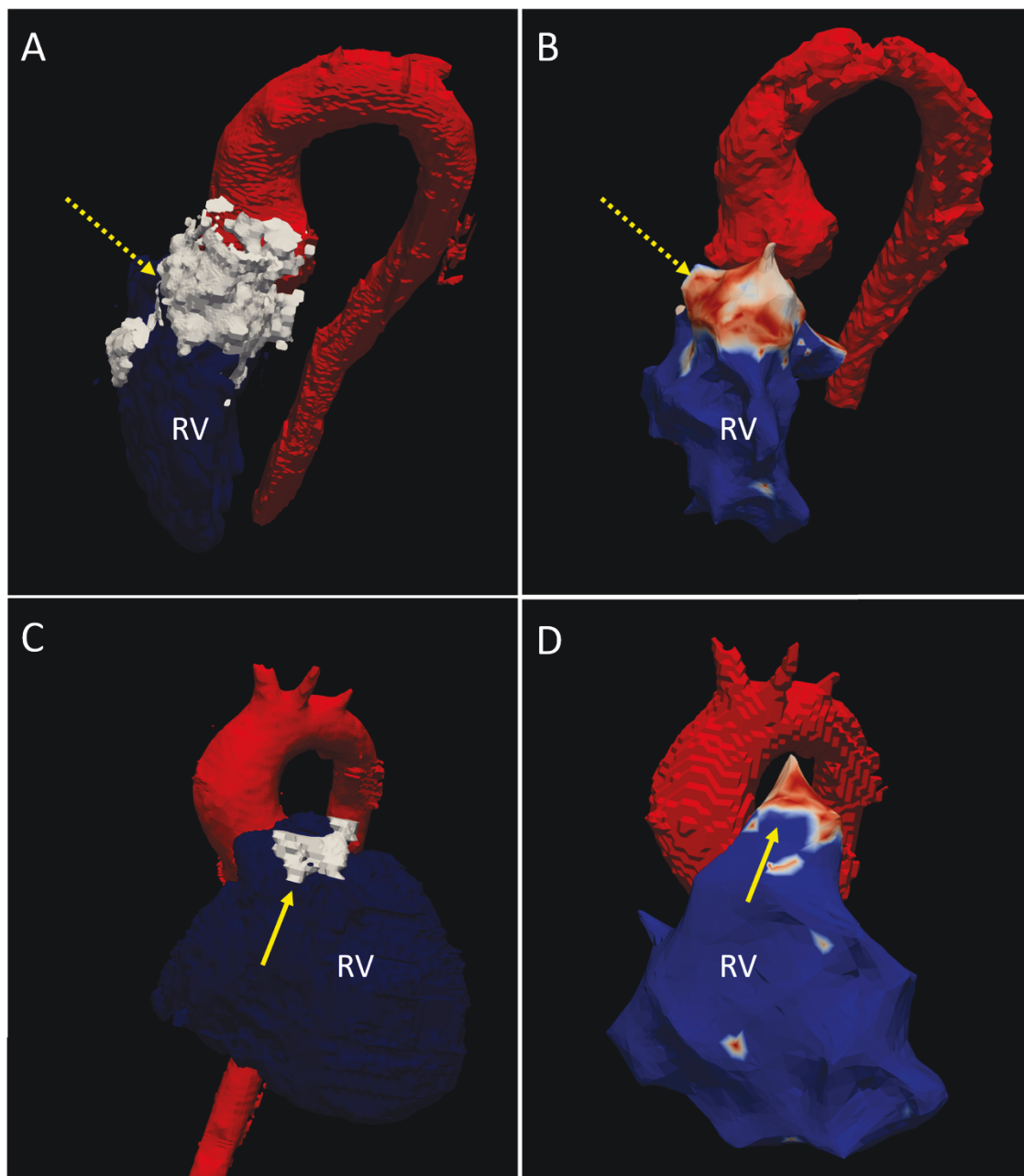
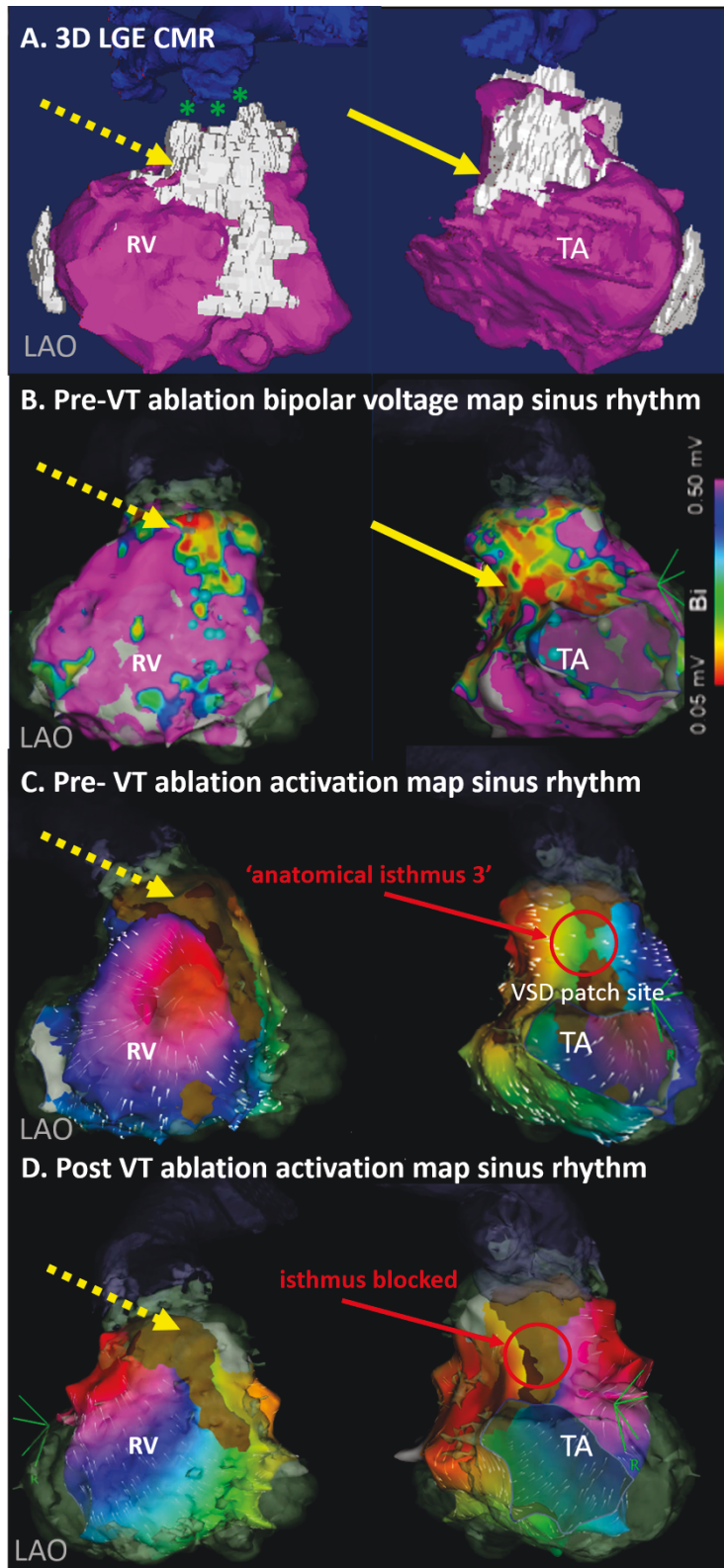


Figure 3.3: Correlation of scar defined by 3D LGE CMR with corresponding bipolar voltage mapping. 3D reconstructed LGE scar is shown as grey in the RVOT (A-dotted yellow arrow) and RV side of the septum (C yellow arrow). Healthy RV myocardium is reconstructed in blue. 3D LGE reconstructions (**A, C**) are superimposed on to low-voltage signal from corresponding bipolar voltage maps following registration of the two models together (**B, D**). Good geographical match is seen on the overlapped models (B, D) between regions of 3D LGE and low-voltage signal in both the RVOT (B-dotted yellow arrow) and RV septum (D-yellow arrow). Image adapted from Ghonim *et al* 2020¹⁹⁴

Figure 3.4: 3D LGE correlation with voltage and activation mapping preceding induction of VT



A) 3D LGE and 3D bSSFP CMR reconstruction of the RV and pulmonary artery (PA) in blue showing LGE defined scar (grey) and healthy tissue (purple). Metallic artefact from a previous Mosaic valve has caused the signal dropout and absence of segmented tissue between the pulmonary artery and RV seen (green asterisks).

Good geographical correlation is seen between 3D LGE scarred regions (A) and low-voltage scar seen on bipolar voltage maps taken using CARTO 3 version 7 (B) in the RVOT (yellow dotted arrow) and RV septum (yellow arrow). Scar on the bipolar voltage maps is demonstrated by red/yellow/green colours (0.5mv- 1.5mv) and healthy myocardium is represented as purple.

Similarly a good match with LGE-defined scar was seen with activation mapping where brown areas showed slow or no conduction (SNO zone) in the RVOT free wall and septal surface (C) A re-entry mechanism was induced through an 'anatomical isthmus' that is between the pulmonary valve annulus and VSD patch with clockwise activation. Critical activation across the isthmus (red circle) induced a well-tolerated monomorphic VT (TCL 280ms).

D) VT ablation was carried out. Subsequent mapping showed

complete isthmus line block (red circle) causing a change in the direction of activation to travel inferiorly around the tricuspid annulus (TA) and on to the anterior RV with late activation (red) occurring adjacent to the ablation line as a result.

Images adapted from Ghonim *et al* 2020¹⁹⁴

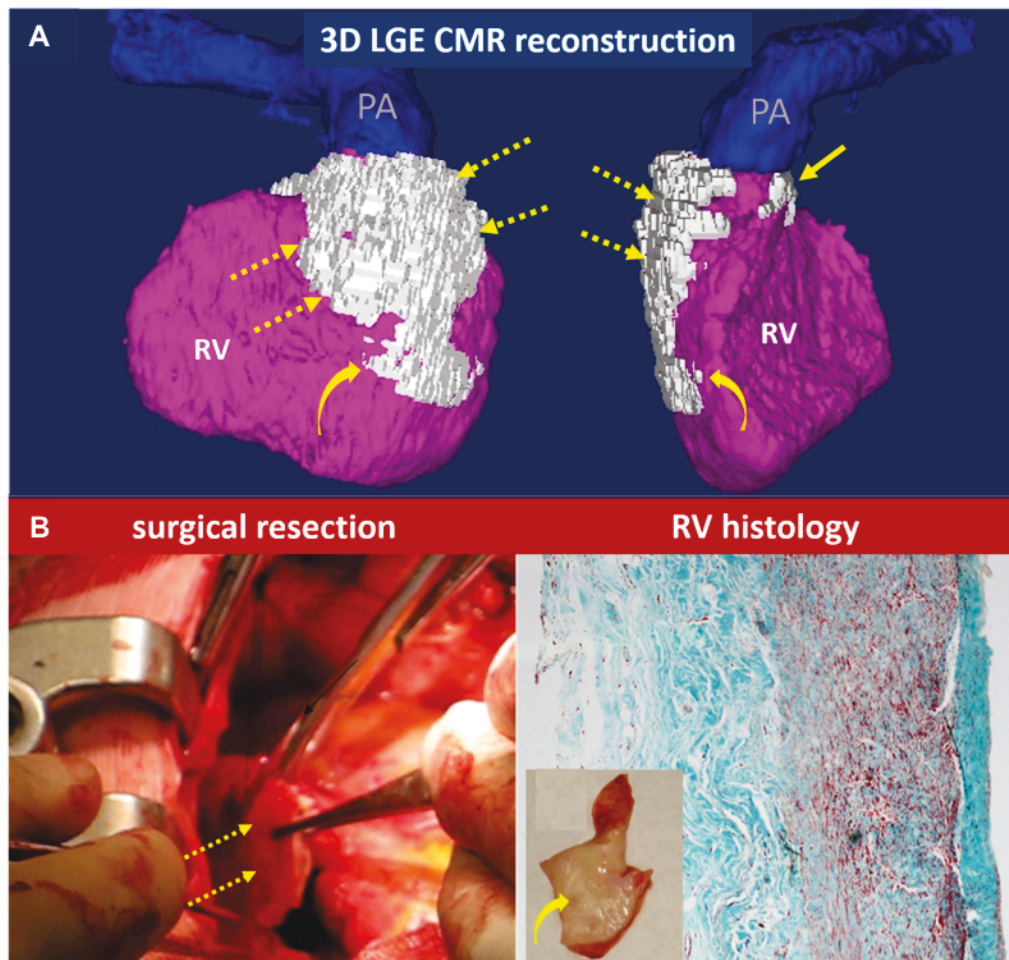


Figure 3.5: 3D Reconstruction of LGE scar in a patient with rTOF who presented with sustained VT correlated with macroscopic and microscopic fibrosis from RVOT specimen resected at time of pulmonary valve replacement. **(A)** demonstrated scar (grey) in the VSD site (yellow arrow), RVOT patch site (dotted yellow arrows) with endocardial native RVOT scar (curved yellow arrow). Prior to surgical valve replacement, the patient underwent ablation for VT originating from the RVOT. **(B)** During surgery scarred RVOT patch (dotted yellow arrows) with native RVOT were excised. **(C)** The native RVOT tissue (inset; curved yellow arrow) was histologically examined with Masson's trichrome stain (magnification x20) where fibrosis appears blue and healthy myocytes red. The endocardium is to the left of the stained tissue and it is the RVOT endocardial surface that is arrowed. Figure adapted from Ghonim *et al* 2020¹⁹⁴

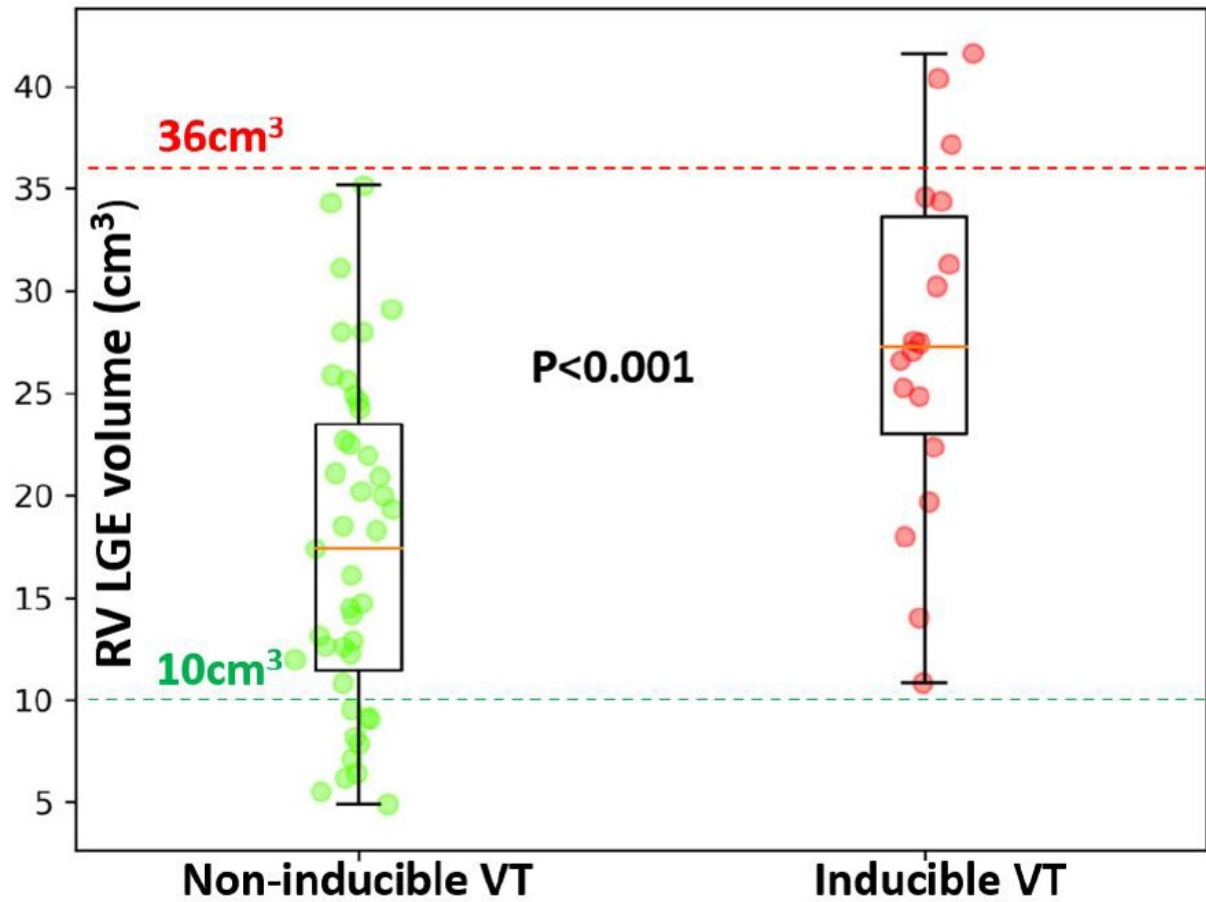
Table 3.2: Univariable predictors of inducible VT.

Univariable model for inducible VT	HR (95% CI)	P value
Age at CMR, per decile	1.6 (1.1-2.3)	0.01
Age of repair, y	1.03 (0.97-1.01)	0.2
Palliative shunt,	0.9 (0.3-2.6)	0.9
NYHA>II	1.6(0.57-4.5)	0.4
Syncope	0.78(0.18-3.2)	0.7
Clinical sustained atrial arrhythmia ≥30 s	0.48 (1.15-1.56)	0.2
Non-sustained VT	3.5 (1.2-10.1)	0.02
Clinical sustained VT ≥30 s	12.8 (2.4-67)	0.003
BNP, per 10ng/L	1.02(0.96-1.08)	0.59
QRS> 180ms	3.5 (1.16-10.7)	0.02
Peak VO ₂ , mL/kg/min	1.05 (0.9-1.1)	0.55
VE/VCO ₂ slope	1.1 (0.97-1.2)	0.14
RV LGE volume, cm ³	1.15 (1.06-1.25)	0.001
Supramedian RV LGE volume, cm ³	3.4 (1.2-9.7)	0.02
LV LGE volume (non-apical vent), cm ³	1.09(1.02-1.17)	0.008
LV LGE volume (non-apical vent/papillary muscle) cm ³	1.1 (1.03-1.17)	0.007
Remote to surgical sites RV LGE volume	1.62 (0.58-4.5)	0.35
Akinetic length of RVOT, mm	1.03(0.99-1.06)	0.07
RVEDVi, mL/m ²	1 (0.99-1.01)	0.6
RVESVi, mL/m ²	1 (0.98-1.02)	0.4
RVEF, %	0.98(0.92-1.04)	0.1
RAAi, cm ² /m ²	1.02 (0.9-1.13)	0.6
RAAi ≥ 16cm ² /m ²	1.81 (0.63-5.2)	0.27
LVEDVi, mL/m ²	1.02 (0.98-1.05)	0.1
LVESVi, mL/m ²	1.02 (0.97-1.05)	0.2
LVEF, %	0.99 (0.94-1.05)	0.6
LAAi, cm ² /m ²	1.06 (0.89-1.3)	0.5
Pulmonary regurgitation %	1 (0.97-1.03)	0.8
Restrictive LV filling pressure	2 (0.5-7.4)	0.3
Restrictive RV physiology	0.4 (0.1-2.03)	0.3

Table 3.3: Bivariable predictors of inducible VT.

Bivariable model for inducible VT		
RV LGE volume cm³	1.12 (1.03-1.23)	0.007
Age at scan (per decile)	1.4 (0.85-2.3)	0.2
RV LGE volume cm³	1.14 (1.05-1.24)	0.002
QRS duration >180ms	1.9 (0.48-7.8)	0.35
RV LGE volume cm³	1.14 (1.1-1.24)	0.001
Non-sustained VT	3.2 (0.8-12.3)	0.1
RV LGE volume cm³	1.14(1.04-1.24)	0.003
Clinical sustained VT ≥30s	8.02(1.26-51.2)	0.02
RV LGE volume cm³	1.14(1.04-1.24)	0.004
LV LGE volume cm ³	1.1(0.9701.23)	0.12

Figure 3.6:



RV LGE volume is a significant predictor of inducible VT as illustrated by this dot graph. All rTOF patients who had RV LGE volumes $\leq 10\text{cm}^3$, $n=10$ (below the green line) were non-inducible for VT and all patients who had $\geq 36\text{cm}^3$ of RV LGE ($n=3$; above the red line) were inducible for VT.

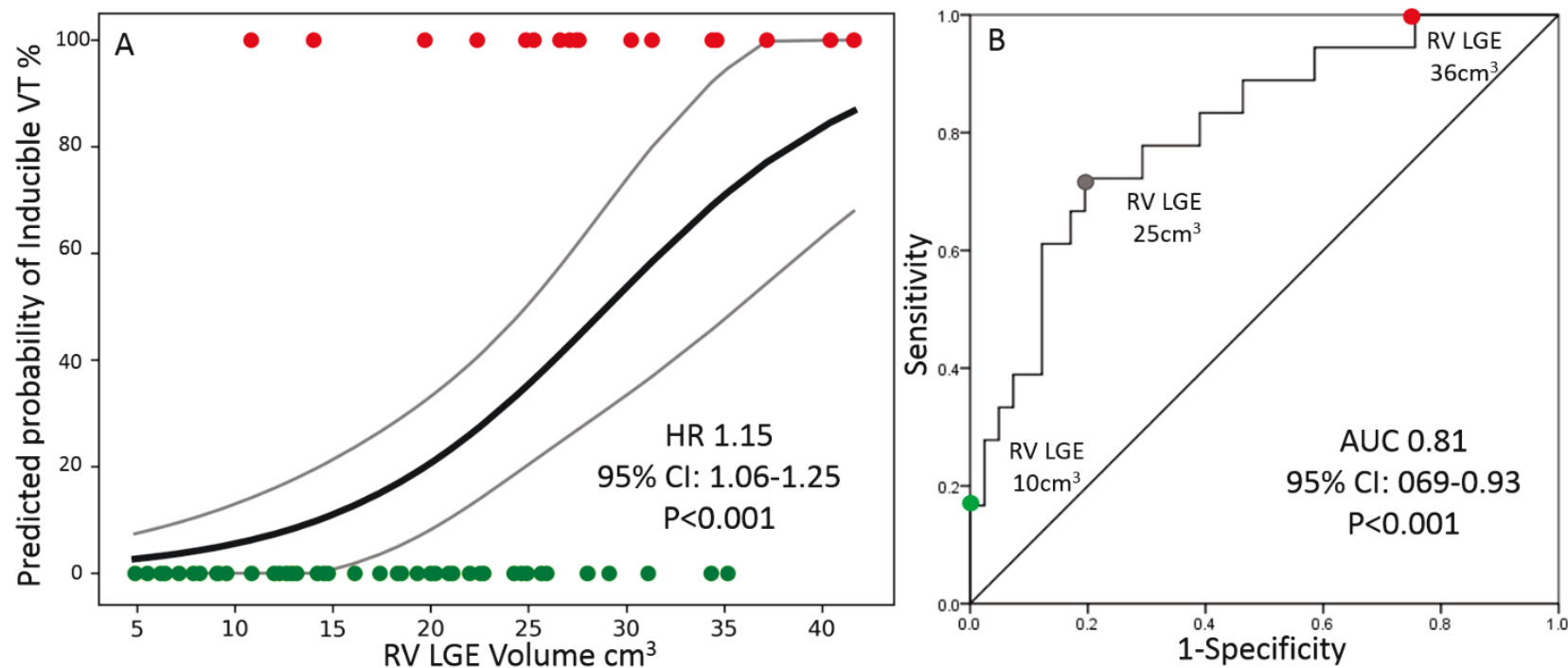
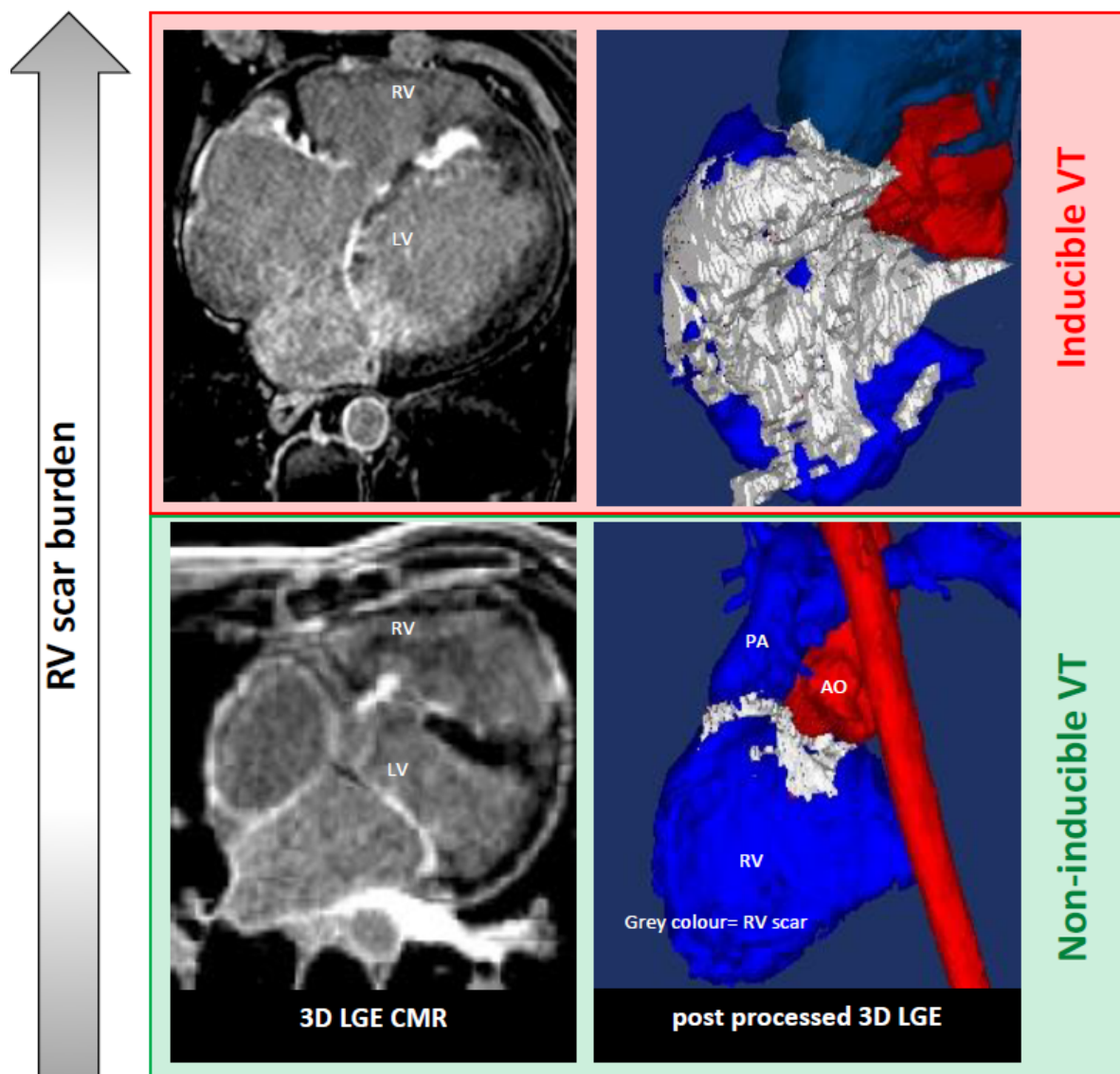


