The Hepatic Effects of Right Heart Lesions in Adult Congenital Heart Disease

Observational Study of Novel Measures of Hepatic Stiffness in Right Heart Valvular Regurgitation

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1. Abstract

Background: The optimum timing of pulmonary valve replacement for pulmonary regurgitation in adults (following repair of Tetralogy of Fallot or Pulmonary Stenosis) is under constant refinement. Right ventricular failure causes hepatic congestion which may lead to hepatic fibrosis. Non-invasive methods of measuring hepatic stiffness, a surrogate for hepatic fibrosis, have been developed. The main study aims were to determine if subclinical abnormalities of hepatic structure were present within this population, and whether this correlated with established prognostic markers including right ventricular ejection fraction.

Methodology: This observational cross-sectional study examined markers of hepatic stiffness using three modalities, including two imaging modalities – Fibroscan and Magnetic Resonance Elastography (MRE), and one serum test – the Enhanced Liver Fibrosis panel (ELF score). Cardiovascular magnetic resonance imaging, electrocardiography, cardiopulmonary exercise test, and NT-Pro-BNP were also utilised. Participants were divided into three study groups. Group 1 contained participants with Moderate-Severe Pulmonary Regurgitation, Group 2 Mild Pulmonary Regurgitation, and Group 3 Tricuspid Regurgitation related to Ebstein's Anomaly (comparator group).

Results: ANOVA testing detected no significant difference between study groups across all three indices of hepatic stiffness. Univariate linear regression was performed using the hepatic primary outcomes as the dependent variable in participants with pulmonary regurgitation (Groups 1&2). ELF score significantly correlated (Pearson's correlation coefficient 'r') with right ventricular ejection fraction ($r = -0.37$, $p = 0.03$), QRS duration ($r = 0.51$, $p = 0.002$), and NT-Pro-BNP ($r = 0.39$, $p = 0.02$). MRE significantly correlated with right ventricular end diastolic volume ($r = 0.42$, $p = 0.01$), QRS duration ($r = 0.043$, $p = 0.017$), and NT-Pro-BNP ($r = 0.48$, $p = 0.006$). Fibroscan significantly correlated with NT-Pro-BNP ($r = 0.46$, $p = 0.01$).

Conclusion: Measurements of hepatic stiffness correlate significantly with established prognostic markers, and may be useful in the clinical surveillance of patients with pulmonary regurgitation.

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2. Declaration of Originality

The work reported in this thesis is original and was performed by myself in collaboration with colleagues at the Norfolk and Norwich University Hospitals NHS Foundation Trust and University of East Anglia. All work done by others and reported in this thesis is referenced.

3. Introduction

3.1 Rationale and Clinical Need

The incidence of congenital heart disease (CHD) is 6.9 – 8 per 1000 live births (Peterson S 2003, The Somerville Foundation). Of those born with CHD at least 85% now survive into adulthood (British Cardiac Society Working Group, 2002). There are currently more than 150,000 adults with congenital heart disease in England (Peterson S 2003), of whom 2,800 are under the care of Norfolk and Norwich University Hospitals and Papworth Hospitals collectively (at the time of this study).

Adults with congenital heart disease are a growing population thanks to improvements in initial diagnosis and management. As adults they are often faced with progression of disease and/or the sequelae of their initial surgery. A prime example of this is pulmonary regurgitation as a result of repair of Tetralogy of Fallot or following intervention to treat pulmonary stenosis. Timing of pulmonary valve replacement in adults, particularly in asymptomatic patients, is challenging, with clinical guidelines under constant refinement.

The aim of this study is to examine novel variables that may contribute to the surveillance of patients with pulmonary regurgitation in particular, which may therefore assist in the timing of pulmonary valve replacement. This study is particularly concerned with congenital conditions of the right heart, particularly those that cause regurgitation from the pulmonary and tricuspid valves. Both conditions are tolerated well for many years. Since the liver is directly connected to the right atrium via the inferior vena cava and thus the right ventricle, markers of liver dysfunction may provide early evidence for a compromised right ventricle – a significant marker in both conditions. The association between cardiac dysfunction and hepatic disease is well

established, and this has been assessed particularly in the Fontan circulation. This study will question whether that association extends to other subgroups within the adult congenital heart disease population. It will address the hypothesis that novel biomarkers of liver dysfunction and fibrosis, which may not be reflected on standard liver function tests, add to the overall assessment of patients with significant pulmonary and/or tricuspid valve regurgitation. In particular if such biomarkers may then contribute to the decision making around valve replacement or repair in adulthood.

3.2 Pulmonary Regurgitation

3.2.1 Pathophysiology

Significant Pulmonary regurgitation (PR) most commonly occurs as a result of intervention to the pulmonary valve and/or right ventricular outflow tract. It may also occur in the setting of the rare absent pulmonary valve syndrome. Acquired/iatrogenic pulmonary regurgitation occurs most commonly in two circumstances – repair of Tetralogy of Fallot (TOF), or relief of congenital pulmonary stenosis (PS). Over time this may ultimately lead to progressive right ventricular (RV) dilatation and impairment of function (Murphy JG 1993, Gatzoulis MA 2000, Oechslin EN 2000, Verheugt CL 2011). Given the improvement in surgical technique and survival (particularly in TOF), there is now a substantial adult population faced with the sequelae of surgical palliation. The question of if and when to replace the pulmonary valve, and which variables to consider, alongside the optimal timing, have therefore gathered much interest.

3.2.2 Tetralogy of Fallot

Tetralogy of Fallot is one of the most frequent forms of congenital heart disease, and is one of the most common reasons for intervention in the first year of life (Report of the New England Regional Infant Cardiac Program 1980). It is characterized by the following four anatomical abnormalities: 1) Ventricular septal defect (VSD), 2) Right ventricular outflow tract obstruction (RVOTO), 3) Overriding aorta, and 4) Right ventricular hypertrophy (Figure 1, Ho SY 1995).

The precise embryological mechanism that leads to this pattern is unknown however genetic and environmental factors are thought to be contributory. There is initially anterior and cephalad deviation of the infundibular (outlet) septum. This results in the listed abnormalities 1-3, with hypertrophy of the right ventricular occurring as a result of increased workload, as a consequence of right ventricular outflow tract obstruction (Al Habib HF 2010). The outflow tract obstruction is highly variable and can be subvalvar (infundibular stenosis being the most common), valvar or supravalvar. There may also be associated pulmonary artery stenoses within the main vessels and beyond, patent foramen ovale, atrioventricular septal defects (particulary associated with Trisomy 21), secundum atrial septal defects, and right sided aortic arch. A right ventricular-pulmonary artery valved conduit may be used to avoid the most common coronary artery anomaly – the left anterior descending artery arising from the right coronary artery and traversing the right ventricular outflow tract (Babu-Narayan SV 2010)

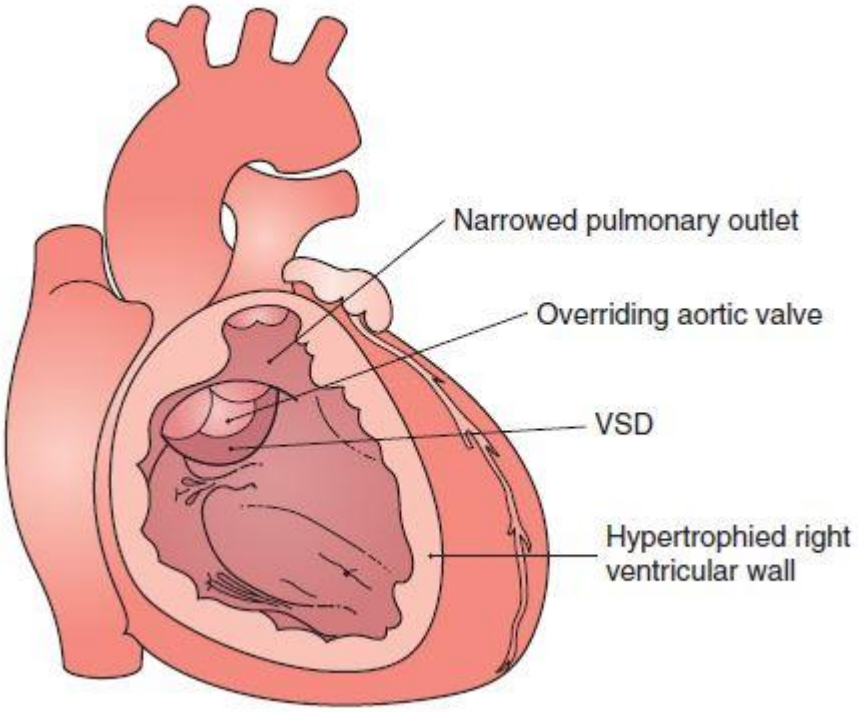


Figure 1: Anatomy of Tetralogy of Fallot. Courtesy Ho SY, 1995

3.2.3 Surgical History

The first successful palliation in TOF occurred in 1944 using a Blalock-Taussing shunt where the subclavian artery was directly anastomosed to the pulmonary artery (Blalock A 1945). This procedure was later modified to a shunt (usually Gore-tex) connection rather than a direct anastomosis to protect the pulmonary circulation from systemic arterial pressures. Within 10 years the first successful open repair was carried out (Lillehei CW 1955). Open surgical repair involves closing the ventricular septal defect and relief of the right ventricular outflow tract obstruction. Historically this involved a technique known as transannular patch repair. A large incision was made in the right ventricle and the VSD was closed from this approach. The pulmonary stenosis was relieved by means of valvectomy and the defect repaired using a patch across the pulmonary valve annulus. This technique has fallen out of favour, as it causes

obligatory severe pulmonary regurgitation and rendered a large section of the right ventricle dyskinetic. It has been replaced by a technique that repairs the VSD through the right atrium, and RVOTO through the pulmonary artery through a minimal incision. Mild to moderate RVOTO is accepted over significant pulmonary regurgitation as this is better tolerated in the long term (Babu-Narayan SV 2010).

3.2.4 Congenital Pulmonary Stenosis

Obstruction to the right ventricular outflow tract caused by a stenotic pulmonary valve may be detected either as a single entity (more commonly) or as part of more complex congenital heart disease. The treatment of choice for isolated pulmonary stenosis in the 1960s and 1970s was surgical valvotomy. This has largely been replaced with percutaneous balloon valvuloplasty, first described in 1982 (Kan JS 1982). Both surgical and percutaneous techniques are associated with subsequent pulmonary regurgitation, although less so with the percutaneous approach in studies of mid to late follow up (Rao PS 1988, McCrindle BW 1991, O'Connor BK 1992, Masura J 1993, Rao PS 1998, Garty Y 2005). Current Guidelines recommend catheter intervention in non-dysplastic pulmonary valves and in those who may require stents to treat pulmonary artery peripheral branch stenosis. A surgical approach is deemed necessary for dysplastic valves, those with infundibular stenosis or other associated lesions (Baumgartner H 2010).

3.2.5 The Natural Course of Pulmonary Regurgitation

Although pulmonary regurgitation is tolerated well in the early and short term phase, chronic volume overload of the right ventricle causes progressive dilatation and right ventricular failure (Gatzoulis MA 1995). As the right ventricle gradually dilates and eventually fails, there are a plethora of consequences for the patient including sustained ventricular tachycardia, sudden cardiac death, atrial arrhythmias, right ventricular hypertrophy and left ventricular dysfunction (Valente AM 2014, Nollert G 1997), shown in Figure 2 (Kim YY 2016). During the long term follow up of these patients, clinicians need to remain vigilant to symptoms related to arrhythmia or heart failure to judge the optimum timing for pulmonary valve replacement.