Figure 3.7: 3D RV LGE volume cut offs that predict inducibility of VT. **A** 3D RV LGE independently predicts inducible VT. Binary logistic regression curve illustrates higher predicted probability of inducible VT is related to higher RV LGE extent. **B** Receiver-operating characteristic curve show that RV LGE of 25 cm^3 (grey dot) had the most optimal sensitivity (72%) and specificity (81%) for predicting inducible VT (Youden index). A RV LGE of 10 cm^3 were 100% sensitive and 17% specific for inducible VT (green dot) whereas a RV LGE of 36 cm^3 (red dot) was 25% sensitive and 100% specific for inducible VT.

Figure 3.8:



Graphic to summarise the main findings of the study; the risk of inducible ventricular tachycardia (VT) is greater with increasing RV scar burden. 3D LGE CMR (left) and post processed segmented 3D LGE (right) are shown. An example of a patient with inducible VT and who had a high RV scar burden is shown in the top red panel. In contrast, an example of a patient in whom VT was non-inducible and with minimal **ventricular** scarring is shown in the lower green panel.

Discussion

This is the first prospective study to date that examines the association between ventricular myocardial scar burden defined by state-of-the-art 3D LGE CMR and VT inducibility in a highly-phenotyped cohort of adult rTOF patients.

The major and unique findings are that RV LGE burden independently predicts inducible VT over and above well-established risk factors, namely, older age and prolonged QRS duration and remained independently associated even when tested against sustained VT pre-PES. This association is summarised in an infographic (**see Figure 3.8**). A small increase in LGE volume is associated with a large increase in risk of inducible VT whereby for every 1 cm³ of LGE there is a 15% increased risk of inducible VT. We propose thresholds for RV LGE volume that discriminate between patients at higher and lower risk. These findings may help avoid needless invasive studies, better select rTOF patients for further invasive risk stratification testing and help in the planning of VT ablation where relevant.

i. Inducible VT in repaired tetralogy of Fallot

Inducible VT was a strong independent predictor of clinical sustained VT and SCD during follow-up in a multicentre study⁵⁹, one of very few studies on outcome in ACHD. This study with its clinically important endpoints has influenced clinical practice and contributed to guidelines^{1, 27, 196, 197}. Therefore, we chose inducible VT as a justifiable proxy endpoint to evaluate patient risk using 3D LGE-defined scar. There are notable differences between this previous study and our own. Their patients were younger (mean age 16 years versus 40 years), more had at least moderate PR (74% versus 20%), more had history of clinical sustained VT or syncope (17% versus 14%, 24% versus 16%, respectively) and they included patients who already had AICDs at baseline⁵⁹, implying in combination, that PES was being

performed in an even higher risk rTOF population in this older study than the described here. Since then, the approach to managing significant pulmonary regurgitation has also evolved with efforts concentrated on proactive pulmonary valve implantation before symptom onset¹⁹⁸. Our study cohort reflects this more contemporary practice.

In our centre, patients who were deemed clinically to be of at least 'intermediate-risk' were individually selected for PES after multidisciplinary input. Typically, selected patients would have 2 or more of the following: QRS duration ≥ 180 ms, mild LV impairment, moderate RV impairment or cardiac syncope. Given the high negative predictive value of inducible VT in rTOF patients⁵⁹, it is often speculated that a negative PES given its negative predictive value is more useful as a 'rule-out' test to reassure patients that a primary prevention AICD is not required. Conversely, an inducible VT compared with a sustained clinical VT is not highly specific and hence testing is avoided in those with low pre-test probability.

ii. 3D LGE predicts inducible VT

We found that 3D LGE extent was strongly associated with inducible VT and was stronger than established predictors, including age, QRS duration > 180 ms^{21, 50} and non-sustained VT²³ which did not remain significant when tested in combination with RV LGE in bivariable analyses. Even when combined with clinical sustained VT which, as expected, was independently predictive of VT inducibility in univariable analysis, RV LGE volume still remained an independent predictor in bivariable analysis.

In recent large cohort studies other non-invasive risk factors of adverse outcome have been reported in rTOF including sustained atrial arrhythmia, RV ejection fraction, LV ejection

fraction¹⁷, maximal right atrial area, RV outflow tract akinetic region length³⁶, BNP⁴⁸ and peak VO₂¹⁹⁹. These were not found to be predictors of inducible VT outcome in this smaller cohort.

RV LGE volume had a high negative predictive value of 100% vs lower positive predictive value of 37% (at RV LGE 10cm³ cut-off). This suggests that RV LGE volume may have a role as a 'rule out' test to reassure patients, potentially sparing low-risk patients from having an invasive test in future or perhaps be a 'rule in' test for those in whom VT ablation is considered an option. In our study, 10 of the 49 patients (20%) with non-inducible VT had an RV LGE volume below the 'rule-out' cut-off of 10cm³. Out of these 10 patients identified, only 1 (10%) had syncope, 2(20%) had QRS duration ≥180ms and all had preserved RV/LV EF when compared to the remaining 39 patients in whom 10 (15%) had suffered syncope, 17 (25%) QRS duration ≥180ms, had normal-mild LV impairment [LVEF 58(49-65%)] and mild-moderate RV impairment [RVEF 45(40-54%)]. In hindsight, the 10 patients identified with minimal RV scarring (RV LGE ≤10cm³) had weaker clinical indication for PES. By using 3D LGE to help support clinical decision making, an invasive PES could have been avoided in these patients without change in their clinical management.

i. 3D LGE-defined anatomical substrates of arrhythmia

The association of 2D LGE-defined scar with adverse clinical features, most importantly history of arrhythmia, has been previously reported from a cross-sectional study⁴⁷. Whilst 2D LGE CMR application in the RV is feasible it requires expertise as it is susceptible to false positive results secondary to partial volume effects from sternal wires, pericardial fat and the thin RV wall of only a few millimetres. It is also susceptible to false negative results due to, for example, inexperience setting acquisition parameters and/or suboptimal timing of

the acquisition following contrast agent administration including finishing inversion recovery acquisitions too early after contrast given⁴⁷.

Two-dimensional (2D) RV LGE quantification remains challenging to translate from highly expert research settings to the real world. Personalised virtual whole heart models that include myocardial scar can now allow better clinical visualisation and robust quantification of the arrhythmia substrate in the RV (**see Figure 3.1**) with the potential for deeper mechanistic insight and integration into invasive procedures (**see Figure 3.4**).

Differences between electrically defined scar and anatomically defined scar are well described²⁰⁰. These may be due to inherent limitations with EAM not being able to assess intramural and epicardial scar and its dependence on good catheter contact. Though it was not the primary intention of this study we demonstrated that RV LGE-defined scar corresponded to RV fibrosis (**see Figure 3.3**) and show a good albeit imperfect geographical match between LGE and low-voltage mapping points. Integration of LGE and EAM is challenging due to differences in (1) the co-ordinate systems, (2) spatial resolutions, (3) timings of the acquisitions within the cardiac and respiratory cycles and (4) the patient condition. These are the most likely reasons why a more exact match was not seen. It is also possible and remains unclear if the small area of disagreement may be attributed to the heterogeneity within the scar regions and at the perimeter of the scar or 'gray' zone. High resolution mapping studies with smaller electrodes will most likely give better insight into this problem.

Agreement between 3D LGE CMR and EAM paves the way for the use of 3D LGE CMR in planning anatomical substrate guided electrophysiology procedures, in rTOF patients likely to require VT ablation. A substrate-based approach facilitated by whole heart 3D LGE CMR

can be particularly advantageous for patients who cannot haemodynamically tolerate VT, for patients with complex scar substrate and for those with atypical substrates that are unexpectedly remote from usual surgical scar sites but may cause VT²⁰¹.

Patients with the highest volume RV LGE and inducible VT (three patients above the red line on **Figure 3.4**) had a tendency towards being older when compared to patients with smaller volumes of RV LGE who were non-inducible [38 (27-58.5) years vs 29 (23-43) years respectively ($p=0.08$)]. Although not demonstrable in these three patients, there was a significant association between earlier surgical eras, higher LGE volume and inducible VT in the entire cohort (see **Figure 3.2**). In future, it is expected that with further advances in patient management and improving risk profile, LGE burden may become less predictive of outcome.

Limitations

It is recognised that VT inducibility as the primary outcome for this study is not a direct indicator of mortality but rather a surrogate marker. Equally, it is considered this to be the most robust secondary marker of mortality available as we wanted to avoid using soft endpoints, like frequent ventricular ectopy, which may have driven the arrhythmic endpoint in other fibrosis rTOF studies¹⁷¹. A large prospective follow-up cohort could inform us of the relative prognostic value of 3D LGE CMR and inducible VT with respect to mortality.

Multivariable analysis was not performed because of the relatively small number of events, and therefore cannot determine whether the presence of RV LGE alone predisposed to inducible VT. The quantity of ventricular fibrosis may be underestimated in several patients in whom there is absence of signal in the region of sternal wire artefact for example as

shown in **Figure 3.4**. In two patients with a relatively low burden of RV LGE (10-15cm³) and also inducible VT, it is possible that the degree of RV LGE was underestimated secondary to signal drop out relating to artefact from a percutaneous pulmonary valve stent inserted within an RV-PA conduit and from a bioprosthetic pulmonary valve demonstrating this limitation.

A small number of Patients with Fallot variant anatomy were included in this study (**see Table 3.1**). Recognising that there is a significant difference in pre-natal and post-natal loading conditions in patients with pulmonary atresia with VSD compared with standard TOF, care was taken to ensure that only patients without MAPCAs and complex pulmonary atresia were recruited. A small number of patients with absent pulmonary valve were included in this study although it is accepted that this condition may be also considered as a separate entity. In this case, the inclusion of 3 patients with absent pulmonary valve is unlikely to have influenced the results.

Conclusion

RV LGE-defined scar burden is a strong and independent predictor of inducible VT in adult patients with rTOF. 3D LGE CMR should be incorporated into the non-invasive assessment of adults with rTOF being considered for programmed electrical stimulation. Not only can it help predict which patients are most likely to need to proceed to VT ablation and or AICD, but it can also help avoid needless invasive procedures.

Chapter 4: Risk score predicts malignant ventricular arrhythmia and mortality in repaired tetralogy of Fallot

For the work presented in this chapter, a detailed background and literature review were presented in the Chapter 1: Introduction and the conclusions are reiterated in the overall conclusions chapter of this thesis (Chapter 8).

Prospective study recruitment had taken place between 2002 and 2019. My exact role was to complete recruitment from 2016-2019. I participated in CMR data acquisition and reporting, LGE scoring, organising echo, ECG, BNP and CPEX investigations. I subsequently analysed the data, designed the risk scores, and wrote the manuscript and its revisions. The manuscript is currently in revision for consideration of publication in *JACC: cardiovascular imaging*.

Introduction and aims:

Patients with rTOF are at risk of premature death. Currently a risk stratification scheme that can robustly predict malignant ventricular arrhythmia (VA) and mortality in rTOF patients does not exist. It is not clear to clinicians how in this condition to use known multiple risk factors together pragmatically to risk stratify patients and the relative clinical weighting of each risk factor remains unknown.

In this study, we sought to identify patients at high risk of clinically important VA and death who may benefit from targeted therapy, by assessing the relative weighting of independent predictors of outcome including LGE CMR and devising an easy to apply clinical risk score.

Methods:

Patients and study design

We prospectively recruited consecutive rTOF patients ≥ 16 years old between 2002-2019 for LGE CMR in addition to standard tertiary care¹⁹⁶ (including 3 with dual chamber permanent pacemaker [1 conditional, 2 conventional])²⁰². Patients with contraindication to MRI or gadolinium were excluded. Patients provided written informed consent. The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

CMR image acquisition and analysis

The standardised CMR protocol for rTOF assessment was acquired in all patients as described in chapter 2: Methods⁴⁷. LGE images were obtained using an inversion-recovery gradient echo sequence with inversion times optimised to null normal myocardium by meticulous visual inspection of each image. Images were repeated in 2 separate phase-encoding directions or cross-cut to exclude possible areas of artefact and to define subtle RVLGE. Ventricular volumes analysis excluded trabeculations from RV and LV blood pool. Maximum length of RV outflow tract (RVOT) akinetic region and indexed right atrial area (RAAi) were measured as described in Chapter 2.

RVLGE was semi-quantified using the previously published segmental scoring system to account for the unique geometry of the RV (**see Figure 4.1**)⁴⁷ by myself and another experienced operator blinded to clinical data. LGE was considered present when in locations that either did not alter when re-imaged in the same plane with a phase-swap or remained visible in a second orthogonal or cross-cut plane. LVLGE was scored using the

standard 17-segment LV model. LVLGE related to apical vent at time of surgery and RV/LV septal insertion points was not included in the analysis as previously described⁴⁷. Inter-study reproducibility of LGE scoring was tested by repeating scans and analysis in 20 patients blind to previous study⁴⁷.

Standard of care investigations

As part of routine care patients undergo periodic 12-lead electrocardiograms (ECG), blood sampling for B-type natriuretic peptide (BNP), echocardiography and cardiopulmonary exercise testing (CPEX)^{1, 26}. RV restrictive physiology was defined by antegrade Doppler flow in the pulmonary artery in late diastole present throughout the respiratory cycle ('a' wave). Tricuspid regurgitation, RV systolic pressure (RVSP), tricuspid annulus planar excursion (TAPSE) and LV diastolic filling parameters were reported from echocardiography. Peak oxygen uptake (peak VO_2) was included when $\text{RER} > 1$. Ambulatory ECG Holter monitoring was performed if clinically indicated. Non-sustained VT was defined as ≥ 3 consecutive ventricular beats ≥ 100 bpm for ≤ 30 seconds duration and sustained atrial arrhythmia was defined as ≥ 30 seconds duration. These were recorded from Holter readings, routine pacemaker device interrogations and medical notes.

Follow-up and endpoints

Follow-up time was calculated from the time of index CMR until an endpoint had occurred or the last contact with the patient. Mortality status was verified from the UK Health and Social Care Information Service. Cause of death was established from death certificates, communication with the patient's primary care physician and review of medical records.

The primary endpoint was all-cause mortality. The secondary endpoint was ventricular arrhythmia (VA) which included SCD, resuscitated ventricular fibrillation (VF) and clinically sustained VT. Only the first event for each patient was included in the analysis. Clinically sustained VT was defined as VT $\geq$ 30 seconds duration or causing haemodynamic compromise requiring cardioversion. SCD was defined as an 'unexpected death' in the absence of progressive cardiac deterioration. Resuscitated VF was defined as an appropriate shock for VF or successful resuscitation following VF cardiac arrest.

Statistical analysis

Continuous data are summarised as means ($\pm$ SD) and medians with interquartile range. Comparison between groups was made by χ^2 , Fisher's exact test or Mann Whitney test. A 2-sided $p < 0.05$ was considered statistically significant. Intraclass correlation coefficient was used to assess reproducibility of LGE scoring. The association between variables and outcome was tested using Cox proportional hazards model. Significant univariable predictors of outcome were converted to categorical variables (based on highest/lowest quartile or decile) to allow for the development of a user-friendly risk stratification score. Relative beta coefficient values of only the variables that remained independently predictive of the outcomes and unrelated to one another in bivariable analysis were used as a guide to assign a relative weighting to each variable. A weighted-risk stratification score was thus derived for mortality and the secondary endpoint VA respectively. Receiver-operating-characteristic (ROC) curves were used to determine whether risk scores for mortality and VA could be used to predict outcome. The thresholds for each risk category for mortality and VA were selected based on sensitivity and specificity for outcome.

Cox-proportional hazards survival plots were generated to illustrate the survival differences between high, intermediate and low-risk categories for mortality and VA. Patients who already had the endpoints were censored at baseline. The performance of the devised risk score to predict mortality was compared using area under the curve (AUC) ROC analysis against other previously proposed models also applied to our cohort

Results

Study population

A total of 550 rTOF patients studied with LGE CMR (median age 32 years [23-42 years]; 57% male) were prospectively followed-up for a mean duration of 6.4 years (± 5.8 years). This represents 3512 patient-years of follow-up. Patient characteristics are summarised in **Table 4.1**. LGE clinical correlates were consistent with our previous description¹⁴. Inter-observer reproducibility of LGE scoring was highly reproducible (intra-class correlation coefficient 0.97 and 1 for the RV and LV respectively)⁴⁷ Clinical events at study end in relation to RVLGE extent are summarised in **Table 4.2**.

All-cause Mortality

During the follow-up period, a total of 27 deaths were recorded (13 SCDs, 12 deaths due to heart failure and 2 non-cardiac deaths). Univariable predictors are summarised in **Table 4.1** and were consistent with previous reports/consensus aside our novel finding that supramedian RVLGE score (≥ 5) is associated with higher mortality. Mortality related to increasing RVLGE extent (**see Figure 4.2**). Non-sustained VT, previous palliative shunt, ventriculotomy and QRS duration >180 ms were not univariable predictors of mortality. In

bivariable analysis, supramedian RVLGE remained an independent predictor of mortality [HR 11.4 (95% CI, 2.7-48.8); $P=0.001$] as did the presence of LVLGE, $RVEF \leq 35\%$, $RVEF \leq 47\%$, $LVEF \leq 55\%$, $LVEF \leq 35\%$, BNP levels $\geq 127\text{ng/L}$, $PVO_2 \leq 17\text{ml/kg/min}$, sustained atrial arrhythmia and age ≥ 50 years (see Table 4.3).

Risk Score for Predicting Mortality

A total weighted-risk score ≥ 51 demonstrated a 93% specificity vs sensitivity 51% for predicting all-cause mortality and was chosen as the lower threshold for the highest risk of death category. Conversely a total score ≤ 20 had a sensitivity 93% vs specificity 42% and was used as the upper threshold for the low-risk category. The applied risk score (see Figure 4.3) was a good discriminator of all-cause death [AUC 0.87 (95% CI 0.78-0.95); $P<0.001$]. For every 1-point increase in risk score there was an associated 7% increased risk of death (HR 1.07 [95% CI, 1.05-1.08]; $P<0.001$). Freedom from this outcome at 3, 5 and 10 years was 89%, 87%, and 64% respectively for the high-risk category, 99%, 97% and 94% for the intermediate risk and 99% up to 10 years for the low-risk category.

Ventricular Arrhythmia (VA)

A total of 29 patients reached the ventricular arrhythmia (VA) composite endpoint (10 SCDs, 3 resuscitated VF events; 2 of whom had appropriate shock for VF, 1 resuscitated VF arrest and 16 with documented sustained VT). Freedom from VA was compromised as RVLGE extent increases. (see Figure 4.2). Univariable predictors are summarised in Table 4.1. In bivariable analyses RVLGE score ≥ 5 remained an independent predictor as did LVLGE, $RVEF \leq 35\%$, $RVEF \leq 47\%$, $LVEF \leq 55\%$, $LVEF \leq 35\%$, $PVO_2 \leq 17\text{ml/kg/min}$, BNP levels $\geq 127\text{ng/L}$, RVOT akinetic length $\geq 55\text{mm}$ and RVSP $\geq 47\text{mmHg}$ (see Table 4.3).

Risk Score for Predicting VA

A total weighted-risk score ≥ 40 demonstrated the most favourable specificity 91% vs sensitivity 52% for predicting VA, hence was chosen as the lower threshold for the highest risk of VA category. Conversely a total score ≤ 20 with the most favourable sensitivity 90% over specificity 42% was used as the upper threshold for the low-risk category. The applied risk score (**Figure 4.4**) was also a good discriminator of the VA composite endpoint [AUC 0.79 (95% CI 0.69-0.88); $P < 0.001$].

A 1-point increase in risk score (**Figure 4.4**) was associated with a 7% increased chance of reaching the ventricular arrhythmia composite outcome (HR 1.07 [95% CI, 1.05-1.09]; $P < 0.001$). Freedom from this outcome at 3, 5 and 10 years for patients in the high-risk category was 81%, 79% and 76% respectively compared to 98%, 97% and 93% in the intermediate-risk and 99%, 99% and 97% in the low-risk category.

Histological assessment

In one patient (RV LGE score 4) who suffered a clinically sustained VT followed by sudden cardiac death, the heart was explanted and underwent detailed histopathological examination. There was excellent visual correspondence between the location and extent of replacement fibrosis seen on the explanted heart and *in vivo* LGE CMR. In a second patient, the RVOT patch was excised at the time of PVR and underwent microscopic assessment. There was also excellent agreement between microscopic fibrosis seen over the epicardial surface of the patch and LGE CMR (**see Figure 4.5**)

Table 4.1: Summary of patient characteristics

	All patients*(n=550) median (IQR), no (%)	All-cause mortality Hazard Ratio (95% CI)	P value	Ventricular Hazard Ratio (95% CI)	P value
Age at scan - y	32 (23-42)	1.06(1.03-1.09)	<0.001	1.04(1.02-1.06)	<0.001
Age ≥50y	66 (12%)	4.9(2.2-10.9)	<0.001	3.1(1.3-7.4)	0.01
Male	312 (57%)	1.4(0.6-3)	0.3	0.46 (0.2-1.08)	0.07
Palliative shunt	196 (36%)	2.3(1.1-5.3)	0.03	1.6(0.7-3.4)	0.2
Age of repair- y	4 (1.5-8)	1.06(1.03-1.09)	<0.001	1.04(1.004-1.07)	0.03
Age at repair ≥2y	391 (72%)	1.6(0.5-4.6)	0.4	2.1(0.7-5.9)	0.2
Ventriculotomy	418 (92%)	22.7(0.02-18324)	0.4	1.1 (0.26-4.7)	0.9
Transannular patch	145 (39%)	0.3(0.1-1.05)	0.06	0.6 (0.3-0.5)	0.3
RVOT patch	125 (34%)	1.4(0.5-3.9)	0.5	1.6 (0.6-4.1)	0.3
RV-PA conduit	75 (21%)	0.4(0.5-4.4)	0.5	0.5 (0.1-2.2)	0.4
Redo surgery to implant pulmonary valve [†]	152 (27%)	1.9 (0.8-4.8)	0.1	2.2(1.04-4.8)	0.04
NYHA ≥II	92 (17%)	5.4(2.5-11)	<0.001	3.9 (1.9-8.2)	<0.001
QRS duration [‡] - ms	153 (138-165)	1.01(0.9-1.04)	0.4	1.01(0.9-1.03)	0.3
QRS duration >180ms	46 (8%)	1.2(0.4-3.6)	0.7	1.4(0.5-4)	0.5
BNP [§] - ng/L	39 (23-65)	1.006(1.003-1.009)	<0.001	1.007(1.005-1.01)	<0.001
BNP ≥127ng/l	38	10.2(4.6-22.3)	<0.001	4.6(1.8-11.5)	0.001
RVEDVi - ml/m ²	114 (97-141)	1.01(1-1.01)	0.05	1.01(1.006-1.02)	<0.001
RVESVi -ml/m ²	54 (42-70)	1.01(1.006-1.03)	0.001	1.02(1.01-1.03)	<0.001
RV EF - %	54 (47-59)	0.92(0.8-0.96)	<0.001	0.9(0.8-0.95)	<0.001
RV EF ≤47%	141	3.6(1.7-7.8)	0.001	3.9(1.9-8.2)	<0.001
RV EF ≤35 %	16	5.7(2.2-15.3)	<0.001	6.4(2.4-16.8)	<0.001
RV mass/volume - g/ml/m ²	0.41 (0.36-0.48)	0.46(0.02-11.3)	0.6	1.5(0.06-36.8)	0.8
RVOT akinetic length - mm	34 (24-44)	1.04 (1.01-1.07)	0.003	1.05 (1.03-1.07)	<0.001
RVOT akinetic length ≥55mm	47	3.2(1.4-7.7)	0.008	3.9(1.8-9)	<0.001
RAAI - cm ² /m ²	12 (11-15)	1.3(1.2-1.4)	<0.001	1.2(1.07-1.3)	0.001
RAAI ≥16cm ² /m ²	66	2.4(0.95-5.9)	0.06	2.5(1.09-6)	0.03
LVEDVi - ml/m ²	80 (69-92)	1.02(1.01-1.03)	0.001	1.01(1-1.03)	0.05
LVESVi - ml/m ²	31 (25-40)	1.03(1.01-1.04)	<0.001	1.02(1.008-1.03)	0.002
LV EF - %	61 (56-66)	0.94(0.9-0.97)	<0.001	0.9(0.9-0.96)	<0.001
LV EF ≤55%	129	3(1.4-6.6)	0.004	2.7(1.3-5.8)	0.008
LV EF ≤35%	6	8.7 (2.6-28.9)	<0.001	7.8(1.8-32.9)	0.005
RVLGE score	5(3-7)	1.5(1.4-1.7)	<0.001	1.4 (1.3-1.6)	<0.001
RVLGE score ≥median	322	12.4(2.9-52.8)	0.001	8(2.4-26.7)	0.001
RVLGE score ≥upper quartile	121	22(7.5-64)	<0.001	10.5(4.6-23.7)	<0.001
LVLGE presence	41	7.2(1.7-10.7)	0.002	5.9 (2.6-13.7)	<0.001
Pulmonary regurgitation - %	22 (4-36)	0.98 (0.96-1.01)	0.2	0.99(0.98-1.02)	0.9
Restrictive RV physiology	118 (26%)	1.01(0.3-3.1)	0.9	0.7(0.2-2.2)	0.6
Restrictive RV physiology + RVEDVi ≥ 150ml/m ²	16 (14%)	2.7 (0.6-11.6)	0.2	3.5 (1.04-11.4)	0.04
Restrictive RV physiology + RVEDVi ≤115ml/m ²	55 (47%)	1.1 (0.3-3.8)	0.8	1.9 (0.7-5.7)	0.2
Tricuspid regurgitation ≥moderate	51 (9%)	0.6(0.1-2.5)	0.5	1.2(0.4-3.6)	0.6
RVSP - mmHg	37 (30-47)	0.99 (0.97-1.02)	0.8	1.02(1.004-1.04)	0.01
RVSP ≥47mmHg	113 (21%)	1.4(0.6-3.2)	0.4	2.5(1.2-5.3)	0.01
TAPSE - mm	15 (12-18)	0.95(0.86-1.04)	0.3	0.9(0.8-1.04)	0.3
LV E/A ratio	1.6(1.3-2)	1.5 (0.93-2.5)	0.09	1.3(0.7-2.1)	0.3
LV E/E' lateral wall	6.7 (5.3-8.5)	1.1(1-1.3)	0.05	1.1(0.9-1.2)	0.1
PVO ₂ - ml/kg/min [#]	26.3 (21-31.3)	0.88 (0.81-0.94)	<0.001	0.93(0.8-0.99)	0.02
PVO ₂ ≤17 ml/kg/m ²	50	3.9 (1.8-8.8)	0.001	4.5(1.5-7.9)	0.003
Inducible VT at PES	24/70 (34%)	2.9 (0.5-16.5)	0.2	1.9(0.4-7.9)	0.3
Non-sustained VT [^]	67/550 (12%)	1.1(0.4-3)	0.9	2(0.8-4.7)	0.1
Sustained atrial arrhythmia	62/550 (11%)	6.8(3.2-14.6)	<0.001	2.9(1.3-6.7)	0.009

*Repair for tetralogy of Fallot with pulmonary atresia and no systemic-pulmonary collaterals in 44 (8%), double outlet RV Fallot variant in 13 (3%), with absent pulmonary valve in 10 (2%). †Redo surgical pulmonary valve implantation occurred in 113 at baseline and 118 during follow up. Percutaneous pulmonary valve implantation occurred in 32 during follow up). ‡12-lead ECG was available in 500 (91%). §B-type natriuretic peptide (BNP) was available in 384 (70%). Echocardiography data on restrictive RV physiology was available in 447 (81%), Restrictive RV physiology + top decile RVEDVi ($\geq 150\text{ml/m}^2$) in 16 (14%) and lowest quartile RVEDVi ($\leq 115\text{ml/m}^2$) in 55 (47%). Right ventricular systolic pressure (RVSP) was available in 446 (81%), tricuspid annular planar excursion (TAPSE) in 473 (86%), LV E/A ratio in 509 (93%), and LV E/E' in 477 (87%).

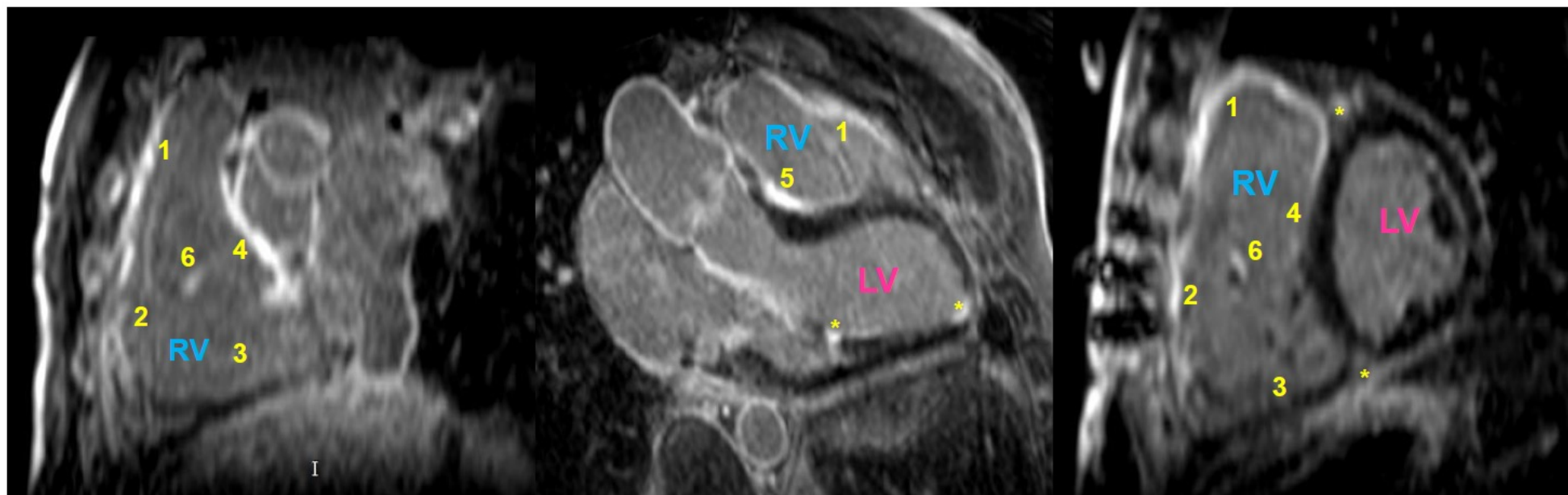
#Peak oxygen uptake (PVO_2) was available in 423 (77%). ^Holter monitoring was available in 142(26%). Non-sustained VT was recorded in 66 patients during follow up (median 12 beats: [8-18], median total cycle length 350ms: [300-3884ms]). EDVi; end-diastolic volume indexed to body surface, EF; ejection fraction, ESVi; end-systolic volume indexed to body surface area, LGE; late gadolinium enhancement, NYHA; New York heart Association classification, PES; programmed electrophysiological study, RAAi; right atrial area indexed to body surface area, RV; right ventricle, LV; left ventricle, RVOT; right ventricular outflow tract.

Table 4.2: Outcomes at study end at mean 6.4 years (± 5.8 years) follow-up from index CMR

Outcomes at study end	All patients	Supra-median RVLGE extent	Infra-median RVLGE extent	P-value
All-cause mortality at study end 	27	25	2	<0.001
Cardiovascular mortality [§]	25	24	1	<0.001
Ventricular arrhythmia - life threatening	29	26	3	<0.001
Clinical sustained ventricular tachycardia*	16	15	1	0.006
Sudden cardiac death	13	12	1	0.02
Heart failure death [‡]	12	12	0	0.002
Resuscitated VF [†]	3	3	0	0.3

*All-cause mortality included 27 deaths (25 cardiovascular deaths and 2 non-cardiac deaths secondary to metastatic breast cancer and a head injury). † There were 25 cardiovascular deaths (13 sudden cardiac death; SCD and 12 heart failure deaths). ‡There were 29 ventricular arrhythmia (VA) composite outcome events (16 clinical sustained VT, 10 SCD and 3 resuscitated VF). §Clinical sustained VT $\geq$ 30 seconds duration or haemodynamically significant VT requiring cardioversion. || Heart failure related deaths included 2 perioperative deaths caused by heart failure. #Resuscitated ventricular fibrillation comprised 1 patient successfully resuscitated from cardiac arrest with ventricular fibrillation and 2 patients receiving appropriate shocks for ventricular fibrillation.

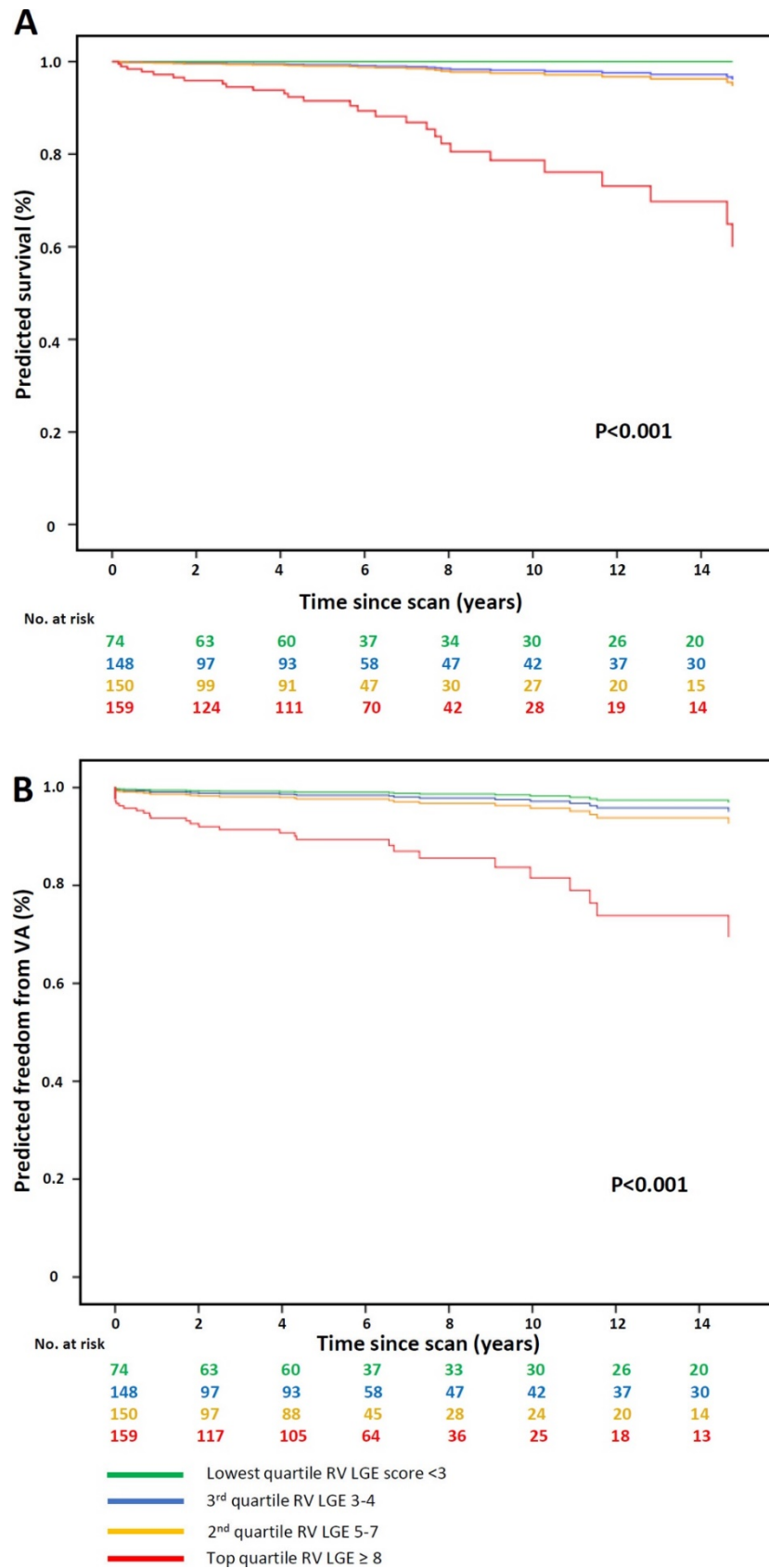
Figure 4.1:



1	Anterior wall of RVOT (0-3)	4	RV surface of septum (0-3)	Mild RV LGE	≤ 2
2	Anterior wall of RV (0-3)	5	VSD patch region (0-3)	Moderate RV LGE	3 or 4
3	Inferior wall of RV (0-3)	6	Trabecular bands (0-3)	Moderate to severe RV LGE	5-7
				Severe RV LGE	≥ 8

Example of severe right ventricle (RV) late gadolinium enhancement (LGE) extent in our Study participant. The total RV LGE score was 12. RV-LV insertion point LGE, left ventricle apical vent site LGE and LV papillary muscle enhancement (asterisks) was not included in the score and was common. Patients with a total RV LGE score ≥ 8 were in the top quartile for RV LGE burden and were graded as severe extent. Patients with a total RV LGE score 5-7 were graded as moderate-severe extent where score of 5 was the median. A total RV LGE score < 5 was deemed as mild or moderate. The segmental system used for scoring RV LGE has been previously published. The RV is divided into 6 segments (yellow numbers 1-6). Regions of RV LGE were scored according to linear extent (0= no enhancement 1 = up to 2 cm, 2=up to 3 cm, 3= 3 or more cm in length) and number of trabeculations enhanced including the moderator band (0= no enhancement, 1 = 1 trabeculation, 2=2-4. Scoring of LVLGE was performed using the universally accepted 17-segment LV model. Points were attributed to the proportion of LGE present in each myocardial segment, as visually judged: 0= No LGE, 1= up to 25% 2=up to 50%, 3= up to 75% and 4= up to and including 100% of the myocardium enhanced

Figure 4.2: RVLGE extent predicts mortality and VA



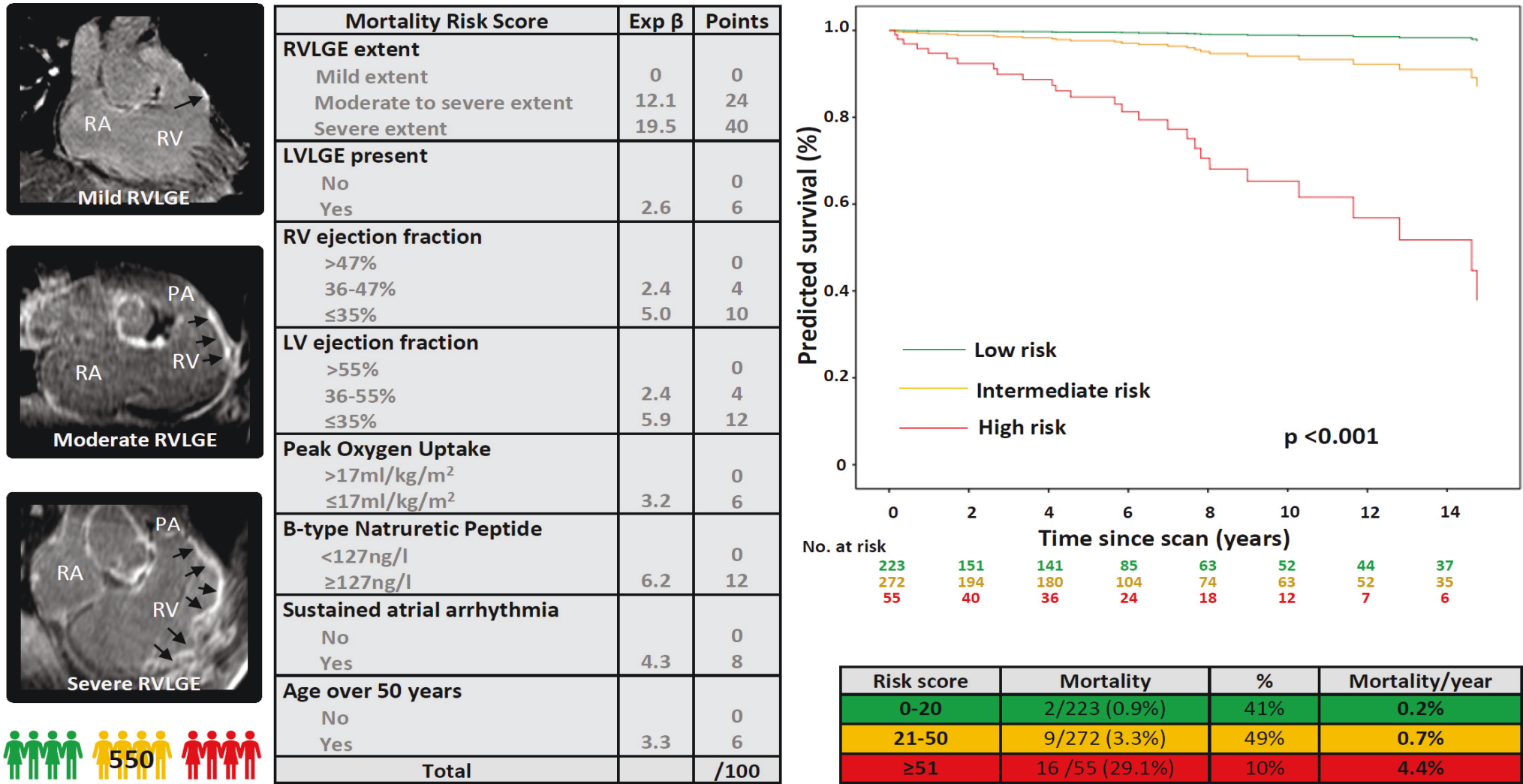
RVLGE extent predicts mortality and VA. Cox proportional hazard survival plots by RVLGE quartiles to predict all cause-mortality (top) and VA (bottom).

Table 4.3: Bivariable analysis.

	All-cause mortality		Ventricular arrhythmia	
	Hazard Ratio (95%	P-value	Hazard Ratio (95%	P-value
RVLGE score ≥median	10.8 (1.05-6.5)	0.001	6.6 (1.9-22)	0.002
LVLGE presence	2.6 (1.04-6.5)	0.03	4 (1.7-9.2)	0.001
RVLGE score ≥median	10.4 (2.4-44)	0.002	7.3 (2.2-24.8)	0.001
Age ≥50 years	3.3 (1.5-7.5)	0.004	2.3 (0.97-5.6)	0.06
RVLGE score ≥median	12.2 (2.8-52)	0.001	7.6 (2.3-25.7)	0.001
Age at repair ≥2 years	1.2 (0.4-3.5)	0.7	1.7 (0.6-4.8)	0.3
RVLGE score ≥median	10.7 (2.5-46.2)	0.001	7.4 (2.2-25)	0.001
Palliative shunt	1.8 (0.8-4)	0.1	1.3 (0.6-2.6)	0.5
RVLGE score ≥median	8.8 (2.04-38.6)	0.004	7.1 (2.1-23.9)	0.002
BNP ≥127 ng/l	6.2 (2.8-13.8)	<0.001	3.2 (1.3-7.9)	0.01
RVLGE score ≥median	9.9 (2.3-43.1)	0.002	6.3 (1.9-21.4)	0.003
RV EF ≤47%	2.4 (1.1-5.3)	0.02	2.8 (1.3-6)	0.006
RVLGE score ≥median	12.1 (2.8-51.7)	0.001	7.8 (2.3-26.1)	0.001
RV EF ≤35%	5 (1.9-13.5)	0.001	5.6 (2.1-14.9)	<0.001
RVLGE score ≥median	11 (2.6-47.6)	0.001	6.8 (2-23)	0.002
RVOT akinetic length ≥55mm	2 (0.8-4.8)	0.1	2.6 (1.1-5.9)	0.02
RVLGE score ≥median	11.7 (2.7-50)	0.001	7.5 (2.2-25)	0.001
RAA ≥16cm ² /m ²	1.6 (0.6-4.1)	0.3	1.8 (0.7-4.4)	0.2
RVLGE score ≥median	11.1 (2.5-47.5)	0.001	7.3 (2.1-24.5)	0.001
LV EF ≤55%	2.4 (1.1-5.2)	0.02	2.2 (1.06-4.7)	0.03
RVLGE score ≥median	11.4 (2.7-48.8)	0.001	7.8 (2.3-26.2)	0.001
LV EF ≤35%	5.9 (1.8-20)	0.004	6.4 (1.5-27.3)	0.01
RVLGE score ≥median	12.2(2.9-52.2)	0.001	7.5 (2.2-25)	0.001
RVSP ≥47mmHg	1.1(0.5-2.6)	0.8	2.2 (1.07-4.7)	0.03
RVLGE score ≥median	11.3 (2.6-48)	0.001	7.7 (2.3-25.8)	0.001
PVO ₂ ≤17 ml/kg/m ²	3.2 (1.5-7.4)	0.004	3.2 (1.4-7.4)	0.005
RVLGE score ≥median	8.7 (2-37.7)	0.004	7.1 (2.1-24)	0.002
Prior sustained atrial arrhythmia	4.3 (2-9.3)	<0.001	2 (0.9-4.5)	0.1
RVLGE score ≥median	12.6 (2.9-53.9)	0.001	7.6 (2.3-25.5)	0.001
Non-sustained VT	0.8 (0.3-2.4)	0.7	1.7 (0.7-3.9)	0.2

BNP; B-type natriuretic peptide, EDVi; end-diastolic volume indexed to body surface area, EF; ejection fraction, ESVi; end-systolic volume indexed to body surface area, LGE; late gadolinium enhancement, LV; left ventricle, PVO₂; peak oxygen uptake; RAAi; right atrial area indexed to body surface area, RV; right ventricle, RVOT; right ventricular outflow tract, RVSP; right ventricular systolic pressure

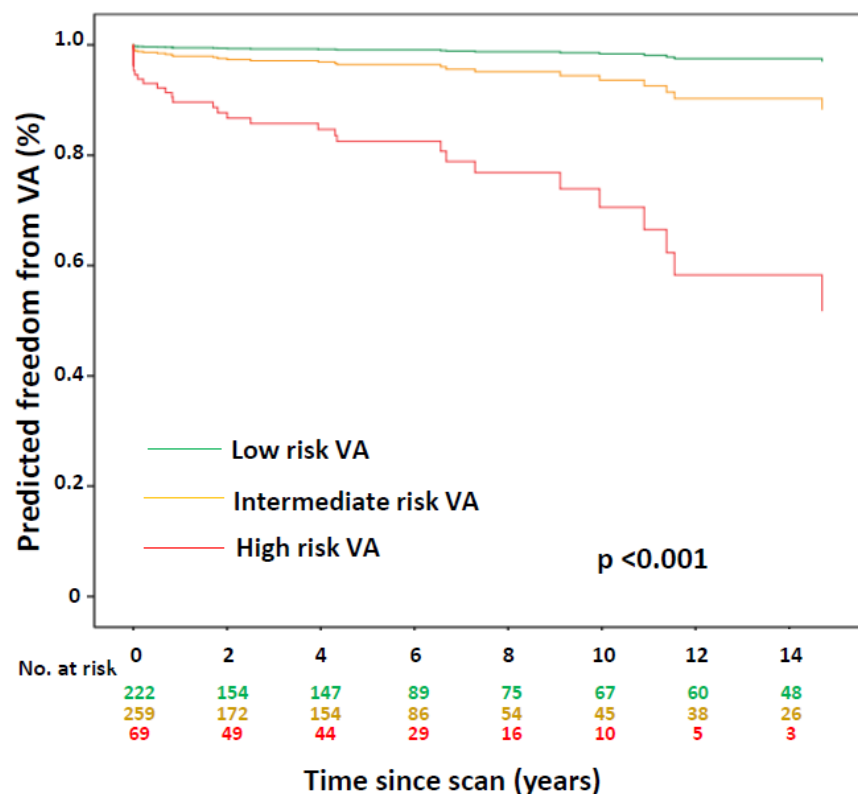
Figure 4.3: Annualised all-cause mortality rate according to risk category



Graphic to illustrate annualised all-cause mortality rate according to risk category. **Left:** risk score with weighted independent predictors of mortality. Points were allocated according to the exponential of the beta coefficient (exp β). **Top right:** cox proportional hazard survival plot showing percentage survival for each risk category. **Bottom right:** Risk categories, red (high-risk), amber (intermediate-risk) and green (low-risk) with corresponding mortality rate and annualised mortality rate.