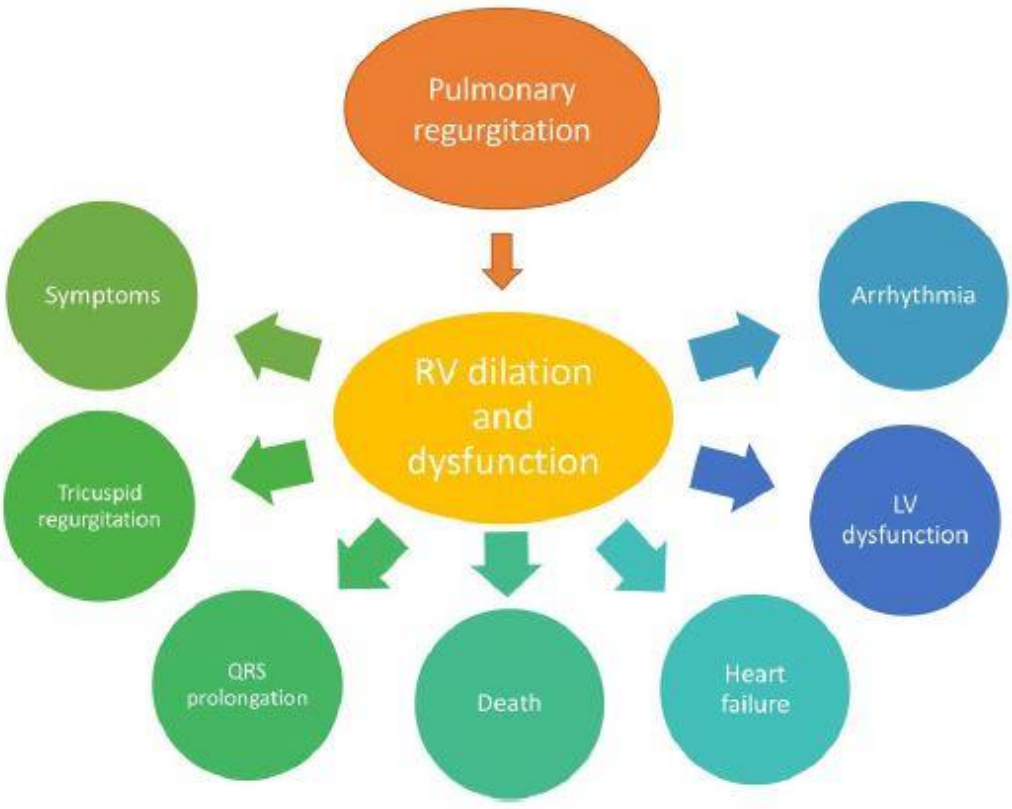


Figure 2: Multifaceted sequelae of chronic pulmonary regurgitation. Courtesy: Kim YY 2016.

3.3 Assessment and Timing of Pulmonary Valve Replacement

3.3.1 Indications for Pulmonary Valve Replacement – Current Guidelines

Guidelines for pulmonary valve replacement (PVR) recommend that symptomatic patients with severe PR should be offered pulmonary valve replacement. Several other variables are considered when the asymptomatic patient is concerned. Current European Society of Cardiology guidelines for PVR are summarised in Table 1 (Baumgartner H 2010).

Recommendation	**Class of Recommendation**	**Level of Evidence**
PVR should be performed in **symptomatic** patients with severe PR and/or stenosis (RV systolic pressure > 60mmHg, TR velocity > 3.5m/s)	**I**	**C**
PVR should be considered in **asymptomatic** patients with severe PR and/or PS when at least one of the following criteria is present • Decrease in objective exercise capacity • Progressive RV systolic dysfunction • Progressive TR (at least moderate) • RVOTO with RV systolic pressure > 80mmHg (TR velocity > 4.3m/s) • Sustained atrial/ventricular arrhythmias	**IIa**	**C**

Table 1: European Society of Cardiology Guidelines for the management of grown-up congenital heart disease; guidelines on PVR (Baumgartner H 2010).
PVR – Pulmonary Valve Replacement, PR – Pulmonary Regurgitation, PS – Pulmonary Stenosis, RV – Right Ventricular, TR – Tricuspid Regurgitation, RVOTO – Right Ventricular Outflow Tract Obstruction.

3.3.2 Right Ventricular Volume and Function

Subjecting patients to the risk of further cardiac surgery, and considering that bioprosthetic or homograft valves have a limited lifespan (10-15 years), decision making surrounding if and when to replace the regurgitant pulmonary valve mandates careful consideration. Optimal timing of pulmonary valve replacement has been the focus of several research studies over the last decade and a half. Therrien and colleagues were the first to suggest that PVR should be performed before right ventricular function deteriorated (Therrien J 2000). This study of 25 patients undergoing PVR showed no significant difference in mean pre-operative versus post-operative right ventricular end diastolic (RVEDV) or systolic (RVESV) volumes measured by radionuclide angiography. Mean RVEDV pre-op 227.1ml versus post op 214.9ml, $p = 0.74$, Mean RVESV pre-op 157.4ml versus post-op 155.4ml, $p = 0.94$. Furthermore there was no significant difference in mean right ventricular ejection fraction (RVEF), remaining reduced post-surgery (35.6% versus 34.7%, p-value = 0.78).

Vliegen and colleagues (Vliegen HW 2002) were the first to show significant improvements in right ventricular volumes and ejection fraction (corrected for right sided valvar regurgitation and shunts) in their cardiovascular magnetic resonance imaging (CMR) study of 26 patients. At a mean follow up of 7.4 ± 2.4 months after pulmonary valve replacement, RVEDV reduced from 305 ± 87 to 210 ± 62 mls ($p<0.001$), and RVESV reduced from 181 ± 67 to 121 ± 58 mls ($p<0.001$).

Several other studies subsequently assessed the response of right ventricular parameters measured on CMR after pulmonary valve replacement. The search for the optimal threshold at which reverse right ventricular remodelling may occur prompted several studies In another study, Therrien and colleagues (Therrien J 2005) studied 17 patients undergoing PVR with CMR pre and post-operatively (mean follow up 21 months). They

reported a significant reduction in RVEDV from 163 ± 34 to 107 ± 26 ml/m2, p <0.001 and RVESV from 109 ± 27 to 69 ± 22 ml/m2, p <0.001. Participants had a mean pre-operative RVEF% of 32 ± 7, and post-operative mean of 34 ± 10, with no significant increase on statistical testing, p = 0.12. They noted that participants with pre-operative indexed values of RVEDV > 170ml/m2 and RVESV > 85ml/m2 did not normalise their right ventricular volumes post operatively. \

Oosterof and colleagues (Oosterhof T 2007) further studied these thresholds in a study of 71 patients, with follow up CMR performed a median of 9 months after PVR. They reported a mean reduction in RV volumes by 28% after surgery. This study also did not show a significant increase in RVEF%, from 42 ± 10 to 43 ± 10 postoperatively, p = 0.34. A pre-operative threshold of RVEDV <160 ml/m^2 was associated with normalisation of the RV (defined as < 108ml/m^2) with a sensitivity of 55% and specificity of 92%. A pre-operative threshold of RVESV of < 82 ml/m^2 was associated with normalisation (defined as < 47ml/m^2) with a sensitivity of 74% and specificity of 82%. Pulmonary valve replacement has also been shown to cause reverse remodelling of the RV if performed below these thresholds in another study by Burchill and colleagues (Burchill L 2011).

Lee and colleagues in a larger study of 170 patients undergoing PVR found that the optimal cut-off points were RVEDV of 163 ml/m2, and RVESV of 80 ml/m2. This was associated with significant decrease in right ventricular volume, and improvement in function as measured by CMR (Lee C 2012). More recently Bokma and colleagues (Bokma JP 2016) examined mid-late term outcomes in 157 patients, and found that pre-operative RVESV < 80ml/m2 was associated with ‘RV normalisation’ (a term used to describe RVEF > 48% and RVEDV < 108 ml/m2). They also showed that pre-

operative RVESV > 95ml/m2 was associated with suboptimal mid-to-late haemodynamic outcomes

In a meta-analysis of 48 studies including 3118 patients undergoing pulmonary valve replacement, this remodelling of the right ventricle (RVEDV and RVESV) was again shown. There was no significant difference in 'uncorrected' right ventricular ejection fraction (measured on CMR) seen post operatively. However when using studies that utilised RVEF corrected for residual shunts & pulmonary and tricuspid regurgitation, an improvement is seen post-operatively. (Ferraz Cavalcanti PE 2013).

In summary, thresholds have been defined at which reverse remodelling of the right ventricle may occur, and there has been a move towards surgical intervention before the right ventricular function (ejection fraction) deteriorates.

3.3.3 Left Ventricular Volume and Function

Although left heart size and function is not a part of the current guidelines on criteria and timing of PVR, several research studies have demonstrated the sequelae of chronic pulmonary regurgitation and response to PVR. Proposed mechanisms of left ventricular dysfunction include the interaction between the geometries and function of right and left ventricles (the Bernheim effect), pulmonary arterial and venous flow, shared myocardium (in particular the ventricular septum), and shared coronary blood flow.

Kondo and colleagues (Kondo C 1995) were the first to demonstrate dynamic left ventricular (LV) dysfunction during exercise in those with repaired Tetralogy of Fallot. They studied 29 patients using radionuclide ventriculography on a bicycle ergometer. Left ventricular ejection fraction was depressed in all participants, and an inverse correlation was seen with RVEDV and degree of pulmonary regurgitation.

Davlouros and colleagues (Davlouros PA 2002) compared 85 patients with repaired Tetralogy of Fallot against 26 controls using CMR. They showed that left ventricular ejection fraction was lower in the study group versus controls ($p = 0.002$) and that right ventricular systolic dysfunction correlated with left ventricular systolic dysfunction.

The left ventricle has been shown to increase in volume and function following PVR. Frigiola and colleagues ([1]Frigiola A 2008) studied 36 patients using CMR in a prospective study which included 25 surgical pulmonary valve replacements and 11 percutaneous pulmonary valve replacements in two groups. In addition to post-operative improvements in right ventricular volumes and function, both groups were shown to have significantly improved left ventricular effective stroke volumes. The same author ([2]Frigiola A 2008, Circulation) conducted a further study on 71 patients undergoing surgical pulmonary valve replacement with CMR pre and post-operatively (1 year). The

previously documented improvements in right ventricular volumes and function were seen, and in addition an increase in left ventricular end diastolic volume (66 ± 12 to 73 ± 13 ml/m^2, P < 0.0001), and improvement in effective left ventricular cardiac output (3.0 ± 0.6 to 3.4 ± 0.7 L/min, P < 0.0001). Several authors stress that clinicians should not misinterpret the increase in left ventricular size after PVR, as this signifies improved filling pressures rather than dilatation/dysfunction.

3.3.4 Tricuspid Regurgitation

One of the consequences of right ventricular dysfunction (Figure 2) of any cause is tricuspid valve annulus dilatation and resultant 'functional' tricuspid regurgitation. This is pertinent to patients with repaired Tetralogy of Fallot. Iatrogenic damage or other pathologies of the tricuspid valve leaflets or annulus may also contribute to tricuspid regurgitation. Tricuspid regurgitation may then worsen right ventricular dilatation and contribute to right atrial enlargement and arrhythmias (Gatzoulis MA 2000, Bonello B 2013).

Pulmonary valve replacement reduces the degree of tricuspid regurgitation in repaired Tetralogy of Fallot. Kogon and colleagues (Kogon B 2010) assessed 35 patients with mean age 30.5 years with pre and post operative echocardiography. They used a scoring system for degree of tricuspid regurgitation, where 0 = none, 1 = mild, 2 = moderate and 3 = severe. In all patients there was a significant reduction in the degree of tricuspid regurgitation 1 month post-operatively (mean score 2.33 vs 1.3, p < 0.0001). Of note, there was no significant difference between those who had pulmonary valve replacement alone versus those that had concomitant tricuspid valve annuloplasty (mean score 1.29 vs 1.31, p = 0.81).

Bokma and colleagues (Bokma JP 2015) examined the impact of pre-operative tricuspid regurgitation in those who underwent pulmonary valve replacement in a retrospective study involving 129 patients. Adverse clinical events were defined as death, arrhythmia (sustained ventricular or supraventricular) and heart failure. In addition to age and right ventricular end systolic volume, severe pre-operative tricuspid regurgitation was a significant predictor for adverse events (hazard ratio 2.49, 95% CI 1.11 to 5.52, p = 0.028) on multivariate cox regression analysis.