Figure 4.4: Annualised rate of VA according to risk category.

Ventricular Arrhythmia Risk	Exp β	Points
RVLGE extent		
Mild extent	0	0
Moderate to severe extent	7.8	22
Severe extent	8.9	24
LVLGE present		
No		0
Yes	4	11
RV ejection fraction		
>47%		0
36-47%	2.8	8
≤35%	5.6	16
LV ejection fraction		
>55%		0
36-55%	2.2	5
≤35%	6.4	17
Peak Oxygen Uptake		
>17ml/kg/m ²		0
≤17ml/kg/m ²	3.2	9
B-type Natruretic Peptide		
<127ng/l		0
≥127ng/l	3.2	9
Akinetic RV outflow length		
<55mm		0
≥55mm	2.6	9
RV systolic pressure		
<47mmHg		0
≥47mmHg	2.2	5
Total		/100



Risk score	VA	%	VA/year
0-20	3/222 (1.4%)	40%	0.2%
21-39	11/259 (4.2%)	47%	0.9%
≥40	16 /69 (23.2%)	13%	3.7%

Graphic to illustrate annualised rate of VA according to risk category. **Left:** risk score with weighted independent predictors of VA. Points were allocated according to the exponential of the beta coefficient (exp β). **Top right:** cox proportional hazard survival plot showing freedom from VA for each category. **Bottom right:** Risk categories, red (high-risk), amber (intermediate-risk) and green (low-risk) with corresponding VA rate and annualised VA rate.

Table 4.4: Comparative performance against other proposed risk models

Risk Model applied for prediction of Mortality	AUC ROC, P value (CI)
Current PhD work mortality model 2020	0.87, P<0.001 (0.78-0.95)
Current model without LGE 2020*	0.81, P<0.001 (0.71-0.91)
Valente RVEF model 2014 [†]	0.64, P=0.02 (0.5-0.77)
Valente LVEF model 2014 [‡]	0.63, P=0.03 (0.49-0.76)
Bokma 2017 [§]	0.64, P=0.01 (0.54-0.75)
Khairy without invasive data 2008	0.56, P=0.3 (0.46-0.65)

*Scores were calculated using the model in **figure 4.2** without the inclusion of LGE CMR given that LGE is not in routine clinical practice for this condition.

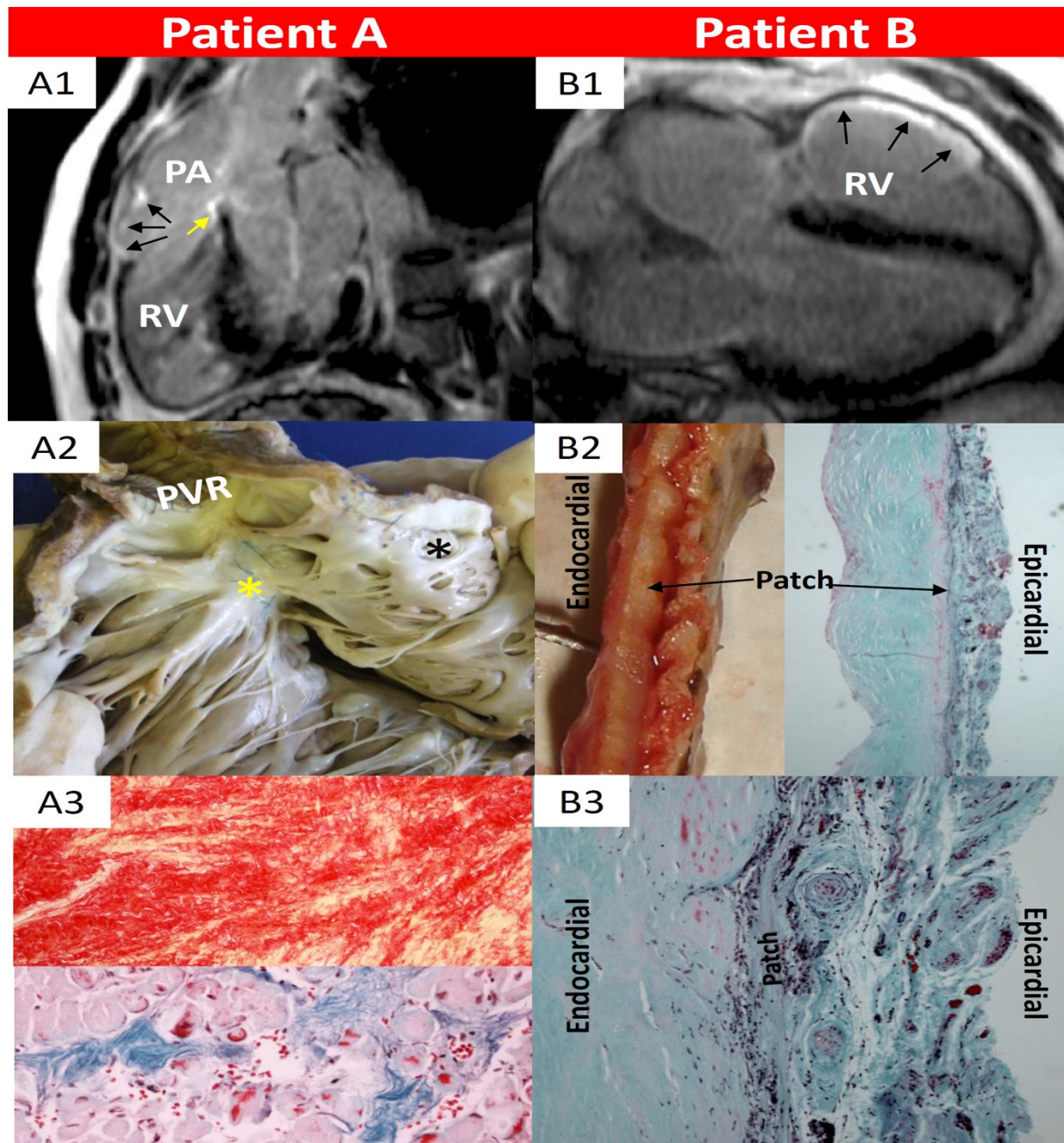
[†] To enable testing of existing models on our study cohort, points were allocated, using a similar approach to our study to the predictive cutoffs reported by Valente et al¹⁷: 3 points = RV mass/volume $\geq 0.3\text{g/ml}$, history of atrial arrhythmia, RVEF <48% in males / <50% in females. 2 points= RV mass/volume $\geq 0.3\text{g/ml}$, RVEF <48% in males / <50% in females. 1 point= RV mass/volume $\geq 0.3\text{g/ml}$

[‡] Points were allocated to the predictive cutoffs¹⁷ as follows: 3 points = RV mass/volume $\geq 0.3\text{g/ml}$, history of atrial arrhythmia, LVEF <55% in males / <54% in females. 2 points= RV mass/volume $\geq 0.3\text{g/ml}$, LVEF <55% in males / <54% in females. 1 point= RV mass/volume $\geq 0.3\text{g/ml}$

[§]Patients were scored using the point allocation prescribed by Bokma et al³²,

^{||} Calculated using the non-invasive parameters only given lack of invasive data available for most patients ²³

Figure 4.5: Correlation of RV LGE with histology.



Patient A (left column) *In vivo* CMR (**A1**) showing LGE in the ventricular septal defect (VSD) patch site (yellow arrow) and right ventricular outflow tract, RVOT (black arrows) below the pulmonary artery (PA). *Postmortem* macroscopic section of RV opened longitudinally (**A2**). VSD patch site (yellow asterisk) and RVOT (black asterisk). Microscopic examination (magnification x200) of the RVOT (**A3**) confirmed the presence of extensive collagen (with Picrosirius Red stain, the collagen stained red and areas with myocardium stained yellow (magnification x100). At higher magnification x200, with Masson's Trichrome stain showing areas of collagen staining blue and myocardium pale red below. **Patient B (right column)**- LGE CMR in a patient with a childhood RVOT patch repair (**B1**) and RVOT LGE (black arrows). Subsequent RVOT patch surgical excision at time of elective pulmonary valve replacement confirmed macroscopic (**B2 left**) and microscopic (**B2 right**; magnification x16) fibrosis (blue regions on the Masson's Trichrome stain) with endothelialisation over the epicardial and endocardial surface of the patch seen at higher magnification (x100)

Discussion

This is the largest prospective study to date that examines the association between LGE extent and long-term outcomes, in an adult rTOF cohort with a considerable follow-up period and hard clinical endpoints. Two weighted-risk scores have been devised that include LGE and other independent predictors of outcome to refine and enable personalised risk stratification in rTOF, to predict mortality and malignant ventricular arrhythmia. For the first time it is shown that the extent of LGE is a significant and independent predictor of mortality calling for its routine and periodic inclusion in the clinical surveillance of adults with rTOF.

Contemporary predictors of death and VA

It is well-recognised that no single risk factor accurately predicts adverse outcome in rTOF patients^{15, 30}. A multi-factorial risk score proposed in 2008 from a retrospective multicentre study was pioneering²³. However, the previous study included patients that were already considered high-risk with significant PR and included recurrent events in those with secondary prevention AICDs, hence limiting its application. Furthermore, invasive tests (LV end-diastolic pressure and VT inducibility) included in this 2008 risk score²³ are not considered pragmatic in unselected patients especially for serial study. The addition of LGE CMR to the risk assessment armamentarium that we propose here is non-invasive, hence safer and more applicable. Additionally, other non-invasive risk predictors have since been described^{17, 36, 48, 49, 199}. In keeping with the largest multicentre observational registry study to date (INDICATOR)¹⁷ atrial arrhythmia and CMR-derived RV and LV ejection fraction were predictors of outcome in our study. Prospective studies have also identified other CMR-

derived RAAi $\geq 16\text{cm}^{36}$ and RVOT akinetic length³⁶, peak $\text{VO}_2^{49, 199}$, BNP⁴⁸ and RVSP¹⁷. These parameters were also univariable predictors in our study. In contrast, QRS duration $>180\text{ms}$, previous palliative shunts or ventriculotomy were not predictive of outcome. This may relate to the changing profiles of more contemporary adult rTOF cohorts over the past few decades^{21, 50}. In the recent era, a more conservative approach to RVOT reconstruction, avoidance of ventriculotomy with a trans-atrial/trans-pulmonary approach and primary repair at a much younger age has evolved. We, like others,^{21, 56} found non-sustained VT (NSVT) to be benign; it was neither associated with mortality nor significant VA calling into question guidelines suggesting NSVT should be considered for AICD^{26, 196}. The secondary composite endpoint for VA intentionally did not include appropriate AICD shock due to concerns that appropriate AICD therapy could be delivered for potentially benign NSVT. Furthermore, the aim of this study was to predict prognosis and not device outcomes. This study's population reflects our tertiary centre's practice of being proactive in treating asymptomatic patients with PR before RV dysfunction ensues as per evolving consensus criteria¹⁹⁸ hence we cannot infer that the lack of association between PR and outcomes implies PR is not a risk factor.

TOF Risk Score for guiding primary prevention AICD

In acquired heart disease, a survival benefit from primary prevention AICD was demonstrated in patients with a minimum 3.5% annual risk of SCD²⁰³. Physical and mental health morbidity²⁴ is significant for ACHD patients living with AICDs mandating its consideration in AICD decision-making. Given that SCD is regularly documented in high-volume centres²¹, it is clear that the current *status quo* is unacceptable. Clinicians and patients want more clarity and precision for the selection of rTOF patients for primary

prevention AICD. In this study we have identified addressable high-risk groups of patients that have an estimated 4.4% annual risk of mortality and 3.7% risk of VA who could be considered for primary prevention with AICD or VT ablation. These patients comprise 10% and 13% of the total cohort of rTOF patients, respectively. On the other end of the risk spectrum, patients in the low-risk category had only 0.2 % annual risk of VA, thus can be reassured. We anticipate that a re-evaluation of risk would be triggered with change in clinical status or when a routinely timed non-invasive test shows change; LGE would not be added to CMR study at every visit.

Risk is a continuum throughout a patient's life and so the inherent limitation of having a categorical risk score design is acknowledged; nevertheless, this is easier to apply in clinical practice.

Limitations

This was a single centre study, yet the cohort was large and followed for a long period with rTOF repair at many centres (7 in the UK and other international) hence reflective of various surgical eras and approaches and representative of this heterogeneous population. RVLGE CMR acquisition requires training to avoid false negative reporting⁴⁷. The previously published RV segmental scoring system for LGE⁴⁷ given its high reproducibility and its simplicity continued to be used for consistency in this prospective study. Signal-intensity based thresholds might be considered an alternative for quantifying RVLGE but are limited by partial volume effects, sternal wire artefact, epicardial fat and the thin RV wall. No studies to date have validated this in the uniquely shaped RV post rTOF repair¹³⁵. It is expected that the mortality and VA risk scores presented may no longer remain relevant to future rTOF populations due to changing risk profiles anticipated with

even further improvement in surgical, catheter techniques and medical management. LGE extent may become less predictive like QRS duration and palliative shunt in our current era. A small number of Patients with Fallot variant anatomy were included in this study (see Table 4.1) however we did not include patients with complex pulmonary atresia with significant MAPCAs. Patients with absent pulmonary valve were included although a very small number unlikely to affect the study outcome

Future direction

External validation of these risk score algorithms is the next step. Applying LGE to the RV is becoming easier and more comprehensive with the advent of operator-independent LGE sequences¹⁸⁷, higher resolution 3D LGE¹²⁶, automatic quantification²⁰⁴ and machine learning which will aid widespread clinical application of this work. Future studies of fibrosis burden will quantify LV interstitial fibrosis (T1 mapping CMR) and require bespoke approaches for the RV.

Conclusions

Most of the growing population of adults living with repaired tetralogy of Fallot can expect long and healthy lives, but a small minority are at much higher risk for premature cardiovascular death. For the first time we show LGE extent is prognostic justifying its inclusion in clinical practice. Weighted-clinical risk scores that include LGE extent are presented to identify patients at high risk for death or life-threatening VT that may benefit from primary prevention AICD or other targeted therapy.

Chapter 5: RV native T1 mapping has incremental value in the early detection of adverse remodelling

For the work presented in this chapter, a detailed introduction and literature review were presented in Chapter 1: Introduction and the conclusions are reiterated in the overall conclusions chapter of this thesis (Chapter 8).

Prospective study recruitment had taken place between 2017 and 2019. I was involved in recruitment into this study from its inception. I participated in CMR data acquisition for patients and for healthy volunteers. I reported the CMR data, performed T1 mapping analysis, segmented the 3D LGE and participated in organising and collating data from other tests including echocardiography, ECG, BNP levels and CPEX. Subsequently, I analysed the data and drafted the manuscript.

Introduction and aims

Diffuse fibrosis, defined by LV T1 mapping, is associated with ventricular arrhythmia and mortality in patients with acquired left-sided heart disease. The potential to reverse diffuse fibrosis with anti-fibrotic therapy and to help time PVR in adult patients with rTOF makes the idea of T1 application in the RV particularly attractive.

Currently there are no T1 mapping sequences available that are dedicated to measuring T1 in the thin walled RV. We sought to apply a native T1 MOLLI sequence specifically developed with the intention of improving sensitivity to diffuse fibrosis and to meet the spatial resolution requirements of the RV in a cohort of adult rTOF patients and healthy volunteers.

Cross-sectional examination between CMR defined fibrosis (both T1 mapping and LGE) with other known parameters of adverse risk in rTOF were examined.

Methods

Patient selection

Adult rTOF patients (>16 years old) were prospectively recruited and consented for T1 mapping and 3D LGE CMR at the Royal Brompton Hospital between 2017-2019. Patients with MRI and gadolinium contrast contraindications were excluded, as well as patients with arrhythmia at the time of scan (to limit artefact from cardiac motion). A reference control cohort of 30 healthy adult volunteers without prior history of cardiovascular disease or diabetes were also recruited for T1 mapping and 3D LGE CMR. The study was locally registered and approved.

CMR acquisition

CMR was acquired on a 1.5 Tesla scanner using a standardised protocol as previously described in Chapter 2: Methods. Native T1 mapping was performed using a modified Look-Locker inversion recovery (MOLLI) sequence 5(4)5. This sequence was optimised in attempt to improve sensitivity to fibrosis by primarily using a higher flip angle²⁰⁵ (typical image parameters: TR 300ms, TE 1.15ms, flip angle 55°, field of view (FoV), 360mm matrix 256 x 200mm) and a narrower slice thickness of 6mm slice to improve spatial resolution. T1 mapping was performed in a mid-ventricular short axis orientation with meticulous care to ensure that the angle of acquisition was directly perpendicular to the thickest part of the RV wall (inferior or anterior wall) in at least two views during mid-diastole (the stillest part of the cardiac cycle) in order to minimise partial volume effect¹⁵². Like-images were

separately obtained for the LV except with ensuring that the angle of acquisition was directly perpendicular to the LV septum. T1 maps for LV and RV were repeated twice.

Extra-cellular volume (ECV) was derived using a second standard 11 heart beat MOLLI sequence^{152, 206} (TR 200, TE, flip angle 35°, FoV 360mm, matrix, 6mm slice thickness) applied pre-contrast (5(3)5) and 14-18 minutes post contrast (0.12mmol/kg) administration (4(1)3(1)2) in the same short axis orientation. Haematocrit was measured at time of CMR from venous blood samples to use in the calculation for ECV¹⁵⁶.

For two-dimensional (2D) LGE images acquired in long and short axis using an inversion-recovery gradient echo sequence (8mm slice thickness), inversion times were optimised to null normal myocardium by close visual inspection. Three-dimensional (3D) LGE using a respiratory-navigated continuous adaptive windowing strategy (CLAWS)¹²⁷ inversion-recovery, gradient-echo sequence was acquired once the optimal inversion time was reached (typically at least 20 minutes post-contrast injection). 3D LGE coverage of the entire heart typically required 64 slices at 1.5x1.5x4mm³ pixel resolution.

Routine clinical data

As part of standard care for patients with ACHD^{26, 196}, interval 12-lead electrocardiogram (ECG), blood samples for b-type natriuretic peptide (BNP), echocardiography, cardiopulmonary exercise testing (CPEX), and CMR are performed. Ambulatory ECG Holter monitoring was performed if clinically indicated by symptoms volunteered or for risk stratification. Prior sustained VT defined as ≥ 3 consecutive ventricular beats ≥ 100 bpm for ≥ 30 seconds and sustained atrial arrhythmia defined as ≥ 30 seconds were recorded. RV restrictive physiology was assessed on echocardiography using diastolic forward flow through the pulmonary artery, PA ('a wave'). Tricuspid

regurgitation, tricuspid annulus plane systolic excursion (TAPSE), right ventricular systolic pressure (RVSP) and peak pulmonary valve velocity were also measured on echocardiography. CPEX was assumed valid within 1 year from the CMR study.

Statistical analysis

Continuous data are summarized as mean ($\pm$ SD) or median (interquartile range) depending on normality. Correlation of ranked continuous data was tested with Spearman's rank correlation (ρ). Comparisons between patient and control groups was made using a Mann-Whitney test, chi-squared test or Fisher's exact test as appropriate. Differences in mean ranks of variables categories of fibrosis severity was tested with a Kruskal Wallis test. Subsequent post hoc analysis with Dunn's pairwise testing was used to determine where significance between each pairing in the group lies. All tests were 2-sided and a P value of <0.05 was considered significant. For the assessment of intra-observer and inter-observer reproducibility, variability was expressed as the coefficient of variation (COV, percent), derived from the within-subject SD divided by the mean multiplied by 100%. Intra-class correlation coefficients (ICC) were calculated using absolute agreement, 2-way mixed model. Statistical analysis was performed with SPSS (IBM Statistics V.22).

Results

Study patients

A total of 148 adult rTOF patients (median age 30 years: 23-41 years, 46% male) and 30 healthy controls (median age 33 years: 29-44 years, 47% male) underwent T1 mapping (both native T1 and ECV) as well as 3D LGE CMR.

Native T1 maps using the optimised high-sensitivity sequence were acquired in 107 (72%) patients, where it was possible to perform analysis successfully for 102 patients (95%) in the RV and in all patients (107,100%) in the LV. In 5 rTOF patients, it was not possible to draw an adequately sized ($\text{ROI} \geq 0.15\text{cm}^2$) in the inferior RV wall or in the anterior RV wall secondary to sternal wire artefact therefore, those T1 maps were excluded from the analysis. In the healthy control group, it was possible to analyse T1 in 15 (50%) volunteers for the RV and in 30 (100%) for the LV.

Haematocrit was available in 132 (89%) for the calculation of ECV. Four haematocrit measurements (3%) were excluded from the analysis due to significant anaemia. RV ECV calculations were possible for 121 patients (95%) whereas LV ECV was possible in all cases 128 (100%) with acceptable haematocrit. 3D LGE was available in 128 (87%).

Patients compared to controls

Baseline demographics and patient characteristics are summarised in **Table 5.1**. There was no significant difference in age, gender, body surface area or haematocrit between patients and controls.

Patients had significantly increased RV native T1 and RV ECV compared to controls (1064ms: 1015 -1103ms versus 995ms:961-1013ms, $P<0.001$ and 33%:30-37% versus 31%:29-33% respectively). However, for the LV there was no significant difference for native T1 and ECV between the patient and control group (992ms:959-1020ms versus 976ms:995-1015ms, $P=0.4$ and 27%:24-28% versus 25%:23-38%, $P=0.3$ respectively).

LGE was found in the RVOT and RV septum in all patients (100 %), in RV trabeculations in 4% and at the site of previous LV apical vent insertion in 36%. There was no LGE found in the control group (insertion point LGE was not included in the analysis due to its clinical insignificance⁴⁷).