3.3.5 QRS Duration on Electrocardiogram

Arrhythmias contribute significantly to morbidity and mortality following repair of Tetralogy of Fallot (Rosing DR 1978, Deanfield JE 1980, Murphy JG 1993). Right bundle branch block is almost universal in patients having undergone repair of Tetralogy of Fallot. The QRS duration has been shown to correlate with degree of right ventricular dilatation and dysfunction. Gatzoulis and colleagues (Gatzoulis MA 1995) studied 178 adult patients with mean follow up of 21.4 years. The 12-lead electrocardiograms of 9 patients with documented ventricular arrhythmia and 4 with sudden cardiac death had QRS duration of > 180ms. The study also showed that patients with restrictive right ventricular physiology had shorter QRS duration (129.3ms ± 20) versus the widened QRS duration of those without restrictive physiology (157.5ms ± 13.2, p < .001).

In a further multi-centre study of 793 patients over a mean follow up 21.1 ± 8.7 years, Gatzoulis and Colleagues (Gatzoulis MA 2000) studied risk factors for arrhythmia and sudden death. They demonstrated that a prolonged QRS duration and the rate of change in QRS duration over a 10-year period were risk factors for adverse arrhythmic events.

QRS prolongation has also been linked with post-operative adverse outcomes other than arrhythmia, including death, re-operation, and heart failure. Scherptong and colleagues (Scherptong RWC 2010) studied 90 patients with previous repair of Tetralogy of Fallot that underwent PVR. They assessed pre and post-operative QRS duration, and adverse events over a mean follow up of 5.5 ± 3.5. 5-year event free survival was better for patients with pre-operative QRS duration ≤ 180ms than for those with QRS duration ≥ 180ms (90% versus 76%, p = 0.037). 5-year event free survival was also better for patients with post-operative QRS duration of ≤ 180ms than for those with QRS duration ≥ 180ms (91% versus 71%, p = 0.004). Failure to reduce the post-operative QRS duration was associated with a hazard ratio of 6.767 ($p < 0.01$), with a post-operative QRS duration of ≥ 180ms associated with a hazard ratio of 3.685 ($p < 0.05$).

In addition to the QRS duration, QT dispersion (difference/range of QT interval measurements across electrocardiogram leads) has also been studied in patients with repair of Tetralogy of Fallot. In their study on 99 patients, Gatzoulis and colleagues (Gatzoulis MA 1997) studied electrocardiographic risk factors for sustained ventricular tachycardia. This included 59 with repaired Tetralogy of Fallot including 10 with documented ventricular tachycardia and 40 controls. A QT dispersion of > 75ms, or QRS dispersion of > 35ms, or QT interval of 500 ms, or JT dispersion > 65ms predicted those with sustained ventricular tachycardia. If those with QRS duration ≥180 ms were considered, either QT dispersion > 60 ms, or QRS dispersion > 35 ms, or JT dispersion > 60 ms would have a high sensitivity (98%) and specificity (100%) in identifying patients with sustained ventricular tachycardia.

Most recently Bokma and colleagues have suggested that fragmented QRS (additional spikes within the QRS complex, related to myocardial fibrosis) may be more sensitive than QRS duration alone in risk stratification, but further studies are required to confirm

this finding (Bokma 2016). Accurate and reproducible measurements of QT dispersion and QRS fragmentation are difficult (Malik M 2000) and these techniques are not used routinely in the clinical care of this population. The relative ease of serial measurements of QRS duration over the long-term follow up of patients following repair of Tetralogy of Fallot is therefore more practical, and is able to both risk stratify and time PVR.

3.3.6 Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CPET) is used to quantify exercise intolerance in adult congenital heart disease, particularly as patients may over time start limiting their activities without an awareness of symptoms. Objective measures of reduced exercise tolerance are more prevalent than either patient or physician may suspect (Diller GP 2005). CPET provides prognostic information in repair of Tetralogy of Fallot which is similar to its role in heart failure.

A symptom limited exercise test is performed with simultaneous measurement of cardiac and respiratory function including MVO_2 (maximum volume of oxygen consumed), VE/VCO_2 slope (minute ventilation to carbon dioxide production ratio), heart rate and blood pressure response, oxygen pulse (surrogate for stroke volume), and electrocardiogram recording.

In a study of patients with prior repair of Tetralogy of Fallot, Giardini and colleagues examined the prognostic value in 118 individuals who underwent CPET (Giardini A 2007). The primary outcome of death or hospitalisation (cardiac causes) occurred in 27 patients after a mean follow up of 5.8 ± 2.3 years. MVO_2 (hazard ratio 0.974) and the VE/VCO_2 slope (hazard ratio 1.076) were predictors of death or hospitalisation. In a subgroup analysis of patients who achieved ≤36% of predicted peak oxygen uptake

versus those that scored > 36%, 5-year mortality was significantly higher, 48% vs 0%, p < 0.00015. Those with high VE/VCO_2 slopes > 39 were at greater risk for cardiac-related death than those with a slope of < 39 (31% vs 0%, p <0.0001).

In a more comprehensive multi-centre European retrospective study of 875 patients, Muller and colleagues (Muller J 2015) examined the predictive value of cardiopulmonary exercise testing and QRS duration on cardiac outcomes. Over a mean follow up of 4.1 ± 2.6 years following CPET, the primary outcome of death or sustained ventricular tachycardia occurred in 30 patients (3.4%). MVO_2, QRS duration and age were all found to be significant predictors of the primary outcome on multi-variate cox regression modelling. Fresh thresholds were determined that conferred the worst risk of achieving the primary outcome including $MVO_2 \leq 65\%$, VE/VCO2 slope ≥ 31, and QRS duration ≥ 170ms.

Pre-operative CPET is also useful in risk-stratifying patients undergoing PVR, with MVO_2, VE/VCO2 slope and heart rate reserve able to predict early surgical mortality (Babu-Narayan SV 2014). Serial measurements in addition to assessment using other modalities described are therefore considered a routine part of the long term follow up and pre-operative evaluation.

3.4 N-Terminal Prohormone of Brain Natriuretic Peptide

N-terminal Prohormone of Brain Natriuretic Peptide (NT-Pro-BNP) and Brain natriuretic peptide (BNP) are well established diagnostic and prognostic markers for heart failure in the general population. Their role in adult congenital heart disease is less well defined.

NT-Pro-BNP has been studied in subjects after repair for Tetralogy of Fallot. Paulino and colleagues (Paulino A 2017) studied 15 paediatric patients and showed that NT-Pro-BNP significantly correlated with CMR derived right ventricular end diastolic volume, right ventricular end systolic volume and pulmonary regurgitant fraction (mean follow up 13.5 years). Right ventricular ejection fraction was not a significant correlator. Another study of 40 paediatric patients with a history of repair of Tetralogy of Fallot (Valverde I 2015) undergoing CMR also reported significant correlations between NT-Pro-BNP and right ventricular end systolic and diastolic volumes, and pulmonary regurgitant fraction. There was no association with right or left ventricular ejection fraction. Pietrzak and colleagues (Pietrzak R 2009) studied 20 children with repaired Tetralogy of Fallot. They showed that NT-Pro-BNP was higher in subjects with severe pulmonary regurgitation versus those with mild to moderate degrees of pulmonary regurgitation. Higher NT-Pro-BNP levels were also detected in those with more severe degrees of tricuspid regurgitation. They also measured QRS duration in lead II before and after a standard Bruce protocol treadmill test. The authors showed that participants with abnormal QRS prolongation after exercise had significantly higher NT-Pro-BNP than those that displayed QRS shortening after exercise.

Norozi and colleagues (Norozi K 2005) compared 50 adult patients with repaired Tetralogy of Fallot to 100 age-matched healthy controls. They underwent measurement of NT-Pro-BNP, echocardiography and cardiopulmonary exercise testing. They showed significantly elevated levels of NT-Pro-BNP in the Tetralogy of Fallot group compared with the healthy controls. In addition, significant correlations were found against right ventricular dimensions (end-diastole, measured using M-mode echocardiography) and estimated right ventricular systolic pressure. A significant negative correlation was also seen between NT-Pro-BNP and peak oxygen uptake. This inverse relationship between

natriuretic peptides and exercise capacity has also been shown in other studies involving subjects with repaired Tetralogy of Fallot (Trojnarska O 2006).

The studies described above show the association between right ventricular volume overload, right ventricular dimensions, exercise capacity and NT-Pro-BNP. The association between NT-Pro-BNP and markers of liver stiffness has not been studied in patients with pulmonary regurgitation.

3.5 Ebstein's Anomaly

Ebstein's anomaly is a relatively rare congenital malformation of the tricuspid valve. It was first described by Wilhelm Ebstein in 1866. There is great variety in morphology but in general the valve leaflets are malformed and apically displaced. The anterior leaflet of the tricuspid valve is abnormally elongated and 'sail-like' and usually arises correctly from the tricuspid annulus. There is failure of delamination of the posterior and septal leaflets resulting in tethering of these malformed leaflets to the endocardium. The posterior and septal leaflets are displaced apically ($\geq 0.8 cm/m^2$ Body Surface Area) and may be attached to the right ventricular endocardium (Baumgartner H 2010). The defect causes the right ventricle to be divided into two; the proximal atrialised RV and the apical functional RV. The tricuspid valve is typically regurgitant into a dilated right atrium. Associated conditions include patent foramen ovale or atrial septal defect (typically secundum ASD), ventricular septal defect, patent ductus arteriosus, coarctation of the aorta, atrial arrhythmias, and rarely left heart lesions. There is an association with accessory pathways, the Wolf-Parkinson-White syndrome in particular, in 25% of cases. The clinical presentation depends on age at presentation and includes cyanosis, heart failure and arrhythmia.

Management and follow up requirements are determined by the clinical manifestation. In the adult this may include management of arrhythmias, pacemakers, heart failure, and referral for surgery to repair the abnormal/Ebstein tricuspid valve when severe tricuspid regurgitation is associated with symptoms and or progressive dysfunction of right sided chambers. Surgical repair of the tricuspid valve has progressed through several techniques – the favoured approach is currently the 'cone repair' first described by da Silva (da Silva 2007). Indications for intervention in Ebstein's anomaly are shown in Table 2 (Baumgartner H 2010). Repair of the tricuspid valve in Ebstein's anomaly is only appropriate if the remaining right ventricular size and function are adequate. Surgery is not without risk of re-operation. A study of 539 patients by Brown and colleagues (Brown ML 2008) undergoing tricuspid valve surgery showed good short (94% at 5-years) and long (76% at 20-years) term survival. However, freedom from re-operation rates of 86% at 5-years fell to 46% at 20 years.

Indications	**Class of Recommendation**	**Level of Evidence**
SURGERY		
Surgical repair should be performed in patients with more than moderate TR and symptoms (NYHA class >II or arrhythmias) or deteriorating exercise capacity measured by CPET	**I**	**C**
If there is also an indication for tricuspid valve surgery, then ASD/PFO closure should be performed surgically at the time of valve repair	**I**	**C**
Surgical repair should be considered regardless of symptoms in patients with progressive right heart dilation or reduction of RV systolic function and/or progressive cardiomegaly on chest X-ray	**IIa**	**C**

Table 2: European Society of Cardiology Guidelines for the management of grown-up congenital heart disease; recommendations for intervention in Ebstein's Anomaly (Baumgartner H 2010).

TR – Tricuspid Regurgitation, NYHA – New York Heart Association Score, CPET – Cardiopulmonary exercise test, ASD/PFO – Atrial Septal Defect / Patent Foramen Ovale, RV – Right Ventricular

CATHETER INTERVENTION		
Patients with relevant arrhythmias should undergo electrophysiologic testing, followed by ablation therapy, if feasible, or surgical treatment of the arrhythmias in the case of planned heart surgery	**I**	**C**
In the case of documented systemic embolism probably caused by paradoxical embolism, isolated device closure of ASD/PFO should be considered	**IIa**	**C**
If cyanosis (oxygen saturation at rest <90%) is the leading problem, isolated device closure of ASD/PFO may be considered but requires careful evaluation before intervention	**IIb**	**C**

Table 2 (cont): European Society of Cardiology Guidelines for the management of grown-up congenital heart disease; recommendations for intervention in Ebstein's Anomaly (Baumgartner H 2010).