Compared to healthy controls, patients had significantly higher indexed RV end-diastolic volumes, RVEDV ($P<0.001$), RV mass ($P<0.001$) and lower RV EF ($P<0.001$). They also had significantly higher indexed LV end-systolic volumes, LVESVi, ($P<0.001$), and lower LVEF ($P<0.001$). **See Table 5.1.**

T1 mapping parameters association with adverse markers of outcome

Linear correlations for both RV/LV T1 parameters (native T1 and ECV) and LGE volume are detailed in **Table 5.2**. There was no significant association between RV/LV T1 parameters and LGE volume ($r=0.1$, $P=0.3$) found. Strong ventricular-ventricular correlation between RV native T1/ECV with LV native T1/ECV ($r=0.4$, $P<0.001$) was demonstrated.

Higher native RV T1 correlated moderately with later repair ($r=0.22$, $P=0.02$) and elevated BNP levels ($R=0.22$, $P=0.03$) however correlated negatively with QRS duration ($r=-0.24$,

P=0.01) and echo markers of RV pressure overload [RVSP ($r=-0.30.3$, $P=0.03$) and peak pulmonary valve velocities ($R=-0.33$, $P=0.002$)]. Native LV T1 correlated with late repair, worse peak VO₂, and smaller RV:LV diastolic volume ratios. LVECV was associated with longer QRS duration and worse peak VO₂.

LGE association with adverse markers of outcome

Focal fibrosis burden defined by 3D LGE volume had more significant and stronger associations with older age ($p<0.001$), late repair ($p<0.001$), longer QRS duration ($p<0.001$), higher BNP levels ($p<0.001$), lower peak VO₂ ($p=0.006$), higher RVSP ($p=0.004$) and peak pulmonary valve velocity ($p=0.01$), larger akinetic length in the RVOT ($p<0.001$), larger RV/LV volumes ($p<0.001$), lower RV EF ($p=0.002$) (**See Table 5.2**).

Patients without previous pulmonary valve replacement (PVR)

A sub-analysis was performed of rTOF patients who had not had a prior PVR since their original repair ($n=87$). Patient demographics and clinical characteristics are summarised in **Table 5.3**. Patients in this subgroup had higher native RV T1 and RV ECV in comparison to the subgroup of patients ($n=62$) who had undergone PVR [Native T1 1074(1037-1110ms) versus 1030 (1002-1098ms) $P=0.001$ and RVECV 35 (31-37%) versus 30 (28-35%) $P=0.001$ respectively]. Like findings in the combined cohort detailed above, elevated RV/LV native T1 and ECV were significantly associated with late age of repair and elevated BNP level. Increased LV ECV was also associated with impaired peak VO₂.

Conversely raised native RV T1 was significantly associated with reduced markers of RV pressure overload (reduced RVSP and PS). Further sub-divisions of this cohort in to 3 groups;

patients with $\leq$ trivial PR and no PS, at least mild PR only and mixed pulmonary valve disease did not relate to significant differences in RV/LV T1 parameters or LGE volume.

Individual and combined value of native T1 and LGE

In the subset of rTOF patients without prior history of PVR (n=87), the associations of top quartile RV native T1 (≥ 1077 ms) and top quartile RV LGE volume (≥ 10.2 cm³) when tested separately and in combination with each other against adverse markers in rTOF are described in **Table 5.4**.

Patients with a combined top quartile native RV T1 and top quartile LGE had late TOF repairs (P=0.01), significantly larger RV volumes (RVEDVi, P=0.002, RVESVi, P=0.005), higher RV mass (P=0.007), larger right atrial area (RAAi, P=0.03) and akinetic RVOT segments (p=0.01) when compared to patients who had both less than top quartile RV T1/ LGE **See Figure 5.1 and Table 5.4**

Patients with only top quartile native RV T1 had significantly larger RVEDVi (p=0.001) and RVESVi (p=0.01) when compared to patients with both less than top quartile RVT1/LGE. Conversely, the presence of top quartile RV LGE in patients with already top quartile RV T1 was significantly associated with later repair (P=0.03), larger RAAi (p=0.01), and longer akinetic RVOT segments (p=0.03). **See Figure 5.1 and Table 5.4**

T1 mapping correlation with histology

In one patient, an RV myocardial biopsy excised from the body of the RV (anterior free wall below the RVOT) during time of their operation was histologically assessed for fibrosis using picrosirius red stain (**see Figure 5.2**). The collagen volume fraction measured was 20.6%. In the similar region to where the biopsy was taken from in the anterior RV wall, a high native RV T1 (1094ms) and RV ECV (29%) was measured in the absence of LGE. Post-operative T1 CMR in a similar region of the anterior RV wall at 7 days following successful PVR to treat significant PR demonstrated a marked increase in native RV T1 (1591) and RV ECV (35%) values followed by a significant reduction in RV native T1 (1145ms) and RVECV (33%) at 6 months post PVR. Pre-operative native LV T1 (953ms) and LVECV (27%) similarly increased at 7 days post operation (984ms and 28% respectively) before decreasing to 900ms and 22% respectively 6 months post PVR.

Table 5.1: Characteristics of patients and controls.

	Patients (n=148)	Controls (n=30)	p-value
Age at CMR, years	30 (23-41)	33 (29-44)	0.1
Male, n	68 (46%)	14 (47%)	0.6
BSA, m ²	1.78 (1.6-1.98)	1.8(1.71-1.95)	0.5
Haematocrit	0.41(0.39-0.44)	0.39 (0.38-0.43)	0.1
Palliative shunt, n	52 (35%)		
Age at repair, years	2 (1-6)		
Transannular patch, n	44 (30%)		
RVOT patch, n	44 (30%)		
RV-PA conduit, n	22 (15%)		
Redo-PVR, n*	61 (41%)		
Sustained atrial arrhythmia, n [†]	14 (9%)		
Sustained VT, n [†]	2 (1%)		
Non-sustained VT, n [†]	7 (5%)		
Hospital admission for heart failure, n	2 (1%)		
NYHA ≥II, n	20 (14%)		
QRS duration, ms [‡]	153 (140-164)		
QRS ≥ 180ms, n	11 (7%)		
BNP ng/L [§]	45 (27-78)		
Peak VO ₂ , ml/kg/min	25 (21-33)		
Percent predicted Peak VO ₂ , %	75 (61-86)		
Restrictive RV physiology, n#	54 (36%)		
TAPSE, mm	15 (12-17)		
At least moderate TR, n	28 (19%)		
RVSP, mmHg	34(25-46)		
FAC %	36 (40-42)		
PV peak velocity	2.1 (2.8-2.7)		
RVEDVi, ml/m ²	113 (96-127)	83 (75-95)	<0.001
RVESVi, ml/m ²	50 (41-61)	48 (40-53)	0.5
RVEF, %	55 (49-60)	65 (63-68)	<0.001
RV mass g/m ²	47 (39-52)	34 (30-39)	<0.001
LVEDVi, ml/m ²	85 (74-95)	79 (71-90)	0.8
LVESVi, ml/m ²	34 (28-41)	26 (21-32)	0.009
LVEF, %	61 (57-65)	68 (64-71)	<0.001
RV:LV diastolic volume ratio	1.3 (1.1-1.5)	0.95 (0.91-1.03)	<0.001
RAAi, cm ² /m ²	13 (11-15)		
LAAi, cm ² /m ²	11 (9-13)		
PR fraction, %	14 (4-31)		
RVOT akinetic length, mm	35 (28-43)		
RV ECV, %	33 (30-37)	31 (29-33)	0.01
Native RV T1 (14HB MOLLI), ms	1064(1015-1103)	995 (961-1013)	<0.001
LV ECV, %	27 (24-28)	25 (23-38)	0.3
Native LV T1 (14HB MOLLI), ms	992 (959-1020)	976 (995-1015)	0.4
RV LGE, cm ³	8.1 (5.8-11.1)	0	<0.001
Total LGE, cm ³	8.3 (6-11.7)	0	<0.001

Repair for tetralogy of Fallot with pulmonary atresia and no systemic-pulmonary collaterals in 12 (8%), double outlet RV Fallot variant in 2 (1%) and with absent pulmonary valve in 1 (0.6%) * 5 patients had percutaneous pulmonary valve implantation with Melody valve. [‡] 12-lead ECG was available 148 (100%), [§] BNP was available in 128 (85%), ^{||} CPEX was available in 122 (82%), [#] echo data was available in 138 (93%).

Table 5.2: Associations of RV and LV T1 mapping parameters and LGE with markers of adverse risk in rTOF.

	Native RV T1 (ms)		RV ECV (%)		Native LV T1 (ms)		LV ECV (%)		LGE (cm ³)
N=148	r-value	P-value	r-value	P-value	r-value	P-value	r-value	P-value	r, P- value
Age at CMR, years	0.03	0.7	0.05	0.6	0.1	0.3	0.07	0.4	0.4, <0.001
Age at repair, years	0.22	0.02	0.08	0.4	0.3	0.005	0.1	0.3	0.3 <0.001
QRS duration ms	-0.24	0.01	-0.07	0.4	-0.1	0.2	-0.1	0.3	0.4, <0.001
BNP n/g/L	0.22	0.03	0.1	0.2	0.2	0.03	0.22	0.01	0.4, <0.001
Peak VO ₂ , ml/kg/min	-0.15	0.1	-0.1	0.3	-0.2	0.08	-0.2	0.03	-0.2, 0.006
Percent predicted Peak VO ₂ , %	0.1	0.3	-0.01	0.9	-0.03	0.8	-0.01	0.9	-0.2, 0.03
TAPSE, mm	-0.01	0.9	0.1	0.1	0.08	0.4	0.2	0.08	0.1, 0.4
RVSP, mmHg	-0.3	0.03	-0.3	0.001	0.08	0.4	-0.03	0.7	0.3, 0.004
FAC %	-0.1	0.3	0.1	0.2	-0.05	0.6	0.2	0.1	-0.2, 0.06
PV peak velocity	-0.33	0.002	-0.3	0.002	0.02	0.8	0.04	0.6	0.3, 0.01
RVEDVi, ml/m ²	0.14	0.2	0.1	0.1	-0.1	0.2	-0.1	0.2	0.2, 0.006
RVESVi, ml/m ²	0.02	0.8	0.1	0.7	-0.02	0.2	-0.1	0.2	0.35, <0.001
RVEF, %	0.1	0.4	0.02	0.8	-0.01	0.9	0.06	0.5	-0.27, 0.002
RV mass g/m ²	-0.2	0.05	-0.16	0.08	-0.05	0.6	0.02	0.8	0.28, 0.002
LVEDVi, ml/m ²	-0.2	0.06	-0.16	0.08	0.08	0.4	-0.03	0.6	0.3, 0.001
LVESVi, ml/m ²	-0.1	0.4	-0.02	0.8	0.06	0.5	-0.12	0.15	0.28, 0.002
LVEF, %	-0.3	0.06	-0.01	0.9	-0.1	0.4	0.1	0.2	-0.2, 0.06
RV:LV diastolic volume ratio	-0.01	0.9	0.01	0.9	-0.22	0.02	0.5	0.6	-0.03, 0.8
RAAi, cm ² /m ²	0.06	0.6	0.02	0.8	0.07	0.5	0.1	0.3	0.4, <0.001
LAAi, cm ² /m ²	0.05	0.6	0.1	0.2	0.1	0.14	0.2	0.08	0.03, 0.8
PR fraction, %	0.1	0.3	0.1	0.3	-0.02	0.8	0.2	0.07	0.07, 0.5
RVOT akinetic length, mm	-0.01	0.5	-0.1	0.2	0.1	0.1	0.1	0.2	0.5, <0.001
RV ECV, %	0.5	<0.001					0.43	<0.001	-0.1, 0.3
Native RV T1 (14HB MOLLI), ms			0.5	<0.001	0.4	<0.001			-0.04, 0.7
LV ECV, %			0.4	<0.001	0.36	<0.001			-0.01, 0.9
Native LV T1 (14HB MOLLI), ms	0.4	<0.001					0.36	<0.001	0.1, 0.3

Table 5.3: Demographics and clinical characteristics for subgroup of patients without previous PVR.

	Without re-do PVR (n=87)
Age at CMR, years	28 (21-38)
Male, n	39
Palliative shunt, n	24
Age at repair, years	2 (1-6)
Transannular patch, n	26
RVOT patch, n	27
RV-PA conduit, n	7
Trivial PR and no PS, n (%)	15 (17%)
At least moderate PR and no PS, n (%)	32 (38%)
Mixed pulmonary valve disease, n (%)	38 (44%)
Sustained atrial arrhythmia, n†	10
Non-sustained VT, n†	1
Hospital admission for heart failure, n	1
NYHA ≥II, n	10
QRS duration, ms‡	151 (133-162)
QRS ≥ 180ms, n	5 (6%)
BNP ng/L§	43(25-71)
Peak VO2, ml/kg/min	25 (21-33)
Restrictive RV physiology, n#	0
TAPSE, mm	15 (13-18)
At least moderate TR, n	0
RVSP, mmHg	30 (24-42)
FAC %	39 (32-44)
PV peak velocity	2 (1.6-2.4)
RVEDVi, ml/m2	115 (97-129)
RVESVi, ml/m2	50 (41-60)
RVEF, %	56 (50-61)
LVEDVi, ml/m2	82 (73-94)
LVESVi, ml/m2	34 (28-43)
LVEF, %	60 (57-64)
RV:LV diastolic volume ratio	1.4 (1.1-1.6)
RAAi, cm2/m2	12 (10-15)
LAAi, cm2/m2	10(9-13)
PR fraction, %	26 (8-37)
RVOT akinetic length, mm	34 (27-39)
RV ECV, %	35(31-37)
Native RV T1 (14HB MOLLI), ms	1074 (1037-1110)
LV ECV, %	27(24-28)
Native LV T1 (14HB MOLLI), ms	994 (964-1020)
RV LGE, cm3	7.3(5.1-10)
Total LGE, cm3	7.6(5.5-10.2)

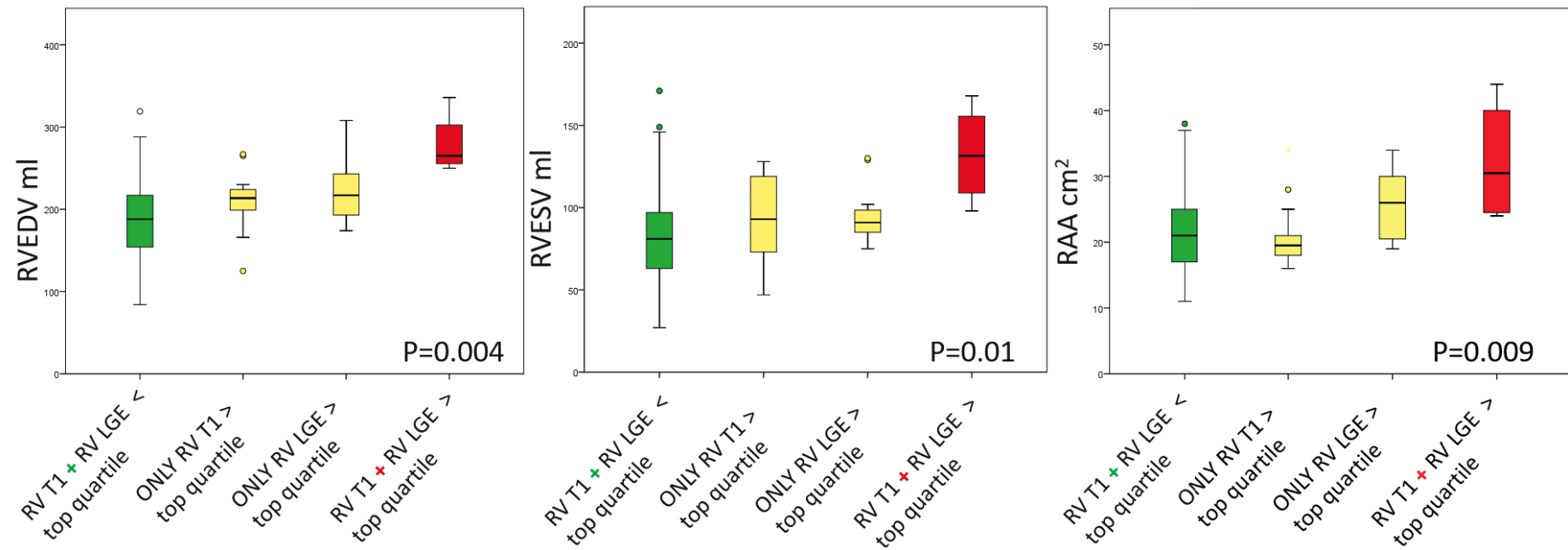
Table 5.4: Associations of T1 mapping parameters and LGE in sub-study of patients without prior pulmonary valve replacement.

Patients without prior PVR (n=87)	Native RV T1 (ms)		RV ECV (%)		Native LV T1 (ms)		LV ECV (%)		LGE (cm ³)
	r-value	P-value	r-value	P-value	r-value	P-value	r-value	P-value	r, P- value
Age at CMR, years	0.1	0.3	0.05	0.6	0.1	0.3	0.07	0.4	0.4, <0.001
Age at repair, years	0.04	0.75	0.08	0.4	0.3	0.005	0.1	0.3	0.3 <0.001
QRS duration ms	-0.24	0.01	-0.07	0.4	-0.1	0.2	-0.1	0.3	0.4, <0.001
BNP n/g/L	0.22	0.03	0.1	0.2	0.2	0.03	0.22	0.01	0.4, <0.001
Peak VO ₂ , ml/kg/min	-0.15	0.1	-0.1	0.3	-0.2	0.08	-0.2	0.03	-0.2, 0.006
Percent predicted Peak VO ₂ , %	0.1	0.3	-0.01	0.9	-0.03	0.8	-0.01	0.9	-0.2, 0.03
TAPSE, mm	-0.01	0.9	0.1	0.1	0.08	0.4	0.2	0.08	0.1, 0.4
RVSP, mmHg	-0.3	0.03	-0.3	0.001	0.08	0.4	-0.03	0.7	0.3, 0.004
FAC %	-0.1	0.3	0.1	0.2	-0.05	0.6	0.2	0.1	-0.2, 0.06
PV peak velocity	-0.33	0.002	-0.3	0.002	0.02	0.8	0.04	0.6	0.3, 0.01
No PR vs Pure at least moderate PR vs mixed pv disease		0.2		0.1		0.7		0.2	P-0.06
PR fraction, %	0.1	0.3	0.1	0.3	-0.02	0.8	0.2	0.07	0.07, 0.5
RVESVi, ml/m ²	0.02	0.8	0.1	0.7	-0.02	0.2	-0.1	0.2	0.35, <0.001
RVEF, %	0.1	0.4	0.02	0.8	-0.01	0.9	0.06	0.5	-0.27, 0.002
RV mass g/m ²	-0.2	0.05	-0.16	0.08	-0.05	0.6	0.02	0.8	0.28, 0.002
LVEDVi, ml/m ²	-0.2	0.06	-0.16	0.08	0.08	0.4	-0.03	0.6	0.3, 0.001
LVESVi, ml/m ²	-0.1	0.4	-0.02	0.8	0.06	0.5	-0.12	0.15	0.28, 0.002
LVEF, %	-0.3	0.06	-0.01	0.9	-0.1	0.4	0.1	0.2	-0.2, 0.06
RV:LV diastolic volume ratio	-0.01	0.9	0.01	0.9	-0.22	0.02	0.5	0.6	-0.03, 0.8
RAAi, cm ² /m ²	0.06	0.6	0.02	0.8	0.07	0.5	0.1	0.3	0.4, <0.001
LAAi, cm ² /m ²	0.05	0.6	0.1	0.2	0.1	0.14	0.2	0.08	0.03, 0.8
RVOT akinetic length, mm	-0.01	0.5	-0.1	0.2	0.1	0.1	0.1	0.2	0.5, <0.001
RV ECV, %	0.5	<0.001					0.43	<0.001	-0.1, 0.3
Native RV T1 (14HB MOLLI), ms			0.5	<0.001	0.4	<0.001			-0.04, 0.7
LV ECV, %			0.4	<0.001	0.36	<0.001			-0.01, 0.9
Native LV T1 (14HB MOLLI), ms	0.4	<0.001					0.36	<0.001	0.1, 0.3

Table 5.5: Clinical associations with top quartile native RV T1 (< 1077ms) and top quartile LGE volume ($\geq 10.2\text{cm}^3$) in isolation and in combination with each other.

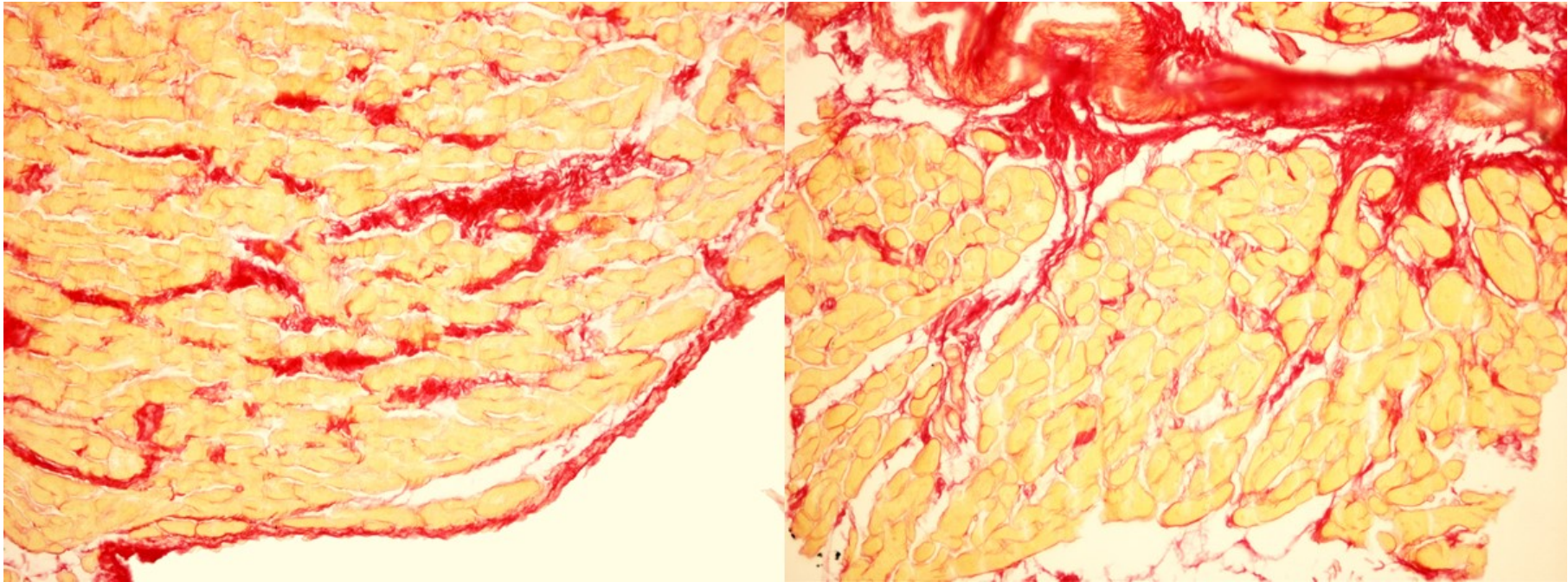
Patients without PVR (n=87)	Both RV T1 + RV LGE < top quartile (n=58)	Only RV T1 $\geq$ top quartile (n=14)	Only RV LGE $\geq$ top quartile (n=11)	Both RV T1 + RV LGE $\geq$ top quartile (n=4)	P-value
Age at CMR, years	29 (22-40)	27 (21-37)	33 (26-40)	48(21-75)	0.1
Male, n	36	8	2	2	0.06
Previous palliative shunt, n	16	2	3	3	0.1
Age at repair, years	2 (1-6)	1.1(1-3)	7 (1-8.5)	23 (4-42)	0.04
Transannular patch, n	15	7	2	2	0.2
RVOT patch, n	20	3	4	0	0.3
RV-PA conduit, n	6	0	1	0	0.6
Sustained atrial arrhythmia, n	7	1	0	1	0.5
NYHA $\geq$ II, n	6	1	2	1	0.6
QRS duration ms	152 (139-164)	146 (121-153)	162(156-174)	153 (152-154)	0.06
QRS $\geq$ 180ms, n	2	0	2	1	0.06
BNP n/g/L	39 (23-63)	49 (30-61)	50 (34-66)	161 (114-208)	0.1
Peak VO_2 , ml/kg/min	26 (20.8-34)	29.5 (22.6-32.7)	24.2 (20.5-34)	17.5 (15.5-21.5)	0.2
Percent predicted Peak VO_2 , %	75 (65-86)	82 (68-94)	70 (53-80)	73 (61.5-75.5)	0.4
Restrictive RV physiology, n	24	9	3	1	0.4
TAPSE, mm	16 (13-18)	16 (13-18)	17 (16-18)	14 (12-15)	0.8
At least moderate TR, n	58	14	11	4	0.3
RVSP, mmHg	27 (22-43)	31 (21-37)	30 (25-36)	46 (36-55)	0.6
FAC %	38 (30-42)	38 (35-41)	35 (32-45)	36 (33-38)	0.9
PV peak velocity, m/s	2 (1.6-2.8)	2 (1.7-2.2)	2 (1.6-2.2)	2.6 (1.9-3.3)	0.8
PV peak velocity $\geq$ 3m/s	5	1	1	1	0.7
RVEDVi, ml/m²	106 (88-121)	120 (105-125)	109 (104-125)	148 (126-169)	0.004
RVESVi, ml/m²	45 (37-56)	52 (40-60)	48 (45-52)	75 (57-81)	0.01
RVEF, %	56 (50-61)	55 (49-62)	56 (53-60)	54 (52-58)	0.9
RV mass g/m²	44 (39-50)	48 (44-59)	45 (38-57)	62 (54-66)	0.02
LVEDVi, ml/m ²	78 (70-89)	78 (72-90)	91 (74-102)	85 (74-93)	0.6
LVESVi, ml/m ²	32 (25-36)	33 (27-38)	34 (25-53)	34 (29-39)	0.8
LVEF, %	61 (58-64)	58 (57-65)	60 (54-66)	58 (56-62)	0.7
RV:LV diastolic volume ratio	1.3 (1.1-1.5)	1.5 (1.3-1.6)	1.4 (1.1-1.6)	1.9 (1.4-2.2)	0.2
RAAi, cm²/m²	11.5 (10-14.4)	10.7 (10.6-11.8)	14.1 (12.1-15.5)	18.1 (14-19.2)	0.04
LAAi, cm ² /m ²	10.5 (9.1-11.8)	10.1 (9.4-10.8)	9.6 (8.8-11.5)	14.2 (8.8-17.8)	0.5
PR fraction, %	24 (5-36)	32 (20-37)	15 (7-40)	40 (30-50)	0.1
PR $\geq$ 20%	32	11	4	4	0.05
RVOT akinetic length, mm	33 (23-37)	33 (28-38)	45 (33-49)	47 (39-56)	0.01

Figure 5.1:



Box plots demonstrating significant difference between patients who have worst quartile degree of RV LGE (focal fibrosis) and RV T1 (diffuse fibrosis) in red versus either worst quartile RV LGE or RV T1 (yellow) and both less than top quartile RV LGE and RVT1 with relation to RV end-diastolic volume (RVEDV), RV end-systolic (RVESV) and right atrial area (RAA)

Figure 5.2:



Picrosirius red staining of the RV biopsy taken from the anterior wall of the RV away from the right ventricular outflow tract at magnification x 20. Diffuse interstitial fibrosis is shown (stained in red) with healthy myocardium stained as yellow. The collagen volume fraction measured was 20.6%.

Discussion

This is the largest study of adult rTOF patients to date that examines the association between CMR defined diffuse and focal fibrosis, with known structural and functional markers of adverse outcome in rTOF patients, using a T1 mapping MOLLI sequence bespoke for the RV and high-spatial resolution 3D LGE CMR.

We show that:

1) Native RV T1 acquired using the bespoke MOLLI sequence correlated with more adverse markers of disease when compared directly with RV ECV suggesting that it is more sensitive at detecting diffuse fibrosis.

2) Greater extent RV diffuse fibrosis (higher native RV T1) occurred in patients with dilated RV with preserved systolic function, whereas more extensive focal fibrosis (RV LGE volume) was associated with RV systolic dysfunction, RA dilatation, raised BNP and reduced exercise capacity. This suggests that RV T1 shows potential to be an early marker of adverse remodelling prior to the onset of ventricular impairment, atrial arrhythmia and symptom development.

3) Finally, milder degrees of diffuse fibrosis (lower native RV T1/RVECV) were found in rTOF patients with prior history of PVR compared to those without previous PVR suggesting that diffuse fibrosis may be reversible with removal of haemodynamic load. Furthermore, in one patient who underwent serial T1 assessment pre- and post-PVR, a significant and marked reduction in RV and LV T1 parameters was measured at 6 months post-operation shedding further insight into reverse remodelling mechanisms.

i. T1 mapping of the RV requires dedicated sequences

Following the success of LV T1 mapping in predicting outcomes independently of LV ejection fraction in acquired heart disease^{140, 154, 207, 208}, there has been increasing enthusiasm in applying T1 mapping to the RV.

Over the past decade, attempts to study this in rTOF patients have been hampered by challenges inherently related to imaging the uniquely shaped RV post-surgical repair. The relatively thin RV wall (only 2-3mm thickness or 2-3mm pixels) and close-proximity to sternal wire artefact, blood pool and pericardial fat, make measurements highly susceptible to partial volume effects. Bearing this in mind, we changed the focus of T1 mapping from being able to accurately determine a true T1 signal, to aiming for greater sensitivity in detecting collagen, by selecting sequence parameters that amplify the magnetisation transfer effect between free water and collagen macromolecules^{140, 178}. We further attempted to improve precision by increasing the number of heart-beats from 11 in the conventional sequence to 14¹⁵², and improve spatial resolution by reducing slice thickness from 8 to 6mm.

In attempt to mitigate partial volume effects, we meticulously ensured that the angle of acquisition was directly perpendicular to the RV inferior or anterior wall and LV septum on 2 views. It was challenging to perpendicularly align for the RV anterior/inferior wall and LV septum simultaneously within a single slice therefore, individually dedicated RV and LV images were chosen. At the analysis stage, strict criteria was applied to only include ROIs of sufficient size $\geq 0.15\text{cm}^2$ as previously described in other well-carried out studies²⁰⁹.

With these special considerations made to tailor native T1 mapping to the RV at the acquisition and image analysis level, we found that native RV T1 was more sensitive in correlating with markers of adverse remodelling in comparison to RV ECV acquired with standard MOLLI imaging. This finding supports the application of dedicated T1 mapping sequences, bespoke to the RV rather than the simple application of standard T1 mapping sequences intended for use in the LV. The 14 heartbeat MOLLI sequence shows promise as being more sensitive to diffuse fibrosis compared to RVECV technique.

As native T1 mapping does not require contrast, it is clinically appealing for ACHD patients who require life-long follow-up.

ii. RV T1 and RV LGE association with different haemodynamic load

The association between type of fibrosis and type of haemodynamic load was more clearly appreciated in the sub-study of patients without prior PVR. Patients without prior PVR had higher PR fraction ($P<0.001$) and lower RVSP ($P=0.002$) and peak pulmonary valve velocity when compared to patients who had at least one previous PVR. In this subgroup, native RV T1 and RVECV as a measure of diffuse fibrosis was also significantly higher. This is consistent with findings from other studies that also have shown this association^{171, 174}. Further subdivision of this group of patients into 3 different sub-cohorts: trivial or no PR or PS, at least moderate pulmonary regurgitation only and mixed pulmonary valve disease in order to explore the relationship between T1 and LGE with each haemodynamic lesion in isolation failed to demonstrate association (**see Table 5.4**).

As opposed to RV T1 defined diffuse fibrosis, more extensive LGE defined focal fibrosis was found in patients with higher RVSP and pulmonary valve gradients although again

subdivision in to 3 distinct haemodynamic sub-cohorts failed to demonstrate association with LGE. The volume of LGE correlated with larger RAA and RV systolic dysfunction (reduced FAC% on echo) **see Table 5.4**. Diffuse fibrosis is associated with larger RVEDVi but also crucially larger RVESVi (a non-invasive marker of RV contractility) in the presence of preserved RV EF (**see Table 5.5**). This suggests it could be an early subtle indicator of impaired myocardial performance before systolic dysfunction develops. The correlation between focal fibrosis with systolic dysfunction, RAAi dilatation, BNP and reduced exercise capacity suggest its effect on the more advanced stages of adverse remodelling.

In keeping with other studies, we have not found any association between T1 mapping markers of diffuse fibrosis and LGE ^{171, 174}. A possible explanation for this could be that focal fibrosis is predominantly related to surgical scar whereas diffuse fibrosis is a response to haemodynamic effects. Although rTOF is often considered a disease of the right heart, it is important not to neglect the assessment of the LV which can be affected by ventricular-to-ventricular interaction. Close correlation between T1 mapping indices in the RV and LV where found similar to in other studies^{171, 172, 174}. Given the challenges involved in acquiring RV T1, it may be argued that the simpler T1 mapping acquisition in the LV could be a still be a useful and more easily obtainable in-direct indicator of RV myocardial health. This is yet to be demonstrated.

The subgroup of patients that had not undergone PVR did not meet standard surgical criteria primarily based on degree of PR and RV volumes¹. Although T1 could be falsely elevated secondary to partial volume affect in more dilated ventricles, in future a robust T1 mapping sequence specific to the RV may help to further guide clinicians with optimal timing for PVR.

Limitations

Despite extensive measures taken in order to minimise partial volume effects, T1 mapping is still very susceptible to signal contamination from surrounding structures and requires significant physics development before it is ready for clinical use. Nevertheless, correlation of native T1 with markers of adverse remodelling and the higher RV T1 found in patients compared to controls with thinner walls (expected to be more susceptible to partial volume effect and falsely elevated T1) suggest that this technique is sensitive at detecting diffuse fibrosis. Although histology was obtained in 1 patient, it is impossible to correlate the location of the sample taken with the region on MRI and therefore we cannot validate RV T1 mapping.

For sampling LV T1, an ROI was drawn in the middle third of the ventricular septum because it has been reported to be the area of greatest precision out of all the septal segments for T1 mapping measurements. The LV free wall is known to be prone to artefact and therefore sampling of T1 there could have increased susceptibility to errors¹⁵⁸. The interventricular septum is generally considered to be part of the LV, although the septum also contains longitudinal fibres belonging to the RV that course in between both ventricles in the superficial and deeper layers and therefore it cannot be analysed in totally separate entities. The contribution of the compact RV myocardium to the ventricular septum is unknown in rTOF although experience gained during analysis of short axis cine images and LGE, suggest that the RV contribution to the septum is mainly in the form of more easily distinguishable trabeculations rather than compact myocardium. Therefore, it is unlikely that the ROI drawn to sample LV T1 in the septum contained a significant proportion of RV compact myocardium.

A small number of Patients with Fallot variant anatomy were included in this study (**see Table 5.1**) however we did not include patients with complex pulmonary atresia with significant MAPCAs. Patients with absent pulmonary valve were included although a very small number unlikely to affect the study outcome.

Future work

A longer prospective study is required to prove that RV native T1 mapping measured using the high-sensitivity MOLLI sequence is a robust biomarker for adverse remodelling. Serial T1 mapping measurements at baseline, early post PVR and at mid and late term follow up post PVR could help us learn if RV native T1 has a role in the optimal timing of pulmonary valve intervention in rTOF.

Conclusion

Our data suggests that diffuse fibrosis detected non-invasively by a novel high-sensitivity native T1 MOLLI sequence specifically tailored to suit the unique properties of the RV, shows promise as an early biomarker of adverse remodelling prior to the establishment of ventricular dysfunction and symptoms.

Chapter 6: Histological fibrosis and biomarkers of collagen turnover in repaired tetralogy of Fallot.

For the work presented in this chapter, a detailed background and literature review were presented in the introduction section (Chapter 1) and the conclusions are reiterated in the overall conclusions chapter of this thesis (Chapter 8).

Prospective study recruitment had taken place between 2008 and 2015. I investigated the role of fibrosis using histology. In addition, I added further analysis of serum markers of collagen turnover, collated, analysed the data and have subsequently drafted the manuscript.

Introduction and aims

Myocardial fibrosis is the final common process that links all chronic cardiac diseases⁶⁴⁻⁶⁸.

Diastolic dysfunction and its sequelae of atrial arrhythmia and decompensated heart failure causes significant morbidity in adult patients with rTOF^{14, 39, 48, 210}. Myocardial fibrosis has been shown to cause diastolic dysfunction by causing adverse remodelling and myocardial stiffness in other conditions^{211, 212}. Most of the histological studies performed in TOF patients have used resected RVOT tissue at time of primary repair as infants^{8, 213, 214}. More data is required to understand the adverse remodelling process in the mid to late term follow up of adult rTOF patients including pre and post PVR. We sought to examine the relationship between structural and functional changes measured by CMR, histologically quantified fibrosis and serum biomarkers of fibrosis in rTOF in order to improve mechanistic insight into the adverse remodelling process.

Methods

Patients and study design

Adult rTOF patient's ≥ 16 years who were clinically scheduled to undergo surgical PVR were recruited to have endomyocardial biopsies taken from the RV and wasted RVOT tissue saved during the time of their operation for the histological quantification of myocardial fibrosis. Patients gave their informed and written consent at least 1 week ahead of their operation date, which was confirmed again on the day of the operation. The study was approved and registered with the local ethics committee.

The indication for PVR was clinically significant pulmonary regurgitation, stenosis or mixed pulmonary valve disease as judged by a multi-disciplinary team. Patients with contra-indication to CMR or gadolinium contrast were excluded from the study.

Patients were investigated with serial CMR studies pre and post PVR. The first study was performed within a year prior to the patients operation as a baseline, the second was performed in the early postoperative recovery period following their surgery (on the day of their discharge from hospital), and the final study was performed to coincide with the patients 2nd outpatient follow-up appointment, where possible. Serum biomarkers for collagen turnover were measured at baseline and fibrosis was quantified histologically. Data from histology and biomarkers was cross-sectionally compared against other known risk factors of adverse clinical outcome in rTOF.

Endomyocardial biopsies, histology analysis and collagen quantification

As detailed in Chapter 2: Methods, tissue samples were taken from the body of the RV and as remote as possible from site of previous surgery in the RVOT. The average size of the RV

specimen was 12x10x3mm. Samples were taken only when the surgeon deemed it was appropriate and technically feasible to do so and under direct vision. Wasted tissue from the RVOT was also taken for histological analysis. The tissues was fixed in 10% buffered formalin and embedded into paraffin. Slices were cut and examined on to slides and collagen was stained with picrosirius red. Histological analysis was performed using a whole slide imaging method where the whole specimen was included in the analysis²¹⁵. On picrosirius red staining, collagen type I and III was stained as a red colour where healthy myocardium stained as yellow. Images were digitalised and were viewed at x 20 magnification using a Hamamatsu Nanosizer 2.0 RS scanner and NDP.scan 3.1 software. For each picrosirius red stain, a process of colour deconvolution^{216, 217} was used to separate out the each colour on the slide and quantify the density of the staining. Each pixel in the digitalized image was allocated a value according to their red-green-blue component. Colour 1 (red) labelled collagen fibers. Colour 2 was used to label the slides background colour and colour 3 was used to identify healthy myocardium (yellow). The collagen volume fraction (CVF) was determined as the total pixel area for colour 1 (collagen) as a proportion of the total pixel area for colour 3 (healthy myocardium) expressed as a percentage.

Serum biomarkers of fibrosis

Venous blood samples were taken for measurement of serum markers of collagen turnover. These included procollagen type I carboxy-terminal telopeptide (PCIP) produced as a by-product of collagen synthesis and matrix metalloproteinase (MMP-2) that degrades collagen in the extracellular matrix (ECM) and its tissue inhibitor (TIMP-1). PCIP was quantified using

an Elisa kit and MMP and TIMP were quantified using a multiplex Quantibody Human MMP array. Blood tests were also analysed for BNP levels.

CMR acquisition and analysis

A standard CMR protocol was performed for functional and structural assessment and acquisition of 2D LGE using the same methodology described in chapter 2. Ventricular volumetric analysis was performed excluding trabeculations and papillary muscle.

Pulmonary artery regurgitant fraction, maximum length of akinetic regions in the RVOT and atrial area were measured as described in Chapter 2, methods. LGE was scored in the RV using the RV segmental scoring system and in the LV based on the 17-segment LV model as previously published ⁴⁷ and also described in the methods chapter 2 and Chapter 4 (Figure 4.1) . LGE at the septal insertion points and LV apical vent sites were not included in the score.

Other non-invasive investigations

Other tests performed included a 12-lead ECG and cardiopulmonary exercise testing. Transthoracic echocardiograms were analysed for restrictive physiology defined by the presence of an 'a' wave on pulse wave Doppler of the pulmonary artery in end-diastole.

Statistical analysis

Continuous data are summarised as means ($\pm$ SD) and medians with interquartile range depending on normality. Comparison between groups was made by χ^2 , Fishers exact test or Mann Whitney test. Correlation of continuous data was tested using the Spearman's rank test. A 2-sided $p \leq 0.05$ was considered statistically significant.

Results

Study population

Twenty-six patients [74% male, median age 35 (25-43), NYHA class $\geq$ II in 17 (63%)] were studied and followed up for 2.4 ± 0.3 years. No patients with other 'Fallot type' anatomy such as DORV, absent pulmonary valve or pulmonary atresia were included in this study.

Baseline patient characteristics are summarised in **Table 6.1**. Biopsies from the RV were analysed in all 26 patients and wasted RVOT tissue was obtained and analysed in 13.

Moderate to severe pulmonary regurgitation resulted in PVR in 22 patients (85%) and in 4 patients (15%) was secondary to mixed pulmonary valve disease. Two patients were smokers and 1 patient had diabetes type II. No patients were taking angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers, beta-blockers or mineralocorticoid receptor antagonist medication.

Fibrosis severity and correlation with invasive biomarkers of collagen turnover

Both replacement fibrosis and interstitial fibrosis was found in all patients. The median histologically quantified collagen volume fraction of RV biopsies obtained was 15 (11.6-27.5 %) and for the RVOT specimens 34.6 (14.2-52.5%).

We were unable to precisely match the site of remote RV biopsies to the corresponding areas on LGE CMR and therefore correlation of LGE with remote RV biopsy histology was not explored. For the RVOT wasted tissue samples however, good correspondence between macroscopic fibrosis with areas of LGE was demonstrated (**see Figure 4.5**). There was a significant association between both supra-median RV biopsy CVF ($\geq 15\%$) and supra-median RVOT CVF ($\geq 34.6\%$) with higher total RV LGE scores ($P=0.1$ and $P=0.03$ respectively). A

higher concentration ratio of MMP-2: TIMP-1 was significantly associated with elevated PCIP levels ($r=0.52$; $P=0.04$) however there was no significant correlation with histological fibrosis as measured by RV CVF ($P=0.5$) and RVOT CVF ($P=0.5$) nor RV LGE extent ($P=0.1$).

CMR findings and correlation with fibrosis severity

All 26 patients underwent LGE CMR at baseline prior to PVR. The median time from baseline CMR to PVR was 32 (1-200) days. LGE was present in the RVOT and VSD patch site in all 26 (100%) and in RV trabeculations remote to previous cardiac surgery in 7 (27%). The median RV LGE score was 5 (3-6).

Post PVR CMR analysis in the early post-operative period was completed in 24 patients (92%) at a median of 6 (5-7) days post-surgery and in 21 patients (81%) at mid-term [median of 1.1 (0.9-2.9) years post PVR]. By mid-term follow-up, 5 patients (19%) did not undergo post PVR CMR because of AICD implantation ($n=4$) and loss to follow-up ($n=1$).

The CMR findings pre and post PVR according to supra-median CVF in the RV and RVOT specimens are summarised in **Tables 6.2 and 6.3** respectively.

Comparing the patients with supra-median CVF with the remainder of the population, restrictive RV physiology was significantly associated with a supramedian RV CVF (in 6 versus 1 patient; $P=0.05$). There was no significant association between clinical characteristics at baseline, QRS duration, BNP level or Peak VO_2 . There was also no significant relation to RV/LV volumes, mass or function and atrial size pre and post PVR.

Serum biomarkers of fibrosis and relation to CMR features

The median ratio of MMP-2: TIMP-1 in the population was 1.5 (1.2-2.1). A higher MMP-2: TIMP-1 ratio was significantly associated with larger ventricular volumes (RVEDVi $r=0.46$; $P=0.05$, LVEDVi $r=0.72$; $P=0.001$, LVESVi $r=0.57$; $P=0.01$) and larger indexed atrial area (right atrial area $r=0.5$; $P=0.03$, left atrial area $r=0.67$; $P=0.006$). The same strong association was also seen at mid-term where patients with higher MMP2-TIMP-1 had still larger ventricles (RVEDVi $r=0.7$; $P=0.01$, RVESVi $r=0.76$; $P=0.006$, LVEDVi $r=0.71$; $P=0.01$, LVESVi $r=0.69$; $P=0.01$). **See Figures 6.1 and 6.2.**

A higher concentration of MMP-2 significantly correlated with the presence of RV restrictive physiology ($p=0.02$). However, the concentration of TIMP-1, PCIP or MMP2-TIMP2 ratio did not relate to RV restriction. There was no association with CMR parameters in the early follow-up stage. There was no correlation with ejection fraction, clinical, BNP, ECG or exercise features at baseline.

A higher PCIP concentration was significantly associated with a lower indexed RV mass/RVEDVi ratio ($r=-0.52$; $P=0.02$), larger indexed right atrial area ($r=0.47$; $P=0.03$) and larger LVEDVi ($r=0.49$; $P=0.03$).

Adverse events

Over the study period, there were as total of 8 (31%) mutually exclusive, major adverse cardiovascular events (MACE) that had occurred (sudden cardiac death in 2, and sustained VT in 6). There were no significant association found between RV/RVOT collagen volume fraction and serum biomarkers of fibrosis with MACE. A total of 6 patients underwent AICD

implantation for primary prevention. Appropriate AICD shock, non-sustained VT and heart failure admissions were not studied. Sustained atrial arrhythmia occurred in 9 (35%) and was significantly associated with supramedian RVOT collagen volume fraction ($P=0.02$) however there was no relation to biomarkers of fibrosis found.

Discussion

This study is hypothesis generating, demonstrating that an association exists between serum biomarkers of collagen turnover with right and left heart remodelling pre and post PVR. The study data furthers our insight into the myocardial health of adult patients with rTOF on an ultrastructure level, suggesting that the implications of an imbalance in the processes that govern collagen turnover can affect the structural phenotype in several ways.

- 1- In this adult rTOF population, the balance between collagen breakdown and the inhibition of collagen breakdown is tipped towards there being an increase in collagen degradation and collagen turnover in the ECM. This is suggested by the presence of a relatively higher MMP concentration compared to TIMP measured in the serum, which also corresponds with an increase in PCIP as a by-product of collagen synthesis. These findings are in support of work by Lai and colleagues²¹⁸, who also reported increased collagen synthesis in adult rTOF patients as reflected by high serum PCIP, and showed that it was associated with RV volume overload and LV mechanical dyssynchrony.
- 2- The imbalance towards the increased turnover of collagen in the ECM is associated with larger biventricular volumes whilst in the presence of preserved ventricular function. This is seen at baseline and post PVR in patients who had failed to reverse

remodel. Furthermore, the same MMP: TIMP profile was associated with larger right and left atriums.

- 3- An increase in histologically quantified fibrosis was found to be associated with RV restrictive physiology and atrial arrhythmia although association with fibrosis biomarkers could not be demonstrated.

Abnormal haemostasis of the extracellular matrix

It is well known that structural and functional changes in all patients with heart disease ultimately reflect pathological changes that are occurring on an ultra-structural level in the ECM⁴¹. ECM haemostasis is controlled by a fine balance between MMPs that digest proteins such as collagen and their tissue inhibitors (TIMPs)^{219, 220}. We have found that increased MMP and decreased TIMP levels, reflecting a more favourable state towards collagen degradation, was associated with dilated RV and LV in patients at baseline before PVR. Patients who had the largest ventricular size post PVR at mid-term follow-up also had the highest MMP: TIMP levels.

This imbalance in MMP and TIMP expression is also seen in patients with dilated cardiomyopathy of any cause (ischaemic, viral and idiopathic)^{82, 221-225} where the disruption of myocardial architecture impairs structural integrity and systolic function. The reverse state (increase in TIMPs relative to MMP) has been reported in patients with hypertrophic cardiomyopathy, where bulking up of the ECM contributes to increased mass/volume, myocardial stiffness and diastolic dysfunction seen in this condition^{76, 226}. Increased MMP relative to TIMP concentration was seen in the variant of hypertrophic cardiomyopathy patients who with time remodelled to having thinner LV walls and systolic impairment²²⁷

further supporting the pathological link between over-activity of MMP and having ‘large and baggy’ ventricles as opposed to ‘smaller and stiffer’ ventricles.