TR – Tricuspid Regurgitation, NYHA – New York Heart Association Score, CPET – Cardiopulmonary exercise test, ASD/PFO – Atrial Septal Defect / Patent Foramen Ovale, RV – Right Ventricular

3.6 Cardiac Investigations

The investigations used to monitor cardiac function in TOF and Ebstein's anomaly include transthoracic echocardiography (TE), cardiovascular magnetic resonance imaging (CMR), electrocardiogram (ECG), and cardiopulmonary exercise testing (CPET).

Echocardiography is the first line diagnostic modality which provides information on pulmonary regurgitation, residual right ventricular outflow tract obstruction, tricuspid regurgitation, and right and left ventricular size and function. There are well known limitations in assessing the right ventricle due to its peculiar tripartite shape. Assessing right ventricular volumes, mass and function necessitates geometrical assumptions which introduces error in its calculation. Echocardiography is also at times limited by poor acoustic windows, with the pulmonary valve a particularly difficult valve to image and assess.

CMR provides more accurate information in many conditions within adult congenital heart disease. In particular, the evaluation of right ventricular volumes and ejection fraction, and pulmonary regurgitation fraction. It is also useful in evaluating the right ventricular outflow tract, conduits between the right ventricle and pulmonary artery, and branch pulmonary arteries (Kilner PJ 2010). It has the advantage of an unlimited view, producing high quality images without ionizing radiation. However not all patients tolerate this technique due to claustrophobia.

Normal values for cardiac indices as determined by transthoracic echocardiography and CMR are discussed in the Methodology section.

3.7 Liver Disease Associated with Cardiac Disease

3.7.1 Congestive Hepatopathy

The liver and heart may influence each other in various disease states. Cardiac and circulatory conditions that affect the liver are of particular interest in this study. These may occur as a result of acute ischaemic hepatitis, or a spectrum from congestive hepatopathy. Heart failure, particularly of the right sided chambers causes venous stasis from high right atrial pressure. Common causes of congestive hepatopathy are ischaemic heart disease, valvar disease, cardiomyopathy, restrictive lung disease (causing pulmonary arterial hypertension and right heart failure), and pericardial disease (Valla D 1991). Any cause of systemic venous hypertension, including from adult congenital heart disease, causes chronic pressure overload of the hepatic venous circulation.

Hepatic venous congestion leads to a series of histological changes which begins with sinusoidal and terminal hepatic venule distention, leading to perisinuisodal oedema and compromised oxygen delivery to hepatocytes. There is leakage of protein rich fluid into the space of Disse (space between sinusoids). This excessive fluid overwhelms the hepatic lymphatic drainage and this fluid leaks into the peritoneal cavity causing a protein rich ascites.

Historically, determining the cause of ascites was based on the distinction between the ascitic fluid protein concentration where a measure of $\geq$ 2.5g/dL would be consistent with an exudate, and < 2.5g/dL, a transudate. This method was shown to be inferior to the measurement of the serum ascites albumin gradient (SAAG). Runyon and colleagues (Runyon BA 1992) tested the two methods in a prospective study of 901 patients with a known cause of ascites. The SAAG method differentiated the cause of

ascites as due to portal hypertension versus other causes in 96.7% of cases. In comparison the ascitic fluid protein concentration was only 55.6% accurate in distinguishing between a transudate and an exudate.

In addition to ascites, there is perisinusoidal collagen deposition and haemorrhagic necrosis (Arcidi JM 1981, Lefkowitch JH 1986, Myers RP 2003). Intrahepatic microthrombi as a result of chronic venous stasis has been shown in an autopsy study to correlate well with areas of fibrous septation, and suggests a contributory role in the development of hepatic fibrosis (Wanless IR 1995).

The spectrum of liver disease that may result from passive congestion includes mild derangement of liver function tests to varying degrees of fibrosis, and cirrhosis. The classical nutmeg appearance of the liver in congestive hepatopathy is shown in Figure 3. In the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program, liver function tests were assessed in those with chronic heart failure. The most common abnormalities seen on liver biochemistry in 2679 patients were low hypoalbuminaemia (18.3%), high alkaline phosphatase (14%), and elevated total bilirubin (which is often unconjugated) (13%). Alanine transaminase and aspartate aminotransaminase were less commonly abnormal. Bilirubin was shown to be the strongest independent predictor of poor prognosis (Allen LA 2009). Patterns of liver involvement in heart failure and recommended further investigation are show in Table 3.

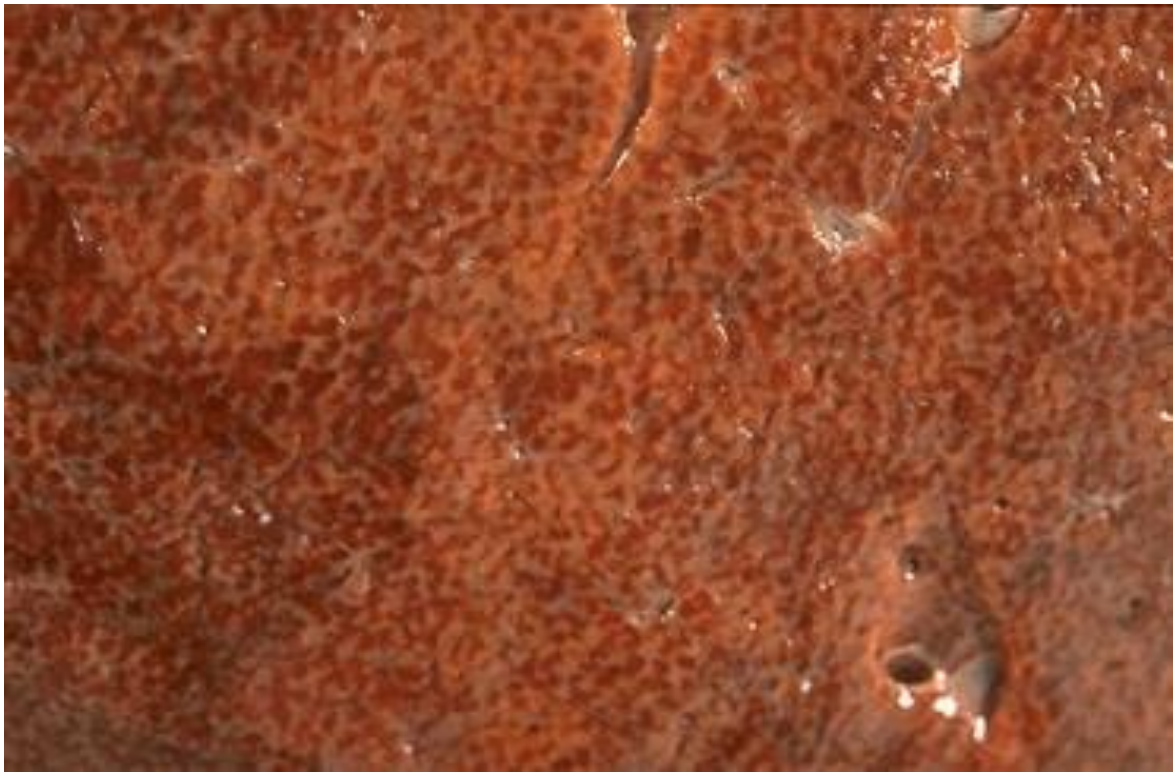


Figure 3: Nutmeg appearance of the liver showing haemorrhagic necrosis – congestive hepatopathy. Courtesy: http://library.med.utah.edu/WebPath/

Condition	**Underlying Pathophysiology**	**Liver Function Tests**	**Further Tests**	**Reversibility**
Congestive Hepatopathy	Right heart volume overload	Modest increase	Rarely required	Yes
Hepatic Fibrosis	Prolonged congestion leading to wound healing response	Modest increase	Consider: HVPG Liver Biospy Ultrasound	Possible
Hepatic Cirrhosis	Advanced fibrosis with synthetic dysfunction and portal hypertension	Modest increase	Consider: HVPG Liver Biospy Ultrasound	No

Table 3: Patterns of liver involvement in heart failure (Sundaram V 2016). *HVPG – Hepatic vein portal vein gradient*.

3.7.2 Assessment of hepatic fibrosis through measurements of tissue stiffness

The gold standard for staging of liver fibrosis is liver biopsy. This technique has several disadvantages including sampling error and inter & intraobserver variability in interpreting and determining stages of fibrosis, particularly in the early stages of fibrosis. Liver biopsy is also associated with risk of pain, bleeding, infection, and damage to internal structures (Bravo AA 2001, Regev A 2002). Therefore, non-invasive tests including fibrosis scores, advanced biochemical markers and imaging studies have been developed as an adjunct or alternative to liver biopsy (Arora A 2012, Lichtinghagen R 2013). Sensitivity, specificity and positive/negative predictive values vary according to the test panel used and the population group screened (Morling JR 2014, Fernandes FF 2015,).

3.7.3 Pathogenesis and Stages of Liver Fibrosis

The pathogenesis of liver fibrosis involves the hepatic stellate cell (previously called perisinusoidal cells). Liver injury of any cause transforms the hepatic stellate cell into fibrogenic myofibroblasts. These activated hepatic stellate cells produce large amounts of extra cellular matrix proteins which leads to fibrosis. This includes hyaluronic acid and amino-terminal propeptide of type III collagen. Matrix metalloproteinases (MMPs) normally act to breakdown this extra cellular matrix in a protective fashion. However, in the fibrotic state the activity of MMPs is hindered by tissue inhibitors of metalloproteinases (such as TIMP-1), also secreted by hepatic stellate cells (Bataller R 2005, Moreira RK 2007).

The degree of fibrosis is important in the staging and therapeutic management of chronic liver disease including assessing response to treatment. Several scoring systems have been developed, including the Knodell (or modified Knodell, called the Ishak score), and the METAVIR. Most of these scoring systems have been developed for portal based aetiologies (eg Hepatitis C), and may not be suitable for processes that damage zone 3 (peri-venular) of the hepatic acinus. Disease specific scoring systems are employed depending on the underlying pathological mechanism. In non-alcoholic fatty liver disease (NAFLD), several scoring methods have been tested since the first system suggested by Brunt and colleagues (Brunt EM 1999). A three-tier 'grade of activity' was proposed which takes into account the extent of steatosis, inflammation, and ballooning of hepatocytes.

Kleiner and colleagues (Kleiner DE 2005) for the NASH Clinical Research Network (NASH CRN) developed this score with the inclusion of degree of fibrosis, and the presence or absence of a further 9 variables. There are several advantages over the older systems including the validation by blinded pathologists, applicability to both adult and paediatric populations, and the single score result which is used to grade activity which has conveniences for statistical comparison in research studies.

3.7.4 Non-Invasive Imaging of Liver Stiffness - Elastography

Elastography is a radiological technique which measures the elastic properties of tissue. As disease processes such as liver fibrosis effect the parenchyma, the tissue hardens and this change in elasticity can be detected by a variety of ultrasound techniques, or magnetic resonance imaging. Both modalities require the generation of a shear wave which distorts the tissue, and this distortion is then measured to give the clinician a

measure of tissue stiffness. The underlying principle of measuring tissue elasticity is based on calculation of Young's modulus, measured in kPa. The equation is $E = 3 (V \times \rho)$, where E is the Young's modulus, V is the shear wave velocity and ρ is the density of the tissue being examined.

The mechanical properties of non-biological materials has been utilised in engineering and construction for decades. Although physicians have been using the clinical skill of manual palpation to determine tissue properties for centuries, the suggestion that the use of acoustic waves may be used to determine the mechanical properties of tissue did not appear until 1986 in Hill's book 'Physical Properties of Medical Ultrasonics' (Hill CR 1986). The advances in the 1990s in ultrasound enabled the current technologies that are able to measure tissue elasticity. Ophir and colleagues (Ophir J 1991) first published the ultrasound based technique using strain elastography, which they tested on animal and 'phantom' models. This was followed by the development of shear wave elastography, with Fibroscan being the first commercially available non-imaging technique available to clinicians (Sandrin L 2003).

3.7.5 Ultrasound Based Techniques

Ultrasound-based modalities are widely used in hepatology to measure liver stiffness. This includes acoustic radiation force imaging (ARFI) which allows measurements of particular areas of interest and simultaneously acquire 2-dimensional images. Transient elastography (TE) is the most common non-invasive imaging technique. Fibroscan (Echosens, Paris, France) is a 1-dimensional ultrasound which measures the velocity of a low-frequency (50 Hz) elastic shear wave propagating through the liver. This velocity is directly related to tissue stiffness, called the elastic modulus. The stiffer the tissue, the

faster the shear wave propagates and therefore a higher Fibroscan value in kPa confers a stiffer and a more fibrosed liver (Boursier J 2008). Normal values for Fibroscan and the corresponding histological stage of fibrosis for the different aetiologies associated with chronic liver disease is shown in Figure 4.

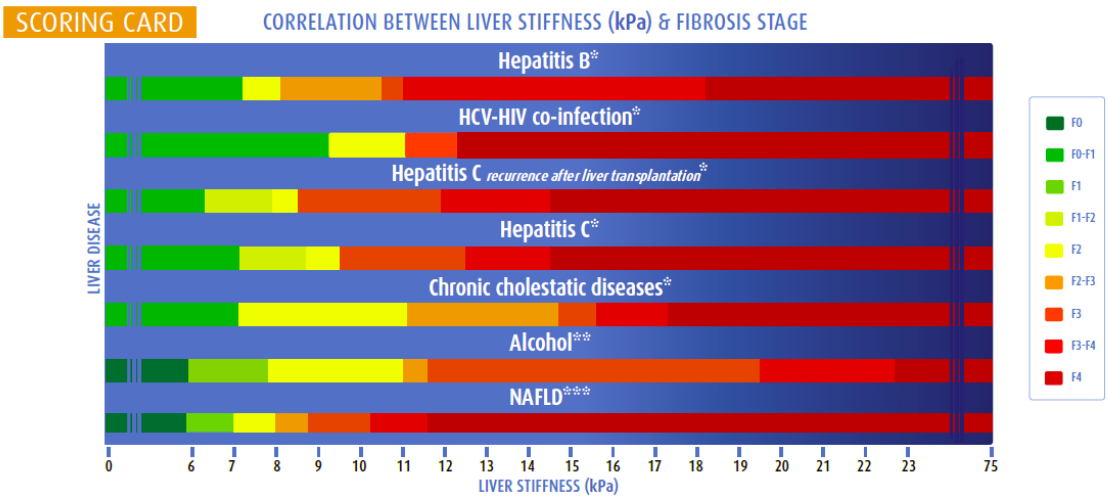


Figure 4: Fibroscan scoring card for stages of fibrosis. Courtesy Fibroscan, Echosens. http://www.fibroscan.com/en/

Advantages of TE include a short procedure time (<5 min), immediate results, and the ability to perform the test at the bedside or in an outpatient clinic. The core of tissue that is examined is a 1 x 4 cm cylinder, and is considerably larger than liver biopsy samples. Drawbacks include difficulty in producing accurate results in ascites and patients with a high body mass index.

Fibroscan has been validated in several studies in chronic liver disease, showing close correlation with histological stages of fibrosis (Sandrin L 2003, Ganne-Carrié N 2006, Foucher J 2006, Castera L 2008, Arora A 2012). In a recent meta-analysis of 57 studies in which a total of 10504 patients were included, high sensitivity (81%) and specificity

(88%) was found in predicting liver fibrosis using transient elastography. It also showed high accuracy in diagnosing liver cirrhosis (Geng X-X 2016).

Fibroscan has been investigated in congenital heart disease, with one recent prospective study of patients using transient elastography to predict central venous pressure. The study contained 13 participants with repaired Tetralogy of Fallot and 7 with prior pulmonary stenosis out of a total of 96 (60 children, 36 adults) undergoing invasive right heart catheter studies. A close and statistically significant correlation ($p < 0.001$) between liver stiffness and central venous pressure (CVP) was seen in both the adult ($r = 0.68$) and paediatric ($r = 0.84$) groups. Subgroup data on specific diagnostic groups was not published, however for the entire cohort, they suggested a Fibroscan threshold of 8.8 kPa would predict a CVP > 10mm Hg (Jalal Z 2015).

The relationship between CVP and liver stiffness (measured by Fibroscan) was examined in a mixed animal and human study by Millonig and colleagues (Millonig G 2010). To model a congested liver, clamping of the inferior vena cava (IVC) in 5 (of a total of 8) anaesthetised landrace pigs resulted in markedly elevated liver stiffness values (3.9 kPa to 27.8 kPa, $p < 0.001$). Unclamping of the IVC returned the liver stiffness to 5.1 kPa, which was also significant at $p < 0.001$. In a further 3 pigs, the input and output vessels of the proximal liver were clamped. By gradually infusing normal saline into the inferior vena cava (to simulate fluid overload) and simultaneously measuring CVP, they were able to show a strong linear correlation, $r = 1$, $p < 0.01$ between liver stiffness and CVP. In the human wing, 10 hospitalised patients with decompensated heart failure were prospectively studied with Fibroscan before and after treatment with diuretics. They showed a significant decrease in liver stiffness by 15.3 kPa, $p = 0.004$.

In another study examining this relationship, Taniguchi and colleagues (Taniguchi T 2014) 31 patients with heart failure were studied with Fibroscan and invasive right heart catheter (RHC) studies. This was a prospective study enrolling patients with acquired heart disease (rather than congenital) who had clinical indications for RHC. The relationship between liver stiffness and CVP was modelled using logarithmic transformation. This model was then validated in a further study group of 49 patients with heart failure. The study reported a Fibroscan threshold of 10.6 kPa would predict a CVP >10 mmHg with high sensitivity (85%) and specificity (93%).

Colli and colleagues (Colli A 2010) studied 27 hospitalised patients with heart failure (acquired left heart disease) with Fibroscan and NT-Pro-BNP. Although they showed significant decreases in liver stiffness ($p < 0.003$) and NT-Pro-BNP ($p < 0.001$) between admission and discharge, a significant correlation between the two tests was not found ($p = 0.0567$).

Further studies have been published in the Fontan group, described in section 3.8.

3.7.6 Magnetic Resonance Elastography

Magnetic Resonance Elastography (MRE) was first developed in 1995 and since then has been validated in several studies for the assessment of liver fibrosis and cirrhosis. The technique involves generating shear waves on the surface of the skin and then imaging the propagation of this shear wave using a modified phase contrast MR sequence. Software using inversion algorithms then transforms this data into elastograms – images/maps depicting tissue stiffness. Tissue stiffness is measured in kPa and correlates closely with the extent of fibrosis. It has the advantage over transient elastography in being able to image a much larger area of liver. It is also considered

highly reproducible in studies of participants with chronic liver disease or normal subjects (Hines CD 2010, Motosugi U 2010, Shire NJ 2011). In a direct comparison with transient elastography, the success rate of MRE (94%) was more accurate than that of transient elastography (84%) (Huwart L 2008). It has the disadvantage of a longer acquisition time, and is considerably more expensive than TE. In a meta-analysis of 12 studies in 697 patients with chronic liver disease undergoing MRE, the following test characteristics were found (Singh S 2015):

- Any fibrosis (F≥1): Optimal cut-off 3.45 kPa, sensitivity 73%, specificity 79 %
- Significant fibrosis (≥F2): Optimal cut-off 3.66 kPa, sensitivity 79 %, specificity 81%
- Advanced fibrosis (≥F3): Optimal cut-off 4.11 kPa, sensitivity 85%, specificity 85%
- Cirrhosis (F4): Optimal cut-off 4.71 kPa, sensitivity 91%, specificity 81%

3.7.7 Serum Liver Fibrosis Markers

In parallel with the development of imaging indices of liver fibrosis, there are several serum assays that have been tested as markers of liver fibrosis. These can be broadly divided into indirect (markers of liver function) and direct markers of fibrosis (markers of hepatic fibrogenesis). The indirect markers include AST-Platelet Ratio Index (APRI), Fibrotest (algorithm using alpha-2 macroglobulin, haptoglobulin, total bilirubin, apolipoprotein-A, Gamma GT, age and gender), FIB4 (panel using Age, AST, platelet count and ALT), NAFLD fibrosis score (glucose, age, body mass index, ALT, AST, platelets and albumin), and Fibroindex (platelet count, AST and Gamma GT). The

direct markers include Hyaluronic acid (HA), amino terminal of serum procollagen III peptide (PIIINP), metallopeptidase inhibitor 1 (TIMP-1), YKL-40 (chondrex) and the Enhanced Liver Fibrosis panel.

The Enhanced Liver Fibrosis panel (ELF panel) is a serological test used as a direct marker of fibrosis. ELF is a proprietary algorithm that takes into account hyaluronic acid level, amino-terminal propeptide of type III collagen level, and TIMP-1. Several other algorithms combining serum markers have been tested in Hepatitis B & C (Imbert-Bismut 2001, Forns 2002, Myers 2003[2], Myers 2003[3], Wai 2003). The ELF panel was the first score using a combination of serum markers to grade liver fibrosis in chronic liver disease of any cause. The original work (Rosenberg 2004) was an international multicentre cohort study that included 1021 patients with chronic liver disease who were undergoing liver biopsy. 921 patients were used in the final analysis (after exclusions due to inadequate tissue or serum samples) which tested an algorithm of age, HA, TIMP-1 and PIIINP. A central pathologist examined all biopsy results which was further examined by 2 pathologists, and then revalidated in a blinded fashion by the same central pathologist. Receiver operator characteristic curve analysis was performed to define thresholds at which there was high sensitivity (90%) and specificity (92%) in the detection of fibrosis. (Rosenberg WM 2004).

In adult congenital heart disease, Guha and colleagues (Guha IN 2013) investigated structural and functional hepatic factors (ELF panel and Indocyanine Green Clearance (IGC)) in stable patients with a Fontan circulation versus those with proven compensated viral cirrhotic patients. 21 Fontan patients (mean age 26.2 years) were compared with 8 patients (mean age 55.5 years) with chronic liver disease (6 with Hepatitis C, 2 with Hepatitis B). All patients underwent blood tests (liver function tests, renal function and full blood count), ELF score, IGC, liver ultrasound and transthoracic

echocardiography. Mean ELF score in the Fontan group was 7.97 ± 1.16 versus 9.0 ± 1.43 in the viral hepatitis group with p = 0.05. They were able to show that both groups had similar global hepatic function and degree of fibrosis. However unlike the viral cirrhotic group, the Fontan group did not display a significant correlation ('uncouping') between global liver function and degree of fibrosis.

The manufacturers of the ELF panel have published reference ranges for patients with liver disease which correlate with degree of fibrosis: < 7.7 none to mild fibrosis, ≥ 7.7 to < 9.8 moderate fibrosis, ≥ 9.8 severe fibrosis/cirrhosis. This has been challenged in several studies, particularly with the threshold for severe fibrosis and cirrhosis. Lichtinghagen and colleagues (Lichtinghagen 2013) studied 79 patients with established Hepatitis C related cirrhosis versus 400 healthy controls. The viral hepatitis group underwent liver biopsy, whereas all participants had the ELF test. They suggested thresholds of < 7.7 had a high sensitivity for exclusion of fibrosis, 9.8 for high specificity (98%) of fibrosis and 11.3 for cirrhosis. The reference range for the normal group was 6.7 – 9.8, with a mean of 8.06, range 5.88 – 10.30. Males had a significantly higher ELF score than females, and slightly higher values were seen in the afternoon versus the morning.

Yoo and colleagues (Yoo EJ 2013) studied 183 healthy participants (made up of 22 liver donors and 161 subjects who attended for health screening) at a single centre in Seoul. They defined the normal range as 5.95 to 8.73 (5^{th} to 95^{th} centile), and again demonstrated slightly higher scores for males versus females (6.72 to 8.93 versus 5.69 to 8.67, p = 0.015).