Due to the small number of patients with restrictive RV physiology, we could not determine whether there was a difference in MMP/TIMP expression between patients with primary RV restrictive physiology and small RVs and secondary RV restriction with larger RVs^{105, 107}. A similar pattern in expression with elevated MMP over TIMP is expected in patients with secondary RV restrictive physiology and dilated RV similar to that reported in ‘burnt-out’ hypertrophic cardiomyopathy phenotype²²⁷.

Myocardial fibrosis and association with RV restriction and atrial arrhythmia

In this study, we histologically quantified myocardial fibrosis acquired from RV biopsies remote from previous surgical sites and RVOT wasted tissue during PVR surgery for significant PR, along with comprehensive CMR volumetric and functional measurements pre-operatively and during follow-up post PVR.

We found that patients with severe myocardial fibrosis were more likely to have sustained atrial arrhythmias and restrictive RV physiology similar to previous reports supporting the pathophysiological link between diastolic dysfunction and fibrosis⁹⁴. We did not perform comprehensive evaluation of diastolic function however, as it was beyond the scope of this study. We did not observe any relationship between the degree of fibrosis with age, age of repair and CMR parameters similar to a previous study with a larger population than ours²⁰⁹. One possible explanation for this lack of relation between histological fibrosis and structural changes seen in this study versus the strong association found with MMP/ TIMP activity, is that cardiac remodelling may be more affected by the way in which myocardial structural

integrity is breached (degradation the ECM scaffolding versus bulking up of the ECM) and not only the volume of fibrosis.

Clinical implications and future direction

This study paves the way for further exploration as to whether the relative profiling of serum MMP and TIMP expression may be used in the surveillance of rTOF patients as an indicator of both the direction and degree of ventricular remodelling as well as to track disease progression. Studies have found that elevated level of MMP-9 in patients with heart failure can predict cardiac dilatation and the degree of adverse remodelling^{228, 229}.

MMP-3 has been reported to predict SCD in patients with hypertrophic cardiomyopathy independent of fibrosis quantified by LGE²²⁶. The role of MMP and TIMP in VT and sudden cardiac death risk stratification in rTOF is yet to be determined and requires further investigation.

Furthermore, the relative over- activity of MMP could potentially be a pharmaceutical target for heart failure therapy. Pharmacological and genetic downregulation of MMP expression has already been found to be successful in the reverse remodelling of the LV in animal studies²³⁰⁻²³².

The imbalance between the activity of MMP and TIMP also begs the question as to what the driving factors for this are. The role of immune system as one driver for myocardial fibrosis and in turn heart failure, has recently gained much interest with a recent study suggesting that patients with a cardio-inflammatory phenotype may benefit from targeted immune

modulation therapy. Early trial data with interleukin-1 blockade in patients with chronic stable heart failure with preserved ejection fraction has shown improvements in cardiorespiratory function as measured by peak oxygen consumption, quality of life measures and reduction in BNP levels^{233, 234}.

A larger prospective study of adult rTOF patients to phenotype MMP/TIMP and inflammatory cytokine expression (Interleukin-1 and high-sensitivity C-reactive protein) with long-term follow-up for clinical outcomes²³⁵, whilst also assessing their correlation with non-invasive T1 and T2 mapping is merited.

Limitations

There are several limitations to this study. This was a small study with a short period of follow-up. Despite the relatively high event rate in comparison to the other studies described in Chapter 3 and 4 (as expected for these selected patients with haemodynamically significant PR) it was not possible to profile MMP/TIMP in all patients and this may have affected association with outcomes. In view of the exploratory nature of this study, it was not possible to perform a power calculation at the start of the study to determine the sample size required. However, a similar study that examined the relationship between RV histology in rTOF and CMR markers of adverse remodelling at early and mid-term follow-up and MACE found associations with a sample size of 53 patients²⁰⁹, although this study did not include measurement of MMP and TIMP expression. Secondly, serum biomarkers of collagen turnover are not specific to the myocardium and their expression can be altered from processes in other organ systems. Therefore, inconsistencies between serum and histology may also reflect other extra-cardiac processes.

Direct staining of the myocardium for MMP and TIMP would be more specific and could be helpful to overcome this issue.

We did not evaluate diastolic function or include aortic cross clamp time²⁰⁹, both known to relate to myocardial fibrosis. Furthermore, our study included patients with predominantly significant PR as the dominant reason for PVR, therefore we cannot assume that myocardial fibrosis will be similar in patients where pulmonary stenosis is the dominant lesion. Patients who had AICDs implanted during the study did not undergo CMR and therefore evaluation of the cardiac remodelling link with histology and biomarkers of fibrosis post PVR was not possible in these high-risk patients.

Conclusions

A high expression of serum MMP relative to TIMP levels that favours a state of ECM degradation and high collagen turnover is associated with larger ventricles, and atria and may be used to identify those patients that will not have complete reverse remodelling post PVR. Atrial arrhythmia and restrictive right ventricle physiology are associated with higher myocardial fibrosis burden suggesting its role in diastolic dysfunction. Further larger and longer prospective study to include diastolic evaluation is required to define the impact on clinically important outcomes.

Table 6.1: Summary of patient characteristics

Patient characteristics	Median (IQR)
Male (n, %)	20 (74%)
Age at PVR (y)	35 (25-43)
Pulmonary regurgitation as dominant reason for PVR (n,%)	22 (85%)
Pulmonary regurgitant fraction (%)	41 (31-50)
Age at repair (y)	4 (2-8)
Palliative shunt (n)	12 (46%)
Transannular patch (n)	13 (48%)
RVOT patch (n)	10 (37%)
RV-PA Conduit (n)	2(7%)
Systolic blood pressure (mmHg)	115 (110-120)
NYHA class $\geq$ II (n)	17 (63%)
QRS duration (ms)	160 (141-168)
BNP (ng/L)	15 (8-28)
Peak VO ₂ (ml/kg/min ²)	22 (16.5-27.5)
Restrictive RV physiology (n)	7 (26%)
Sustained atrial arrhythmia (n)	9 (33%)
Sustained ventricular arrhythmia (n)	7(26%)
RVEDVi (ml/m ²)	149 (126-167)
RVESVi (ml/m ²)	79(59-92)
RVEF (%)	51 (46-57)
Right atrial area indexed (cm ² /m ²)	15 (13-17)
LVEDVi (ml/m ²)	80 (68-88)
LVESVi (ml/m ²)	31 (23-37)
LVEF (%)	62 (58-67)
Left atrial area indexed (cm ² /m ²)	10 (9-11)
RV LGE score	5 (3-6)
LV LGE score	4 (0-4)
RV biopsy collagen volume fraction (%)	15(11.6-27.5)
RVOT specimen collagen volume fraction (%)	34.6(14.2-52.5)
MMP-2:TIMP-1 ratio	1.5(1.2-2.1)

Table 4.2 Clinical correlates with RV biopsy fibrosis (n=26)

Clinical variables	supramedian CVF (≥ 15%)	Inframedian CVF (<15%)	p	RV fibrosis r (P value)
Male (n, %)	11	9	0.1	
Age at PVR (y)	39(25-45)	31(25-40)	0.3	0.22 (0.29)
Pulmonary regurgitant fraction (%)	31(29-49)	40(25-50)	0.8	0.14(0.49)
Age at repair (y)	11(9.5-11.5)	2.6(2-4)	0.2	0.25 (0.2)
Palliative shunt (n)	5	6	1	
Transannular patch (n)	4	8	0.2	
RVOT patch (n)	6	4	0.4	
Conduit (n)	1	1	1	
Systolic blood pressure (mmHg)	119(110-136)	114(110-119)	0.3	
NYHA class ≥II (n)	9	7	0.2	
QRS duration (ms)	152(137-164)	161(159-168)	0.1	0.22 (0.28)
BNP (ng/L)	20(13-28)	10(8-14)	0.6	0.07(0.85)
Peak VO2 (ml/kg/min2)	21(17-27)	25.5(16.3-30.6)	0.9	
Restrictive RV physiology (n)	6	1	0.05	
RV mass indexed (g/m2)	60(53-75)	63(52-70)	0.8	0.3(0.08)
Sustained atrial arrhythmia (n)	4	5	1	
Sustained ventricular arrhythmia (n)	4	3	0.6	
MMP-2: TIMP-1 ratio	1.55(1.4-2.2)	1.4(1-2.5)	0.7	0.2(0.4)
CMR parameters at Baseline				
RVEDVi (ml/m2)	163(101-178)	135(104-162)	0.1	0.21 (0.3)
RVESVi (ml/m2)	66(56-86)	86(66-95)	0.3	0.27 (0.18)
RVEF (%)	51(56-56)	51(45-58)	0.7	-0.29 (0.15)
Right atrial area indexed (cm2/m2)	15(13-18)	14(12-17)	0.5	0.29 (0.14)
LVEDVi (ml/m2)	79(65-96)	81(70-88)	0.8	0.15(0.5)
LVESVi (ml/m2)	30(22-39)	32(24-37)	0.9	0.3(0.2)
LVEF (%)	63(57-67)	61(59-65)	0.9	0.2(0.26)

Left atrial area indexed (cm ² /m ²)	10(9-11)	10(9-11)	0.8	0.02(0.9)
RV LGE score	6 (5-8)	4(3-6)	0.01	0.54(0.005)
LV LGE score	3(0-4)	4 (0-4)	0.8	0.07(0.7)
CMR parameters at early follow-up median 6 (5-7) days				
RVEDVi (ml/m ²)	89(78-129)	108(83-130)	0.6	-0.17(0.48)
RVESVi (ml/m ²)	51(41-67)	60(41-89)	0.9	-0.146(0.5)
RVEF (%)	45(44-49)	44(36-54)	0.9	0.14(0.5)
LVEDVi (ml/m ²)	65(59-72)	73(62-79)	0.5	0.5(0.15)
LVESVi (ml/m ²)	24(21-30)	30(24-35)	0.4	0.3(0.3)
LVEF (%)	62(58-64)	63(56-67)	0.8	-0.4(0.2)
CMR parameters at mid-term follow up median 1.1 (0.9-2.9) years				
RVEDVi (ml/m ²)	96(76-143)	99(82-137)	0.5	-0.185(0.46)
RVESVi (ml/m ²)	42(35-71)	47(42-73)	0.2	-0.346 (0.16)
RVEF (%)	53(51-59)	49(46-56)	0.1	0.23(0.36)
LVEDVi (ml/m ²)	81(62-97)	86(74-103)	0.2	- 0.14(0.5)
LVESVi (ml/m ²)	32(23-36)	34(29-37)	0.4	0.1 (0.7)
LVEF (%)	63(60-66)	64(55-69)	0.7	0.39 (0.12)

The association between supramedian collagen volume fraction (CVF) $\geq 15\%$ measured from RV muscle biopsies was examined against clinical variables and CMR data at baseline, early follow-up 6 (5-7) days and mid-term follow-up 1.1 (0.9-2.9) years.

Collagen volume fraction (%) MMP-2: TIMP-1 ratio; ratio of matrix metalloproteinase 2 expression to tissue inhibitor of matrix metalloproteinase 1

Table 4.3 Clinical correlates with RVOT specimen fibrosis (n=13)

Clinical variables	supramedian CVF ($\geq 35\%$)	Inframedian CVF ($<35\%$)	p	RV fibrosis r (P value)
Male (n, %)	6	4	0.5	
Age at PVR (y)	41 (25-46)	35 (19-41)	0.3	0.3 (0.2)
Pulmonary regurgitant fraction (%)	38 (31-50)	34 (31-49)	0.6	0.1 (0.6)
Age at repair (y)	12.5 (12-13)	4 (2.4-8.5)	0.5	0.2(0.4)
Palliative shunt (n)	3	2	0.7	
Trans-annular patch (n)	3	2	0.7	
RVOT patch (n)	1	3	0.2	
Conduit (n)	5	6	0.5	
Systolic blood pressure (mmHg)				
NYHA class $\geq$ II (n)	4	4	0.7	
QRS duration (ms)				
BNP (ng/L)				
Peak VO ₂ (ml/kg/min ²)				
Restrictive RV physiology (n)	2	0	0.07	
RV mass indexed (g/m ²)	65 (50-83)	55 (49-75)	0.7	0.1 (0.6)
Sustained atrial arrhythmia (n)	4	0	0.02	
Sustained ventricular arrhythmia (n)	3	2	0.7	
MMP-2:TIMP-1 ratio	1.9 (1.4-2.4)	1.4 (1-1.5)	0.6	0.2(0.5)
CMR parameters at Baseline				
RVEDVi (ml/m ²)	161 (144-206)	136(108-174)	0.2	0.4 (0.1)
RVESVi (ml/m ²)	69 (47-89)	84 (62-117)	0.3	0.48 (0.09)
RVEF (%)	48 (43-56)	55 (42-58)	0.4	-0.44 (0.1)
Right atrial area indexed (cm ² /m ²)	16 (13-18)	13(13-15)	0.2	0.56(0.04)
LVEDVi (ml/m ²)	82(76-98)	71 (55-91)	0.2	0.38 (0.1)

LVESVi (ml/m2)	34 (25-37)	23 (17-33)	0.3	0.3 (0.3)
LVEF (%)	62 (58-68)	65 (63-71)	0.4	-0.1(0.7)
Left atrial area indexed (cm2/m2)	10 (9-11)	10 (9-12)	0.9	-0.3(0.2)
RV LGE score	11 (8-13)	3 (3-4)	0.03	0.64 (0.01)
LV LGE score	3 (1-4)	4 (3-4)	0.4	0.1(0.5)
CMR parameters at early follow-up median 6 (5-7) days				
RVEDVi (ml/m2)	146 (125-167)	104 (87-107)	0.3	0.5 (0.1)
RVESVi (ml/m2)	83 (73-94)	49 (42-60)	0.4	0.5(0.1)
RVEF (%)	43 (41-47)	49 (43-51)	0.9	-0.2(0.5)
LVEDVi (ml/m2)	78 (64-92)	57 (54-62)	0.1	0.5(0.2)
LVESVi (ml/m2)	30 (22-38)	19 (18-19)	0.3	0.3(0.3)
LVEF (%)	62 (59-65)	64 (64-98)	0.3	-0.4(0.2)
CMR parameters at mid-term follow-up median 1.1 (0.9-2.9) years				
RVEDVi (ml/m2)	140 (115-196)	103 (83-117)	0.2	0.7 (0.07)
RVESVi (ml/m2)	75 (54-101)	47 (37-58)	0.4	0.6(0.2)
RVEF (%)	51 (47-55)	55 (52-57)	0.6	-0.4(0.4)
LVEDVi (ml/m2)	95 (86-100)	65 (61-84)	0.6	0.2(0.6)
LVESVi (ml/m2)	32 (28-41)	19 (18-28)	0.4	0.4(0.3)
LVEF (%)	65 (59-68)	66 (65-71)	0.6	-0.3(0.4)

The association between supramedian collagen volume fraction (CVF) $\geq 35\%$ measured from RVOT wasted specimens was examined against clinical variables and CMR data at baseline, early follow-up 6 (5-7) days and mid-term follow-up 1.1 (0.9-2.9) years.

Collagen volume fraction (%) MMP-2: TIMP-1 ratio; ratio of matrix metalloproteinase 2 expression to tissue inhibitor of matrix metalloproteinase 1

Table 4.4: Clinical correlates of MMP-2:TIMP-1 and PCIP concentration.

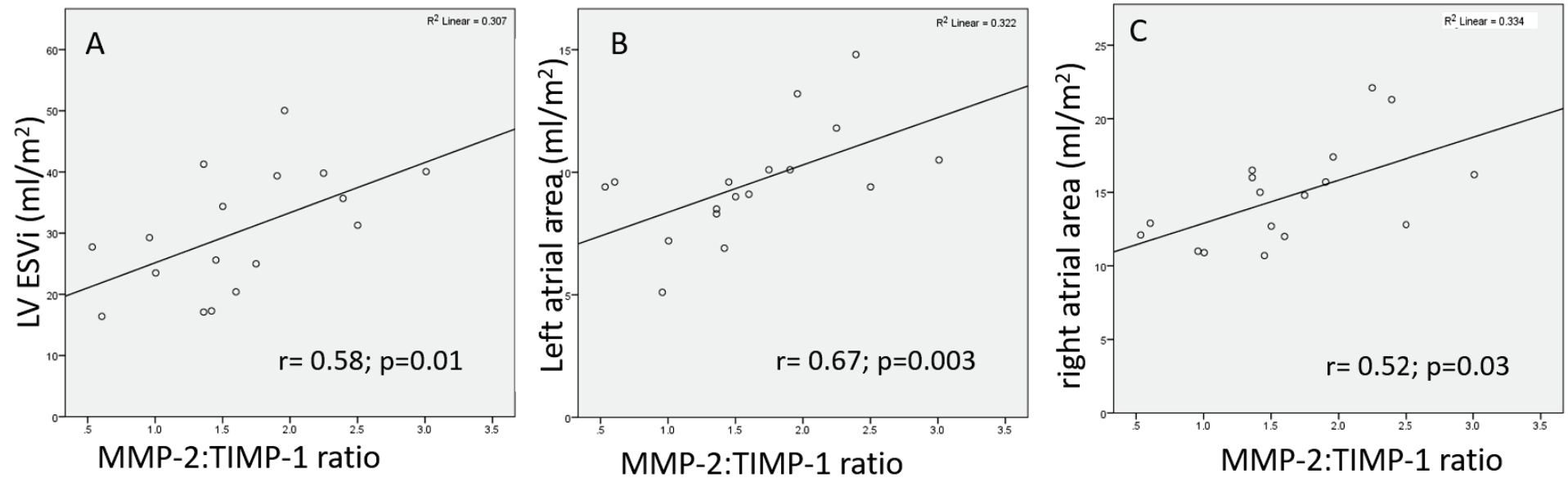
Clinical variables	MMP-2:TIMP-1 concentration, r (P value)	PCIP concentration r, (P value)
Age at PVR (y)	0.04 (0.9)	0.4 (0.07)
Pulmonary regurgitant fraction (%)	0.09(0.7)	0.1(0.5)
Age at repair (y)	0.05 (0.9)	0.3(0.1)
Systolic blood pressure (mmHg)	-0.8(0.001)	0.28(0.3)
QRS duration (ms)	0.3(0.3)	-0.2(0.3)
BNP (ng/L)	0.6 (0.2)	0.77(0.07)
Peak VO2 (ml/kg/min2)	0.27(0.3)	0.05(0.8)
RV collagen volume fraction %	0.28 (0.5)	0.4(0.08)
RVOT collagen volume fraction %	0.2(0.5)	-0.04(0.9)
MMP-2:TIMP-1 concentration		0.52 (0.04)
PCIP concentration	0.52 (0.04)	
CMR parameters at Baseline		
RVEDVi (ml/m2)	0.46(0.05)	0.3(0.2)
RVESVi (ml/m2)	0.4(0.1)	0.2(0.3)
RVEF (%)	0.2(0.38)	0.08(0.7)
Right atrial area indexed (cm2/m2)	0.5 (0.03)	0.47(0.03)
LVEDVi (ml/m2)	0.72(0.001)	0.49 (0.03)
LVESVi (ml/m2)	0.57(0.01)	0.3(0.2)
LVEF (%)	-0.14(0.6)	0.05(0.8)
Left atrial area indexed (cm2/m2)	0.67 (0.003)	0.4 (0.06)
RV LGE score	0.39(0.1)	0.39 (0.09)
LV LGE score	-0.6(0.006)	-0.2(0.4)
RV mass indexed (g/m2)	0.4 (0.1)	0.5(0.8)
RV mass/ RVEDV (g/ml/m ²)	-0.33(0.3)	-0.52(0.02)

CMR parameters at early follow-up median 6 (5-7) days		
RVEDVi (ml/m2)	0.39 (0.2)	0.2(0.38)
RVESVi (ml/m2)	0.3(0.3)	0.3(0.2)
RVEF (%)	0 (1)	-0.3 (0.4)
LVEDVi (ml/m2)	0.2 (0.4)	0.18(0.5)
LVESVi (ml/m2)	0.08 (0.8)	0.1(0.7)
LVEF (%)	-0.2(0.5)	-0.18(0.5)
CMR parameters at mid-term follow-up median 1.1 (0.9-2.9) years		
RVEDVi (ml/m2)	0.7 (0.01)	0.5(0.07)
RVESVi (ml/m2)	0.76 (0.005)	0.2(0.5)
RVEF (%)	-0.5 (0.09)	0.09(0.7)
LVEDVi (ml/m2)	0.71(0.01)	0.38(0.2)
LVESVi (ml/m2)	0.69 (0.01)	0.46(0.1)
LVEF (%)	-0.38 (0.2)	-0.15(0.6)

The association between matrix metalloproteinase 2 expression relative to tissue inhibitor of matrix metalloproteinase 1 (MMP2: TIMP 1) and procollagen type I carboxy-terminal telopeptide (PCIP) levels were examined against clinical variables and CMR data at baseline, early follow-up 6 (5-7) days and mid-term follow-up 1.1 (0.9-2.9) years.

Figure 6.1:

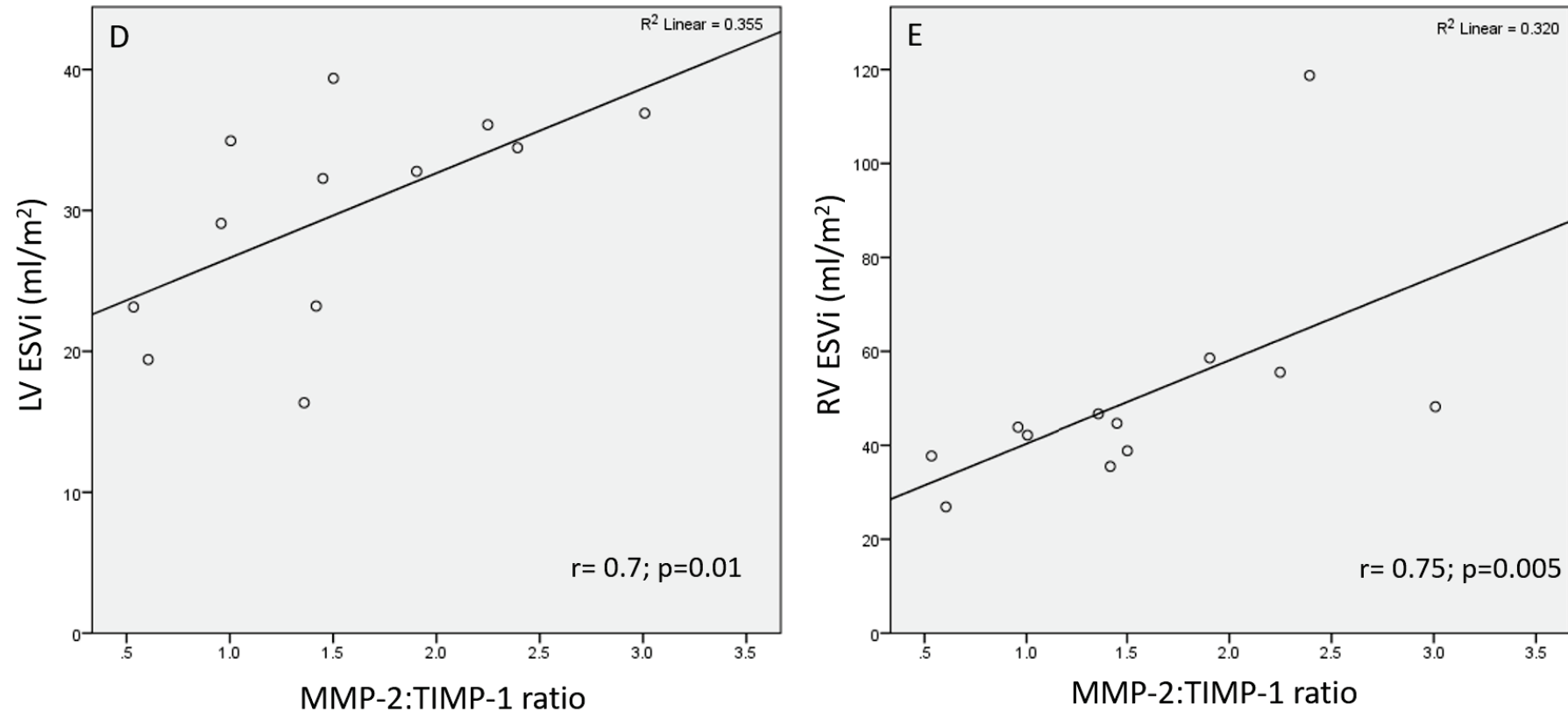
Correlations of MMP2:TIMP1 concentrations with CMR parameters at Baseline



Scatter plots showing significant correlation between increasing MMP2:TIMP1 expression and A) increasing indexed LV end systolic volume (LVESVi), left atrial area (B) and right atrial area (C) measured at baseline.