3.8 Liver disease in Adult Congenital Heart Disease (ACHD)

3.8.1 The Fontan Circulation

In adult congenital heart disease, there has been increased attention in liver disease related to the Fontan circulation. The unique physiology of the Fontan circulation was first described in 1971 and is surgically created in individuals for a number of conditions (essentially any anatomical defect that causes single ventricle physiology) that necessitate the separation of systemic and pulmonary circulations by redirection of returning venous blood directly to the pulmonary circulation (Fontan F 1971). In order for the circuit to function, an adequate transpulmonary gradient (mean pulmonary artery pressure less left atrial pressure) is required to generate a sufficient preload to the systemic ventricle. With the lack of a subpulmonary pump, the major determinant of this transpulmonary gradient is therefore an elevated central venous pressure. This surgical technique has had several advances in terms of surgical technique and survival has dramatically improved in once fatal conditions. However the long term sequelae of chronic venous hypertension includes several multi-system manifestations. This includes plastic bronchitis (airway obstruction due to expectoration of casts), protein losing enteropathy, and hepatic dysfunction.

Hepatic dysfunction in this group may be present as synthetic dysfunction with prolonged prothrombin time and impaired galactose elimination (Narkewicz MR 2003). In addition, structural changes may occur in the liver parenchyma. Most commonly this is in the form of liver fibrosis but more advanced disease is increasingly seen. The association of hepatic cirrhosis and hepatocellular carcinoma was first described in a case report of a patient with chronically elevated central venous pressure related to long standing constrictive pericarditis (Ho SS 1990). Hepatic fibrosis, cirrhosis and

hepatocellular carcinoma was also described in an autopsy study of 9 deceased patients with a Fontan circulation (Ghaferi AA 2005). This was further investigated by Kiesewetter et al who showed that advanced stages of fibrosis and cirrhosis on liver biopsy were correlated with duration since Fontan operation (Kiesewetter CH 2007).

3.8.2 Non-invasive Measurements of Liver Stiffness in ACHD

Given the documented drawbacks of liver biopsy previously described, there has been a move towards non-invasive measurements of liver stiffness as a surrogate for fibrosis in this patient group. This has included a study by Ginde and colleagues (Ginde S 2012) where 16 adult patients underwent serum FibroSure testing (composite of 6 serum markers which can be used to predict histological stage of fibrosis/cirrhosis) and contrast computed tomography (CT) scans. They showed that duration since fontan surgery was significantly correlated with raised Fibrosure scores and typical CT scan findings. The CT findings were present in all 16 participants and included heterogenous enhancement of the liver (69%), varices (38%) and liver nodules (31%).

Non-invasive techniques have also been used to predict unfavourable Fontan haemodynamics, which is attractive to the clinician as it may indicate when more invasive testing is required. In a study of 45 predominantly paediatric patients (median age 13.1 years) undergoing invasive catheterisation, Transient elastography (mean liver stiffness 21.4 ± 10.8 kPA) was shown to have significant correlations with age, duration since Fontan surgery, Fontan pressure, cardiac index, pulmonary vascular resistance, systemic arterial oxygen saturation, and platelet count (Wu FM 2014). In another study on paediatric patients, raised serum fibrosis markers (FibroTest and ActiTest) and TE were also shown to correlate with the age of the patient, confirming the hypothesis that

the haemodynamic abnormality has a cumulative effect. Whilst normal values in this population have not been defined, the authors suggest that longitudinal alterations in fibrosis markers may indicate progressive liver disease and may be used to detect a failing Fontan circulation (Friedrich-Rust M 2008).

MRE has also been tested in adult congenital heart disease. In a study of 14 patients with a Fontan circulation undergoing both CMR and MRE, cardiac index was found to decrease as the age and duration since Fontan surgery increased. Furthermore there was an inverse correlation seen between liver stiffness (median 4.0 kPa) and cardiac index (p 0.02) (Wallihan DB 2014). A more comprehensive study was published by Sugimoto and colleagues (Sugimoto M 2016). 57 paediatric patients underwent MRE, cardiac catheterization and the ELF panel. They were divided into 4 groups including 27 that had undergone intracardiac repair (ICR, for a range of conditions including ventricular septal defect, atrial septal defect, Tetralogy of Fallot), 16 post Fontan surgery, (7 atriopulmonary and 9 total cavo-pulmonary circulation), 14 with congenital heart disease without intervention, and 2 controls with Wilson's disease related cirrhosis. As with the other studies, liver stiffness was shown to increase with duration since Fontan surgery. Central venous pressure as measured by cardiac catheterization was shown to correlate strongly with, and was the only significant predictor of liver stiffness (MRE) on a step-wise multivariate regression analysis. Although the ELF panel was significantly greater in the control group (cirrhosis), no significant difference was seen between the other groups.

3.9 Study Hypothesis and Aims

There is currently a spectrum of adult congenital heart disease lesions which ultimately lead to impairment of right ventricular function. Hepatic dysfunction is regarded as a later manifestation in patients with right-sided heart failure. The primary pathophysiology involved in these cases is hepatic dysfunction due to passive congestion. The optimal time to replace or repair either or both the pulmonary and tricuspid valves to provide competency - in the hope of preventing right ventricular failure - is still not clear. Pulmonary regurgitation in the presence of normal RV systolic function and no more than mild tricuspid valve regurgitation should not cause significant chronic elevation of systemic venous pressure and is not associated with clinically apparent hepatic dysfunction. However, the only published study in patients with repaired Tetralogy of Fallot used standard liver function tests and no structural or functional measures of fibrosis or cirrhosis (Kordybach-Prokopiuk 2015). Whilst there are clear imaging indices in echocardiography and cardiovascular magnetic resonance imaging that describe right ventricular haemodynamics and function, at present there have been few studies which have attempted to identify novel biomarkers of right ventricular dysfunction. The development of hepatic biomarkers that may help in the assessment of these patients and the timing of cardiac surgery is therefore highly desirable.

Study Aims:

1. Are right heart lesions associated with subclinical abnormality of hepatic structure and/or function?

2. If so, whether the presence of hepatic abnormality correlates with right ventricular function (as assessed by Cardiovascular Magnetic Resonance Imaging).

3. For participants with Pulmonary Regurgitation, whether any hepatic abnormality correlates with established prognostic markers such as degree of right ventricular dilatation and peak oxygen uptake on exercise. As such may this contribute to definition of the optimal timing of pulmonary valve replacement?

4. Methodology

4.1 General Methods

4.1.1 Concept and Study Design

The data presented in this thesis is derived from the 'Hepatic Effects of Right Heart Lesions in Adult Congenital Heart Disease' study. This was a prospective observational single centre study of 40 participants. A cross sectional design was used to compare participants divided into groups, by type and degree of valvular regurgitation.

The study was designed by the author and co-investigators / clinical supervisors: Dr. Leisa Freeman (Consultant Cardiologist), Dr. Catherine Head (Consultant Cardiologist), Dr. Simon Rushbrook (Consultant Hepatologist), and Professor Marcus Flather (Honorary Consultant Cardiologist / Academic Supervisor). Further clinical supervision was provided by Dr. Paul Malcolm (Consultant Radiologist). All members of the research team had attained the Good Clinical Practice research training certification. The study protocol was peer reviewed by the adult congenital heart disease teams at Papworth Hospitals NHS Foundation Trust and Guy's and St.Thomas' Hospital NHS Foundation Trust. Recommendations, particularly surrounding the study groups and inclusion/exclusion criteria, were incorporated into the approved study protocol (Version 9, Appendix A).

The author acted as Chief Investigator and was responsible for the project management of the study including protocol development, recruitment, data entry and management, and reporting of incidental findings. Statistical analysis was carried out with supervision from Mr. Ian Nunney – statistician from University of East Anglia.

4.1.2 Study Site and Sponsorship

The primary location for all study related matters was the Norfolk and Norwich University Hospital NHS Foundation Trust (NNUH), which is a secondary care non-surgical cardiology centre (Level 2 centre for adult congenital heart disease). This included patient recruitment and study visits. Sponsorship was provided by University of East Anglia.

4.1.3 Funding

The majority of the funding was contributed by the NNUH based charitable funds E49 & F19. Successful applications for funding were also submitted to The Norfolk Heart Trust, and The Norfolk and Norwich Bicentenary Trust. There was no commercial involvement in the design, funding or running of the study.

4.1.4 Ethics & Research and Development Approval

Ethics approval was granted on 20th January 2016 by East of England – Cambridgeshire and Hertfordshire Research Ethics Committee (Appendix B). Local Research & Development management approval was granted on 18th March 2016 at the Norfolk and Norwich University Hospitals NHS Foundation Trust. Written consent was obtained from all study participants. The study was conducted in accordance with the Declaration of Helsinki (World Medical Association 2013).

4.1.5 Sample Size

A power calculation was considered not essential given the exploratory nature of the study. This is the first piece of research examining this particular combination of novel techniques in measuring liver stiffness in chronic pulmonary and tricuspid regurgitation, and consequently we do not have any prior literature to calculate a sample size. Approval was granted to recruit 40 patients with permission to recruit up to 60 if there were patients whose results could not be used – either because they were not able to fully participate or there were technical problems. The sample size target was determined by feasibility. The intention is that the data collected from this study and conclusions drawn will allow power calculations for any future studies.

4.1.6 Participants & Study Groups

The study selected patients with repaired Tetralogy of Fallot, prior pulmonary stenosis (requiring pulmonary valvotomy) or Ebstein's anomaly, from the adult congenital heart disease Norwich-Papworth adult congenital heart disease database (NORPAP). Patients with adult congenital heart disease are added to the NORPAP database either at the time of referral or once seen as an inpatient or outpatient. The database contains both demographic and clinical data, and is updated on a weekly basis.

The database has 2800 patients of whom ~10% have a diagnosis of Fallot's Tetralogy or pulmonary valve disease. Tricuspid regurgitation was recognised as an important factor that would contribute to systemic venous pressure, and therefore patients with Ebstein's anomaly were chosen as a comparator group. Selected patients were divided into three groups based on previous investigations:

- **Group 1**: Moderate to severe pulmonary regurgitation
- **Group 2**: No more than mild pulmonary regurgitation
- **Group 3**: At least mild tricuspid regurgitation associated with Ebstein's anomaly

The aim was to enrol fifteen participants in each of Groups 1 & 2, with a target of ten participants in Group 3. Once the required number of participants was recruited in one group, priority was given to recruitment to other groups. A total of three hundred and thirty patients were screened; eighty three patients met the inclusion and exclusion criteria and were invited to participate (Flow Chart - Appendix D). A total of forty participants were recruited. Details on those not recruited (n=43) including diagnoses, planned study group allocation, demographics, and reason not recruited are given in Appendix D.

Recruitment began on 30th March 2016 and was completed on 28th December 2016. In the sections that follow, the study groups will be denoted as:

- **Group 1** (Mod-Sev PR)
- **Group 2** (Mild PR)
- **Group 3** (Ebstein)

4.1.7 Inclusion Criteria

1) All participants in Groups 1 (Mod-Sev PR) & 2 (Mild PR) will have a prior diagnosis of Tetralogy of Fallot, or pulmonary stenosis treated with valvotomy/valvuloplasty with subsequent pulmonary regurgitation.

2) All participants in Group 3 will have a diagnosis of Ebstein's anomaly of the tricuspid valve associated with at least mild tricuspid regurgitation.

4.1.8 Exclusion Criteria

1) Any other haemodynamically significant lesion* with Vmax > 3m/s, with the exception of tricuspid regurgitation.

2) Contraindications to MRI **

3) Pre-existing chronic liver disease ***

4) Currently Pregnant ****

5) Unable to complete the study protocol for non cardiac reason

6) Age <17 or >70

7) Lack of capacity to consent

* Including any cause of right ventricular outflow tract obstruction for example pulmonary stenosis, sub-pulmonary stenosis, supravalvar pulmonary stenosis, branch pulmonary artery stenosis.

** Non-compatible at 3 Tesla. Includes cardiac pacemakers, some coronary and pulmonary artery stents, intracerebral or eye metallic clips

*** Pre-existing liver disease will be defined as known diagnosis of Cirrhosis of any cause, Non-alcoholic fatty liver disease, alcoholic liver disease, viral hepatitis (hepatitis B or C infection), haemochromatosis, alpha-1-antitrypsin disease, primary biliary cirrhosis, primary sclerosing cholangitis or autoimmune liver disease.