Figure 6.2:

Correlations of MMP2:TIMP1 concentrations with CMR parameters at mid-term follow up (2.4 ± 0.3 years)



Scatter plots showing significant correlation between increasing MMP2:TIMP1 expression and D) increasing indexed LV end systolic volume (LVESVi and increased indexed RV end-systolic volume, RVESVi (E)

Chapter 7: General discussion

Overview of thesis

Overall, this thesis aimed to explore unmet needs in the contemporary management of adult patients with rTOF namely, the improvement of risk stratification for malignant VT and SCD and the selection of patients for primary prevention AICD. The risk stratification theme was addressed over two prospective studies, where CMR had a role in the non-invasive evaluation of myocardial fibrosis and was tested as a predictor of outcome. In chapter 5, the role of RV T1 mapping showed promise as an early biomarker of adverse remodelling. In chapter 6, histologically quantified fibrosis and collagen biomarkers furthered mechanistic insight into the adverse remodelling process post PVR, further feeding into the risk stratification theme and the drive towards delivering personalised and targeted therapy.

Identifying patients at high-risk of life-threatening VT and SCD

The accurate identification of patients at serious risk of malignant VT and SCD has long been considered by some to be the ‘holy grail’ of ACHD electrophysiology. Since the very initial report in 1972 by Wolff *et al*²³⁶ which raised the alarm that premature VT/SCD was prevalent in the adult rTOF population, numerous studies attempting to address risk have been hampered by small study size and with too few events that made it:

- A) challenging to find accurate and robust predictors of outcome
- B) challenging to extrapolate results to all patients.

It became apparent that no single risk factor on its own could accurately predict VT/SCD. It was Bricker *et al* in 1995³⁰ that calculated that a study as large as 1700 patients with follow-

up of 10 years would be required to predict the 0.15% annual incidence of SCD using multiple variables.

Over the last two decades there has been a ramping up of efforts to produce risk scores that encompass well recognised, 'traditional' risk factors like for example: QRS duration > 180ms identified in the landmark study by Gatzoulis *et al* in 1995²¹, as well as other factors previously reported to be significant such as prior palliative shunt, ventriculotomy and non-sustained VT^{23, 32}. The application of these scores in particular, the highly referenced score produced by Khairy and colleagues, includes invasively acquired data such as LVEDP and programmed VT stimulation²³, has been difficult to apply in real-life practice and in unselected patients. Although programmed VT stimulation has proven prognostic value in prior prospective study²³, clinical guidelines^{26, 196} are unclear on how patients should be selected for this procedure as part of the risk-stratification work-up and are also somewhat elusive on recommendations for primary prevention AICD decision.

In this thesis I have for the first time shown that the burden of RV scar defined by LGE CMR predicted inducible VT over and above conventional risk factors such as age and QRS duration in a selected group of patients deemed to be of at least intermediate risk, in whom invasive programmed VT stimulation would be typically considered²⁷. Using 3D high-resolution LGE CMR, we were able to quantify the extent of RV LGE and found that a cut-off of 25cm³ predicted inducible VT with a sensitivity and specificity of 72% and 81% respectively. We also found that all patients who had RV LGE below 10cm³ were non-inducible for VT (sensitivity of 100% at 10cm³). Although we have identified these cut-offs for predicting VT inducibility from our study cohort, these were for illustrative purposes

rather than to be prescriptive. The real clinical role for 3D LGE will be to identify those patients with minimal RV scar (RV LGE less than 10cm^3) but in whom PES is being considered as part of their risk stratification work-up. Patients with RV scarring under the 10cm^3 threshold may potentially avoid an invasive procedure. It is acknowledged that this study was not designed to test whether RV LGE was a predictor of mortality but rather predicted inducible VT as a proxy endpoint for mortality. We therefore, could not also determine whether RV LGE was superior to programmed VT stimulation for risk stratification and so do not suggest that LGE CMR replaces it as a prognostic test.

The question remained as to whether RV LGE was prognostic in rTOF. In order to determine this, we conducted the largest study to our knowledge, which investigated the relationship between LGE CMR with all-cause mortality as a primary outcome and clinically important ventricular arrhythmia/SCD as the secondary outcome, with a follow-up time of up to 16 years. We demonstrated that RV LGE was a strong predictor of both mortality and VT/SCD.

In this thesis, I have shown how RVLGE could be clinically utilised amongst the armamentarium of other clinical tests to assess risk, by determining its weighting against other positive predictors of outcome in our study. All independent predictors were included in a weighted risk score to predict either mortality or VT/SCD. Our scores integrated patients' clinical characteristics, RV LGE extent, simply the presence of any non-apical ventricular LGE, CMR derived RV/LV ejection fraction and RVOT akinetic length, peak oxygen consumption, BNP level and RVSP from echocardiography. From this we were able to identify a high-risk subgroup of patients who had a 4.4% annualised risk of death and 3.7% annualised risk of VT/SCD. We do not have available randomised control trial data (RCT) in rTOF that can help us determine the degree of risk at which a patient will start to receive

benefit from an AICD. However, if we consider as a guide the 3.5% annual risk threshold beyond which patients with ischaemic cardiomyopathy patients²⁰³ begin to receive benefit from AICDs, this suggests that we have identified an important high-risk group that warrants consideration for primary prevention therapy.

The advantages of our risk scores are that they are practical, simple and ready to apply now in clinical practice. They also perform better in predicting mortality when compared with previously proposed risk models applied to our cohort, likely owing to the strength that RV LGE has as a predictor of mortality and clinically important VT/SCD. Our scores are yet to undergo external validation and we acknowledge that our study was only single centre. Despite this, it is anticipated that these results will be applicable to the general unselected rTOF patient, as we included patients who had undergone TOF repair spanning several surgical era's (1960's to 2000's), in seven different centres including international ones with surgery performed by at least seven different cardiac surgeons therefore, representing well the heterogeneity of this condition. In our study, we gained confidence that we found parameters: RVEF <35%, LV EF <35%¹⁷, BNP⁴⁸, PVO₂^{49, 199}, RVSP¹⁷ and prior history of atrial arrhythmia¹⁷, reported as important risk factors by other groups, also to be independent predictors in our study and hence their inclusion in the scores.

In contrast to earlier reports, we found that prior palliative shunt, previous ventriculotomy²³ and QRS duration²¹ non-predictive of outcome. This is likely to represent more modern surgical approaches with a change towards single stage repairs and RVOT reconstruction via a transpulmonary/transatrial approach. We also did not find that non-sustained VT was a predictor of mortality, supporting the findings of several others^{21, 56} who first alerted us to

this lack of association two decades ago. This fuels concerns that despite these findings that suggest that non-sustained VT is benign, non-sustained VT is still being used to make up study composite endpoints^{33, 171} which may be misleading. Furthermore, non-sustained VT was a predictor of appropriate AICD shock in a recent large registry study. As it is challenging to ensure that only AICD shocks delivered where for sustained VT rather than non-sustained VT that would have self-terminating if left without therapy for 30 seconds, we also chose not to include appropriate AICD shock as an endpoint in our study, making sure only to study hard clinical endpoint.

Identifying myocardial disease early

In chapters three and four of this thesis, the focus was on identifying patients at high-risk of mortality and malignant VT. Despite this, four patients who were categorised using the scores, as having intermediate and low-risk, had suffered unfortunately devastating events. This begs the question as to whether we are missing subtle, early signs that can identify patients who will go on to have an adverse trajectory prior to them developing potentially irreversible adverse remodelling e.g. systolic impairment. The second challenge is finding these patients who are 'like a needle in a haystack' amongst a much larger group of low-risk patients.

Diffuse or interstitial fibrosis is known to go undetected by LGE CMR^{41, 155}. Diffuse fibrosis is successfully measured in the LV using non-invasive T1 mapping CMR sequences where it has shown prognostic value in acquired heart conditions^{139, 140, 148, 161, 163}. We have found that elevated LV T1 parameters, suggestive of a high burden of diffuse fibrosis in rTOF, correlated with late repair, impaired exercise tolerance and prolonged QRS duration in

support of previous reports^{164, 171}. Applying T1 mapping to the RV however, has significant technical challenges particularly in ACHD patients where it is easy for the T1 signal to be contaminated due to partial volume effects from sternal wires, adjacent pericardial fat, and blood pool, highly mobile and uniquely shaped RV. Despite several reports of its application in the RV¹⁷¹⁻¹⁷⁴ using the same sequences intended for the LV T1 mapping, it remains inconclusive whether they can accurately detect RV diffuse fibrosis or whether they are measuring contaminated signal. What is clear from our findings, is that RV T1 mapping should be approached with dedicated RV T1 mapping sequences to mitigate technical challenges specific to the RV and it requires meticulous care in its application. Using a MOLLI sequence with parameters optimised to improve sensitivity to diffuse fibrosis and spatial resolution, we set out to acquire T1 mapping bespoke to the RV.

Firstly, we found that the patient group had higher RV T1 mapping parameters compared to the healthy control group. As it is significantly more challenging to measure RV T1 in healthy volunteers with a RV wall thickness of 1-2 pixels (only possible to analyse in 50% of patients¹⁷⁴), we were expecting higher signal contamination and therefore falsely elevated T1 in the healthy volunteers. The fact that the reverse finding was true, gave us confidence in that we were able to measure a meaningful signal. We also found that the optimised MOLLI sequence for acquiring native T1 correlated more sensitively with other markers of disease compared to standard ECV. The significant practical advantages of this are that native T1 measurements does not require contrast administration for its application and is quicker to perform¹⁵⁵. This makes native T1 appealing for its use in clinical practice.

We found that native RV T1 showed promise as being an early, subtle non-invasive biomarker of RV adverse remodelling prior to the establishment of systolic dysfunction. In particular, the positive correlation with RV ESV yet in the presence of normal RV EF emphasises that not only can native RV T1 can give an indication of RV chamber dilatation but also serves as a warning of intrinsic RV myocardial dysfunction²³⁷.

RV ESV in itself is known to correlate closely with RV contractility identified by gold-standard invasive pressure-volume loops²³⁷.

When compared to RV LGE, high RV native T1 signals correlated only with dilated RV (both RVEDV and RVESV), whereas RV LGE correlated with dilated atria, increased BNP levels and impaired exercise capacity in addition to RV volumes. This further supports native RV T1's potential to detect adverse remodelling in its early stages before it can be detected by LGE CMR.

Despite the prospective design of this study, we were unable to show an association with sustained atrial arrhythmia, sustained VT or SCD potentially due to the small number of events that had occurred over the short follow-up time. It is envisaged that native RV T1 needs to prove its prognostic value in a larger study with longer follow-up period before it can be incorporated into clinical protocols. If successful it may have a role in helping to further risk stratify patients, monitor disease progression and response to therapy. It may also aid with the optimum timing of PVRs. It will be challenging to histologically validate diffuse fibrosis on biopsies with native RV T1 as despite it being a diffuse fibrotic process, regional differences have been reported and it will be difficult to exactly match the location of biopsies with the CMR¹³⁸.

Although RV native T1 mapping has shown potential as a non-invasive biomarker of adverse remodelling, there are further technical hurdles that need to be addressed before it is ready for 'prime time' clinical use namely: it is technician dependent, scanner dependent and it is very sensitive to artefact caused by cardio-respiratory motion and inhomogeneity in the magnetic field. It is also important to recall that T1 is not a direct marker for fibrosis but rather a surrogate marker of this by measuring the expansion of the ECM¹⁵⁵. For these reasons, a robust biomarker of fibrosis is still desired.

Our newly gained mechanistic insight, from the study of serum biomarkers of fibrosis reported in chapter six, was that an active process of collagen degradation and high collagen turnover appears to dominate in adult rTOF patients with sufficient RV dilatation or dysfunction or symptoms to be put forward for surgery to replace their pulmonary valve. We found that there was a high expression of MMP (which digest collagen) relative to its tissue inhibitor TIMP present in this patient population. We further demonstrated a strong correlation between high MMP:TIMP expression with dilated RV and LV volumes and dilated atria although no association with ejection fraction was found. This association was present both at baseline before patients underwent PVR, as well as in patients who failed to reverse remodel following PVR. Importantly, we were unable to mirror this association with histologically quantified collagen.

These results suggest that the profiling of serum MMP:TIMP expression, like native RV T1 mapping, may be early subtle indicators of adverse remodelling and intrinsic myocardial impairment prior to the establishment of systolic dysfunction. These results pave the way for further investigation into whether these or other serum biomarkers preferably identified

from assessment of the failing myocardium itself, can help find ‘the missing needle in a haystack’ i.e. the patient who is seemingly low-intermediate risk but will go on to have a devastating event. These data suggest that they may also have a role in the tracking of disease progression, response to treatment and may help to plan the optimum timing for PVR.

Future work

Can 3D LGE non-invasively determine other high-risk electrophysiology features?

Recognising that it may not only be the burden of RV scar that predisposes a patient with rTOF to developing life-threatening VT but also other scar features, the role of 3D LGE to further non-invasively characterise scar substrate remains to be explored. Previous studies have demonstrated the presence of four anatomical isthmuses^{61, 62} in rTOF patients that allow macro-re-entry VT to circuit through. Recent electrophysiological research⁶³ have shown that the narrower the anatomical isthmus, the slower the conduction through the isthmus and increased risk of VT occurring. Further study is required to explore whether 3D LGE CMR can tease out further scar properties such as the presence of the anatomical isthmuses and their width. This work combined with computer simulation may enable the testing of VT inducibility off-line and outside of the catheter lab, potentially giving the operator a huge advantage in the planning of ablation procedures. Work to automate the segmentation of LGE (which was performed manually in this research and was labour intensive) could enable the wider uptake of 3D LGE in clinical practice.

An important obstacle that faces the incorporation and wider uptake of LGE CMR and RV T1 mapping in real world clinical practice is that it is operator dependent and requires initially a steep learning curve to ensure that it can be robustly applied at every attendance. The acquisition of LGE during this work required meticulous care to ensure the optimum Ti was selected so that normal myocardium was optimally nulled by visual inspection. This meant that LGE image acquisition continued until typically 20-30 minutes following contrast administration in order to avoid false negative results. Areas suspicious for LGE were carefully interrogated to minimise false positive results. The development of less operator dependent LGE sequences such as PSIR¹²⁴ and dark blood imaging²³⁸ could make the incorporation of LGE in to clinical work flow easier. The volumetric quantification of LGE using 3D LGE CMR was a manual process that was labour intensive. Artificial intelligence and machine learning in future could help to automate the segmentation and measurement of LGE volume thereby further enabling its use in clinical practice.

Although we have found that LGE CMR has a significant role in the risk stratification for life-threatening VT and death in rTOF patients from our current contemporary era, it is unlikely that it will stand the test of time. With further advances in surgical, catheter based intervention and medical management anticipated, LGE CMR is expected to be less predictive of outcome. Risk scores in future will need to be dynamic to adjust for the patients evolving risk profiles identified from big data research.

It is understandable that the identification of patients at high risk of life-threatening VT and death could also have an adverse impact on patients' mental health, particularly when management options are limited and patients may go on to follow a poor trajectory despite

ICD implantation. However, when we applied our risk scores in clinical practice, patients fed back that they found that having a visual risk scheme helpful for their understanding of their current clinical status and for understanding the medical debate. Being better informed also helped patients to participate in the decision making about their own health. High-risk patients are counselled with regards to their risk of VT and death alongside support from a clinical psychologist that works with the team.

Enabling the wider application of the risk scores to predict VT and SCD

LGE in adult rTOF patients has prognostic value and therefore, it is justifiable to include this as part of the clinical CMR study protocol for adult rTOF patients. We estimate that risk stratification with LGE may be considered in adults who begin to develop other risk factors, and repeated selectively due to new symptoms, or following an intervention such as PVR which would potentially alter scar distribution and typically no more frequently than every five years. Currently the acquisition of RV LGE requires expertise to ensure that the myocardium is meticulously nulled in order to avoid false positive and false negative results. The improvement of LGE sequences towards ones that are less operator dependent such as PSIR¹²⁴ and dark blood late enhancement imaging²³⁸ could help facilitate the wider application of RV LGE assessment. Further work is required to externally validate the risk scores that we have created to identify the patients at highest risk of mortality and VT/SCD. Despite these risk scores, we were unable to correctly identify all patients who had devastating events. Further research is still justified to understand whether other myocardial scar characteristics, diffuse fibrosis and active processes on an ultrastructure level could explain why patients who are of seemingly low or intermediate risk can still suffer events.

Determining the prognostic value of RV T1 mapping

A number of T1 mapping sequences have been applied to the RV previously by other groups [shortened MOLLI (ShMOLLI)²³⁹, MOLLI¹⁵⁰, saturation recovery single-shot acquisition (SASHA)²⁴⁰, accelerated and navigator-gated look-locker imaging for cardiac T1 estimation (ANGIE)²⁴¹] although it remains inconclusive which method gives the most accurate and robust assessment of diffuse myocardial fibrosis. We have shown that native RV T1 using an optimised T1 mapping MOLLI sequence dedicated to the RV, has potential as an early biomarker for myocardial disease in rTOF patients however this method has several imperfections as described in Chapter five. Further work towards developing sequences specifically tailored for the RV T1 are still required. Furthermore, the prognostic value of native RV T1 mapping is yet to be determined calling for its further evaluation in larger prospective studies. Future prospective study of RV T1 mapping could help to determine if RV T1 has a role in helping to further define the optimum timing for pulmonary valve intervention in rTOF and whether it can be used to non-invasively monitor progress of patients on heart failure therapy such as ACE inhibitors and mineralocorticoid receptor antagonists which are known to have anti-fibrotic effects.

Evaluating the role of the immune system in promoting myocardial fibrosis

It has been shown that inflammation has a key role in the development of heart failure. For patients with heart failure and preserved ejection fraction, recent pharmaceutical trials of anti-inflammatory therapy such with interleukin antagonists have so far demonstrated improvement in exercise capacity.²³³⁻²³⁵ The over-activation of macrophages and inflammatory cytokines has been identified in heart failure with preserved ejection fraction

to be a driver for myocardial fibrosis²⁴². This association has not been explored in patients with CHD. I have planned further work to deepen understanding of biomarkers of fibrosis and the effects of inflammation on adverse remodelling in rTOF patients. I plan to study if there is correlation between histological fibrosis, macrophage, interleukins, MMP and TIMP activity measured by the direct staining of the myocardium alongside high sensitivity C - reactive protein and troponin measured on peripheral blood samples.

Chapter 8: Overall conclusions

To conclude, myocardial fibrosis defined by CMR has a central role in the risk-stratification for mortality and life-threatening VT in adult patients with rTOF.

Focal fibrosis in the RV, quantified non-invasively using high-sensitivity 3D-LGE CMR, can predict VT inducibility as a surrogate marker of mortality. Secondly, RV LGE burden can be used practically as a 'rule-out' test to help low-risk patients with minimal fibrosis avoid invasive programmed ventricular stimulation.

RV LGE strongly predicts mortality and clinically important VT. It can be used pragmatically alongside all other independent predictors of outcome within a weighted risk score to identify high-risk patients who warrant closer consideration for advanced therapy such as with primary prevention AICDs.

Diffuse fibrosis defined by native RV T1 mapping, shows promise as an early biomarker for adverse remodelling. On blood serum testing, increased degradation of collagen and high collagen turnover presides in patients with rTOF and corresponds with adverse remodelling. The profiling of MMP/TIMP may have potential as an early biomarker of disease.

Overall, tissue and imaging biomarker candidates may help us further differentiate risk in patients in the 'intermediate' risk group for VT and death and could have a role in tracking disease activity and response to therapy.

Appendices

Abstracts, publications and prizes related to this work

Abstracts & Presentations:

Ghonim S, Gatzoulis MA, Heng EL, Smith GC, Keegan J, Alpendurada FA, Ernst S, Li W, Moon JC, Pennell D, Babu-Narayan SV. Independent and relative value of Late gadolinium enhancement in predicting compromising ventricular arrhythmia or mortality in adults with repaired tetralogy of Fallot.

Oral presentation and early career award in: SCMR 23rd annual scientific sessions. Orlando, FL: SCMR; 2020.

Ghonim S, Ernst S, Keegan J, Giannakidis A, Spadotto V, Voges I, Smith GC, Boutsikou M, Montanaro C, Wong T, Ho SY, McCarthy KP, Shore DF, Dimopoulos K, Uebing A, Swan L, Li W, Pennell DJ, Gatzoulis MA and Babu-Narayan SV. Late Gadolinium Enhancement CMR Predicts Ventricular Tachycardia Inducibility in Repaired Tetralogy of Fallot.

Oral presentation at: SCMR 22nd annual scientific sessions. Seattle: SCMR; 2019.

S Ghonim, M A Gatzoulis, G C Smith, E Heng, S Ernst, W Li, J Keegan, G P Diller, K Dimopoulos, J C Moon, D J Pennell, S V Babu-Narayan
2395 LGE CMR predicts sudden death and VT in adults with repaired tetralogy of Fallot - a prospective study with 3500 patients follow up years, *European Heart Journal*, Volume 40, Issue Supplement_1, October 2019, ehz748.0148.

Oral Presentation at: ESC congress 2019 together with the World congress of cardiology 2019, Paris, France.

S Ghonim, P D Gatehouse, G Giblin, J Keegan, G C Smith, G C Mathews, S Jenkins, F Alpendurada, K Dimopoulos, D J Pennell, W Li, J C Moon, M Gatzoulis, S Babu-Narayan, 277 Can RV optimised native T1 mapping and ECV add clinical value in repaired tetralogy of Fallot?, *European Heart Journal - Cardiovascular Imaging*, Volume 20, Issue Supplement_2, June 2019, jez121.002,

Oral Presentation at: EuroCMR 2019, Venice, Italy.

Ghonim S, Ernst S, Keegan J, Giannakidis A, Spadotto V, Voges I, Smith GC, Boutsikou M, Montanaro C, Wong T, Ho SY, McCarthy KP, Shore DF, Dimopoulos K, Uebing A, Swan L, Li W, Pennell DJ, Gatzoulis MA and Babu-Narayan SV. Late Gadolinium Enhancement CMR Predicts Ventricular Tachycardia Inducibility in Repaired Tetralogy of Fallot.

Oral presentation at: BCCA Annual conference 2018, Liverpool, UK.

Ghonim S, Jenkins S, Smith GC, Mathew G, Gatzoulis MA, Gatehouse P, Babu-Narayan SV. 9 ECV and T1 mapping in repaired tetralogy of Fallot–CMR diffuse fibrosis measurement needs the right method for the right ventricle. *Heart* 2018;104:A9.

Poster presentation at: British Society of Cardiovascular Magnetic Resonance (BSCMR) 2018, Edinburgh, UK

Publications:

Ghonim S, Ernst S, Keegan J, Giannakidis A, Spadotto V, Voges I, Smith GC, Boutsikou M, Montanaro C, Wong T, Ho SY, McCarthy KP, Shore DF, Dimopoulos K, Uebing A, Swan L, Li W, Pennell DJ, Gatzoulis MA and Babu-Narayan SV. Three-Dimensional Late Gadolinium Enhancement Cardiovascular Magnetic Resonance Predicts Inducibility of Ventricular Tachycardia in Adults With Repaired Tetralogy of Fallot. *Circ Arrhythm Electrophysiol*. 2020;13:e008321.

Ghonim S, Gatehouse PD, Gatzoulis MA, Babu-Narayan SV. Is cardiovascular magnetic resonance measurement of diffuse fibrosis ready for clinical use in the systemic RV? *Int J Cardiol*. 2018 Nov 15;271:66-67. doi: 10.1016/j.ijcard.2018.06.095. Epub 2018 Jun 22. PMID: 29970268.

Ghonim S, Voges I, Gatehouse PD, Keegan J, Gatzoulis MA, Kilner PJ, Babu-Narayan SV. Myocardial architecture, mechanics, and fibrosis in congenital heart disease. *Frontiers in cardiovascular medicine*. 2017 May 23; 4:30.

In revision with JACC Cardiovascular imaging: Predicting survival in repaired tetralogy of Fallot- a lesion specific and personalised approach (relating to work from chapter 4).

Prizes:

Early career award- Clinical science at SCMR 23rd annual scientific sessions. Orlando, FL: SCMR; 2020.

Young investigator award at BCCA Annual conference 2018, Liverpool, UK

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