**** Determined by history during invitation to participate on telephone.

4.1.9 Sampling Method

Potential participants were screened from the NORPAP database by the author/Chief Investigator (CI) using the inclusion and exclusion criteria. All eligible participants were sent an invitation letter, the participant information sheet, and short form-36 questionnaire by the CI. The invitation letter contained details on how to opt out of further involvement in the study. A follow up telephone call was made by the CI allowing a minimum of 3 days after the written information had arrived. Potential participants were offered the opportunity to ask questions. Pregnancy status and further clinical history was clarified to ensure participants were eligible for the study. Fasting requirements for the hepatic investigations were given during this telephone call. Arrangements were then made for a suitable study visit date and the relevant tests were booked. Patients were also approached directly by the cardiology consultant supervisors during routine outpatient clinic appointments.

4.1.10 Study Visits

Participants were invited to attend NNUH for their study visit. It was the intention to perform all hepatic investigations on the same day. Where this was not feasible, a second study visit was organised. Participants were met by the CI on the study day and taken to a clinical room for interview and to sign the consent form. Participants followed a cycle of investigations including blood tests, Fibroscan, Ultrasound liver and

MR Elastography of the liver. The study also utilised the results of investigations performed during routine clinical follow up, including CMR, Cardiopulmonary exercise testing, 12-lead Electrocardiogram, and Transthoracic Echocardiography. Time intervals between cardiac magnetic resonance imaging and the study visit date are shown Table 1 - App.E, in Appendix E.

4.1.11 Incidental Findings

All study participants underwent several imaging and serum tests of liver structure and function. The participant information sheet contained details on the agreed protocol should these investigations raise significant incidental or abnormal findings; participants were asked to consent to this arrangement. In the first instance the findings were discussed with clinical supervisors (mandatory discussion with cardiologist and hepatologist). Further appropriate investigations or clinical review would be arranged. The participant and their GP would also be informed in writing. Participants were given the option of withdrawing from the study should they wish to. All significant incidental findings were recorded in the trial master file.

4.2 Hepatic Investigations

4.2.1 Fibroscan

Fibroscan is performed with the patient lying supine (Figure 5) using the Fibroscan 502 Touch machine, with the right arm elevated which separates the ribs to allow access to the right lobe of the lobe. There are two probes available; the Medium probe is suitable for most patients whilst the Large one is used in patients carrying substantial abdominal

adipose tissue. Ultrasound gel is used on the tip of the probe at the point where a line joining the mid axillary line on the right side meets a horizontal line from the xiphisternum (Figure 6). This is approximately the 9th to 11th intercostal space and is the level where a liver biopsy would be performed. The operator, assisted by a time-motion image, locates a suitable liver portion free of large vascular structures and the diaphragm. The operator then presses the probe button to start the measurements ('shots'). Transient Elastography measures liver stiffness in a volume that is a cylinder of approximately 1 cm wide and 4 cm long, between 25 mm and 65 mm below the skin surface. The software determines whether each measurement is successful or not. When a shot is unsuccessful, the machine does not return a value. The entire procedure is considered to have failed when no value is obtained after ten shots. The final result of a Fibroscan session can be regarded as valid if the following criteria are fulfilled: 1) a number of valid shots of at least 10; 2) a success rate (the ratio of valid shots to the total number of shots) above 60%; and 3) an interquartile range (IQR, reflecting the variability of measurements) less than 30% of the median LS measurements (M) value (IQR/M $\leqslant$0.30%). The results are expressed in kilopascals (kPa). It is required that patients are fasted for 3 hours prior to the Fibroscan (Echosens).

The author underwent training to perform Fibroscan with the liver specialist nurse at NNUH. 60 supervised scans were performed as a part of the training on patients attending the hepatology outpatient clinics. The author attended the formal accreditation course run by Echosens and successfully attained the operator certificate on 21st October 2015.

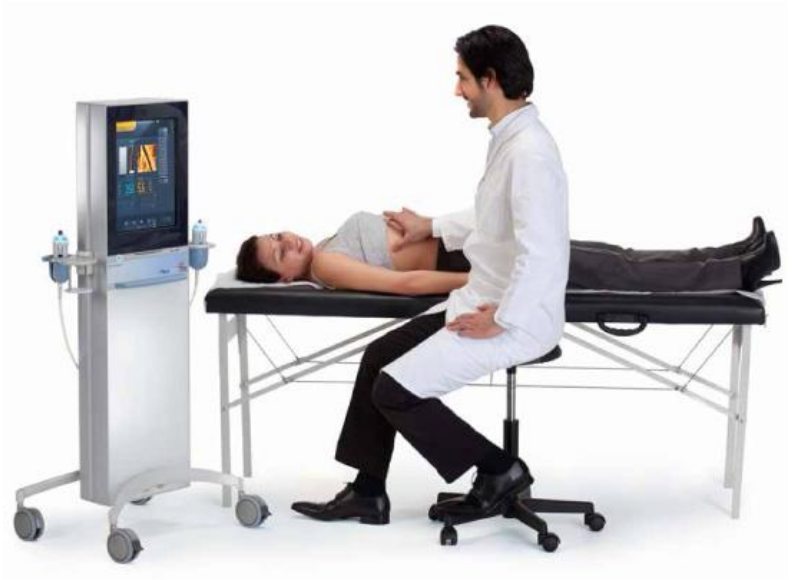


Figure 5: Supine position during Fibroscan. The patient is asked to elevate their right arm above the head.

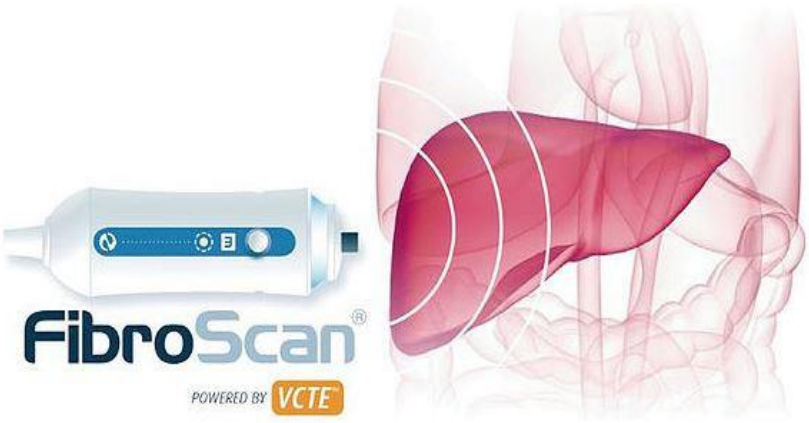


Figure 6: Probe position.

4.2.2 Ultrasound Liver

The patient is in the supine position with their abdomen exposed. A General Electric LogiQ/E9 ultrasound machine was used. A standard curvilinear 2-5 MHz ultrasound probe was used aided by ultrasound gel. Ultrasound of the liver and spleen was performed using standard settings for upper abdominal imaging. The portal and hepatic veins were interrogated with colour duplex at 60° to the angle of flow. Patients were required to fast for 12 hours prior to the scan. All ultrasound scans were performed and reported by Dr. Paul Malcolm (PM), consultant radiologist at NNUH and clinical supervisor on the study. The operator was unblinded to the participants' clinical history. The purpose of performing ultrasound of the liver and spleen was partly to ensure that participants did not have features of chronic liver disease (an exclusion criteria), but also to serve as a correlate with the hepatic primary outcomes. All abnormal results were flagged up to the author and discussed with the Hepatologist supervising this study.

The scanning protocol and reporting template is shown in Table 4.

Item	Outcome
Liver Morphology	Normal / Abnormal
Capsule	Regular / Irregular
Parenchyma	Homogeneous / Heterogeneous
Portal vein	Patent / Occluded Hepatopetal / Hepatofugal Waveform normal / Dampened
Hepatic veins (measured ≈ 5cm from the hepatic venous confluence) Pattern	Dilated / Normal diameter Triphasic / Dampened / Monophasic
Spleen	Length (cm)
Varices	Present / Absent
Upper abdominal free fluid	Present / Absent

Table 4: Scanning protocol for Ultrasound Liver

4.2.3 Magnetic Resonance Elastography of Liver

The patient is in the supine position on a motorised bed within the MRI suite (Figure 7). A pneumatic passive acoustic driver device is strapped to the patient over the right side of the lower thoracic cage, centred approximately over the mid-point of the liver, beneath the MRI coil. When the device is activated, the patient will feel gentle vibrations under this area due to the mechanical vibration waves (shear waves). A MR Phase contrast technique acquires images of wave propagation, and inversion algorithms produce elastograms (tissue stiffness map, Figure 8). Each MR Elastography examination of the liver would take 40 minutes including processing time. The MR

Elastography sequence is acquired on a GE MR750w 3 Tesla MRI Scanner using GEM Anterior Array and Posterior Array coil combination. The Ax MR-Touch Standard BH sequence parameters are as follows: TR: 1000, TE: 59.1, Matrix: 64x64, Flip angle: 90 degrees, NEX: 2, FOV: 135x42 cm, Slice thickness: 7, Slice gap: 2.5, number of slices: 4. All MRE studies were analysed and reported by a single expert radiologist (PM).

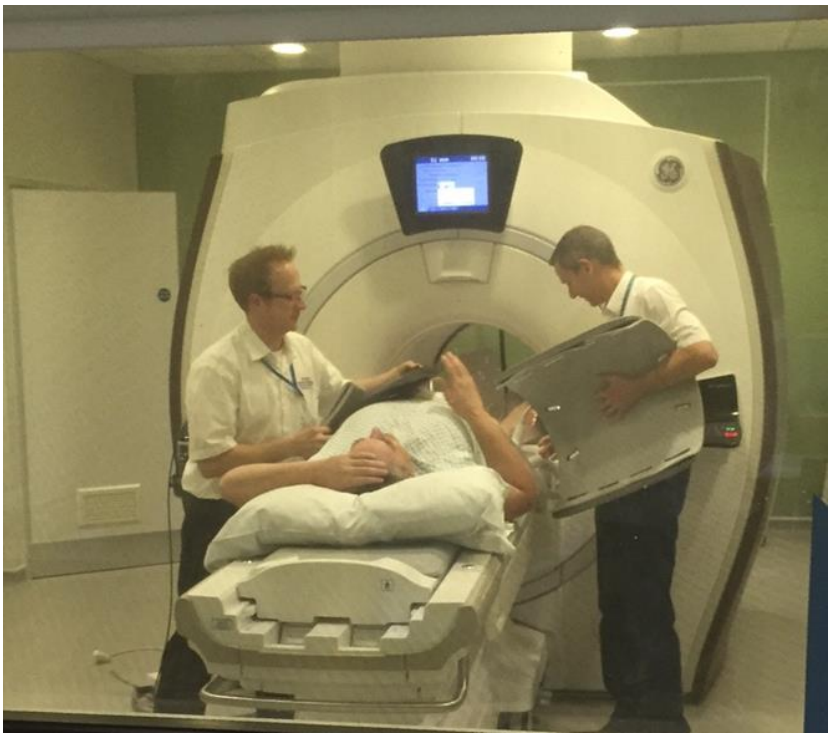


Figure 7: Pneumatic passive acoustic device being strapped to patient before the scanning protocol.

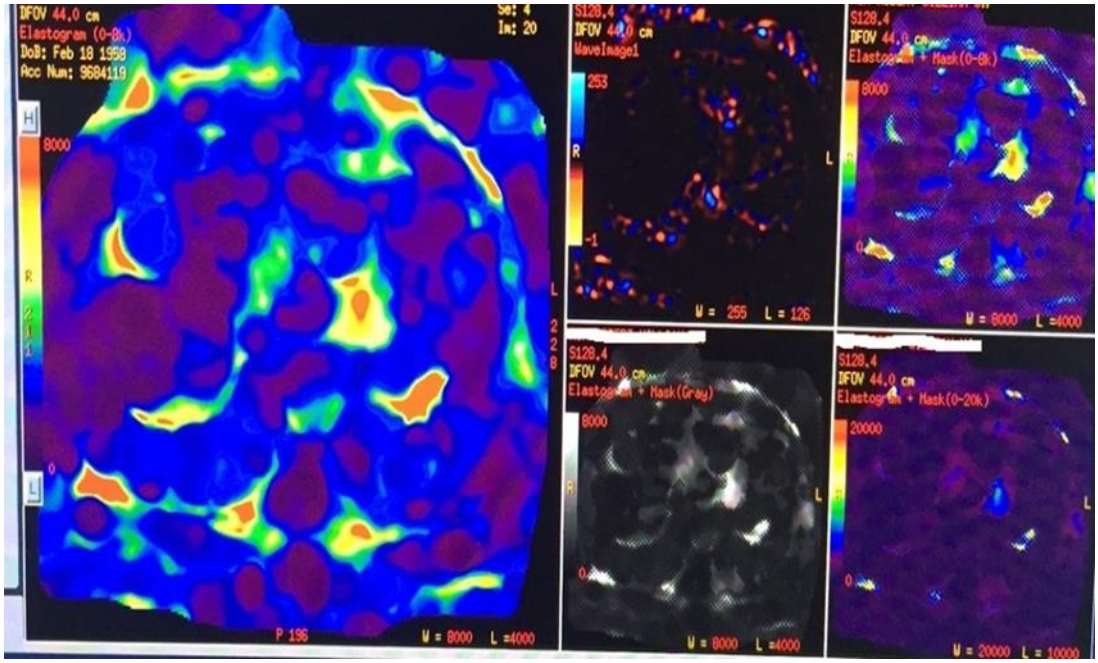


Figure 8: Elastogram – tissue stiffness map.

4.2.4 Enhanced Liver Fibrosis Score

Serum samples underwent standard preparation and freezing at -20 degrees Celsius after phlebotomy on the study day visit. Samples were labelled with the date and study identification number only. These were transported as a set (after recruitment was complete) to the Wolfson Laboratory at the Royal Free Hospital, London. The Wolfson Laboratory use the Siemens Healthcare ADVIA Centaur system to generate an ELF score using an algorithm which combines the measurements of hyaluronic acid (HA), metalloproteinase 1 (TIMP-1), and amino-terminal propeptide of type III procollagen (PIIINP). The ADVIA platforms use acridinium ester chemiluminescence as the signal reporter molecule and paramagnetic solid phase to ensure separation of bound immunocomplex from unbound material. Assay information is given in table 5:

There are two commercially available algorithms involving 3 systems including the ADVIA Centaur XP/XPT, and the ADVIA Centaur XP. The Wolsfon Laboratory uses the ADVIA Centaur XP algorithm:

ELF Score: $2.278 + 0.851 \ln(C_{HA}) + 0.751 \ln(C_{PIIINP}) + 0394 \ln(C_{TIMP\text{-}1})$

Concentrations (C) are in ng/mL.

The Wolfson laboratory uses a Standard Operating Procedure for the ELF panel which is reviewed annually. Quality checks which are designed specifically for the biomarkers of the ELF panel are carried out before any samples are processed. Specifically, HA is calibrated twice a month, TIMP-1 and PIIINP both once a month. Reference ranges are shown in table 6 (Rosenberg 2004).

Assay	**HA**	**PIIINP**	**TIMP-1**
Feature			
Reagent Part Number	10493157	10492440	10492065
Architecture	2-step sandwich assay HA binding protein	1-step sandwich 2 x McAbs	1-step sandwich 2 x McAbs
Pack size (tests)	50	50	50
Wash	Wash 1	Wash 1	Wash 1
Sample size (uL)	20	20	25
TAT (mins)	58	18	18

Table 5: Assay information on TIMP-1 (metalloproteinase 1), PIIINP (amino-terminal propeptide of type III procollagen) and HA (hyaluronic acid).

OBS (Days)	60	60	60
Calibration interval	14	28	28
Probe wash	Ancillary probe wash PN 03395373	Not required	Not required
Sample diluent	Multidil 13 PN 10492364	Multidil 13 PN 10492364	Multidil 10 PN 05440554
ELF Calibrators	Multi-constituent (Lyophilised - HA, PIIINP, TIMP1) PN 10492344	Multi-constituent (Lyophilised - HA, PIIINP, TIMP1) PN 10492344	Multi-constituent (Lyophilised - HA, PIIINP, TIMP1) PN 10492344

Table 5 (cont): Assay information on TIMP-1 (metalloproteinase 1), PIIINP (amino-terminal propeptide of type III procollagen) and HA (hyaluronic acid).

ELF Score	Degree of Fibrosis
< 7.7	None to Mild
≥ 7.7 to < 9.8	Moderate
≥ 9.8	Severe

Table 6: Reference ranges for ELF score.

4.3 Cardiac Investigations

4.3.1 Transthoracic Echocardiography

Echocardiography is used as a first line imaging modality in both general cardiology and adult congenital heart disease. In this study, the results of routine clinical transthoracic echocardiography were used predominantly as a clinical screening tool. NNUH follows the standard British Society of Echocardiography (BSE) scanning protocol for transthoracic echocardiography (British Society of Echocardiography, Wharton 2015).

Right ventricular function was determined by tricuspid annular plane systolic excursion (TAPSE). The normal value, in accordance with the BSE, was considered greater than 1.6cm. Where image quality was not sufficient to determine TAPSE, RVS' cm/s (velocity of tricuspid annular systolic motion) was used to determine right ventricular function, with > 10 cm/s considered as normal. In 13/40 participants, it was not possible to accurately calculate either TAPSE or RV S' due to poor image quality. As this accounted for 32.5% of all participants, echocardiographic parameters of right ventricular function were omitted from the analysis; only CMR data was used.

Pulmonary regurgitation severity classification by transthoracic echocardiography is shown in Table 7:

Parameter	Mild	Moderate	Severe
Pressure half-time			<100ms
Technician qualitative assessment	Mild	Mod	Free/Severe

Table 7: Severity of pulmonary regurgitation by transthoracic echocardiography.

Presence or absence of tricuspid regurgitation (TR) was recorded. Although there are several other variables that may be considered when classifying the severity of tricuspid regurgitation (as recommended by BSE), the research team determined that only two will be considered. Severity classification of tricuspid regurgitation is shown in Table 8.

Parameter	Mild	Moderate	Severe
Colour Flow Jet	Small, central	Intermediate	Very large central jet or eccentric wall impinging jet
Vena Contracta Width mm	Undefined	<7	>7

Table 8: Severity of tricuspid regurgitation by transthoracic echocardiography

Right atrial (RA) size was also recorded, and this was determined by using RA end systolic area (cm^2) as recommended by the American Society of Echocardiography (Rudski 2010). The normal range will be considered as ≤ 18 cm^2.

Right ventricular restrictive physiology was assessed on echocardiography. Both Consultant Cardiologist clinical supervisors (LJF and CH) retrospectively reviewed the most recent echocardiogram of all participants with pulmonary regurgitation (Group 1 Mod-Sev PR and Group 2 Mild PR, n=32). Both Cardiologists were blinded to the participants' clinical history and study group allocation. There was no disagreement between the assessors. Pulsed wave doppler of arterial flow through the pulmonary valve was assessed. Restrictive physiology was recorded as present or not (yes/no), data shown in Table 1 – App.F, Appendix F. Presence of restrictive right ventricular function was defined as forward flow in late diastole throughout the respiratory cycle (Yam Y-Y 2007).

4.3.2 Cardiovascular Magnetic Resonance Imaging

Cardiovascular magnetic resonance imaging (CMR) is the gold standard non-invasive imaging modality for assessing patients with pulmonary regurgitation. Measurement of the 'regurgitant fraction' to grade severity is now routine in this patient group. There are further advantages over echocardiography in measuring right ventricular volumes and function accurately. In repaired Tetralogy of Fallot, serial measurements guide the clinician on decision-making surrounding pulmonary valve replacement. CMR studies performed as a part of routine clinical care were utilised in this research. In order to provide consistency and validity in the reporting of CMR, all studies were re-analysed by a single independent blinded level 3 accredited expert core laboratory observer Dr. Vassilios Vassiliou UEA/NNUH (VV) at NNUH using a semi-automatic software, CMR [42] (Circle CVI, Calgary, Canada). The data used in this study is from this re-analysis, rather than that reported by local radiologists.

All studies were checked for adequate image quality by VV to allow accurate volumetric analysis. No study needed to be excluded due to missing images or artefact. Most studies (37/40) were conducted at NNUH using a Siemens 1.5 Tesla CMR machine using a standardised protocol. One study was performed at Great Ormond Street Hospital, one at Papworth Hospital, and another at Guy's and St. Thomas' Hospital. Images were transferred to NNUH and re-analysed as stated above. The standardised protocol at NNUH involves having the patient laying supine with the external radiofrequency transmitter coil positioned over the heart. The protocol is as follows:

- During free breathing: Half-Fourier Single Short Turbo Spin Echo (HASTE) are initially acquired to produce static images in the transaxial plane during specific phases in the cardiac cycle (ECG gated). Further images are acquired in the coronal and sagittal planes.
- During breathholding: Cine sequences (short 'videos') in the vertical long axis plane (VLA) are then generated using balanced steady state free precession (SSFP).
- During breathholding: 2, 3, and 4 chamber SSFP cine images are then acquired.
- Cine 'stacks' (series of slices) are taken of the left ventricular and right ventricular outflow tracts (LVOT and RVOT).
- Coronal plane stacks of the RVOT are acquired.
- To calculate LV and RV function, cine stacks aligned perpendicular to the long axis of the left ventricle are acquired (6mm thick/4mm gap).
- Right Pulmonary Artery axial & coronal oblique cine images acquired.
- Left Pulmonary Artery axial & coronal oblique cine images acquired.
- Aortic root up to above the Sinus of Valsalva through-plane images acquired.
- Main Pulmonary Artery above the pulmonary valve through-plane images acquired.
- Right Pulmonary Artery flow through-plane images acquired.
- Left Pulmonary Artery flow through-plane images acquired.
- Further through-plane images as required (jet/stenosis) typically of Aortic Valve, Left Ventricular Outflow Tract, Pulmonary Valve & Right Ventricular Outflow Tract.

To calculate RV and LV volumes and function, the endocardial borders were carefully determined in end-systole and end-diastole as shown below in figure 9. LV papillary

muscles and major RV trabeculations were excluded from the blood pool and included in the myocardial mass. The LV epicardial volumes were also determined in diastole to assess diastolic mass. The mitral and tricuspid valves were tracked very carefully to assist with accurate volumetric analysis. All volumetric data was indexed to body surface area and these values were used in the subsequent analysis (ml/m^2). The Mosteller formula (Body surface area = (height (cm) x weight (kg)/3600)^{½}) for body surface area was used for all participants (Mosteller RD 1987).

The European Society of Cardiology has defined normal ranges for right ventricular ejection fraction in their 2013 publication 'Cardiovascular Magnetic Resonance - Pocket Guide' (Herzog 2013). This is based on the work by Maceira AM and colleagues (Maceira AM 2006), outlined in Table 9. Given the small sample size, we determined that adjusting for age and gender may introduce error (and undue complexity) and therefore right ventricular volumes and function would be considered as a continuous variable.

RVEF%	**Age <35**	**Age>35**
Males	57 ± 5 (47-67)	61 ± 6 (49-73)
Females	61 ± 3 (55–67)	64 ± 7 (50-78)

Table 9: RVEF% - Right ventricular ejection fraction in percentage.

Pulmonary Regurgitation:

A region of interest was drawn on the pulmonary artery as shown in figure 10, and from that the forward and backward flow was calculated. Classification of the severity of

pulmonary regurgitation is shown in table 10 and is based on the work by Merca-Rosa L and colleagues (Merca-Rosa L 2012).

Parameter	Mild	Moderate	Severe
PR Fraction	<20%	20-40%	>40%

Table 10: Severity of Pulmonary Regurgitation by CMR. PR – pulmonary regurgitation.

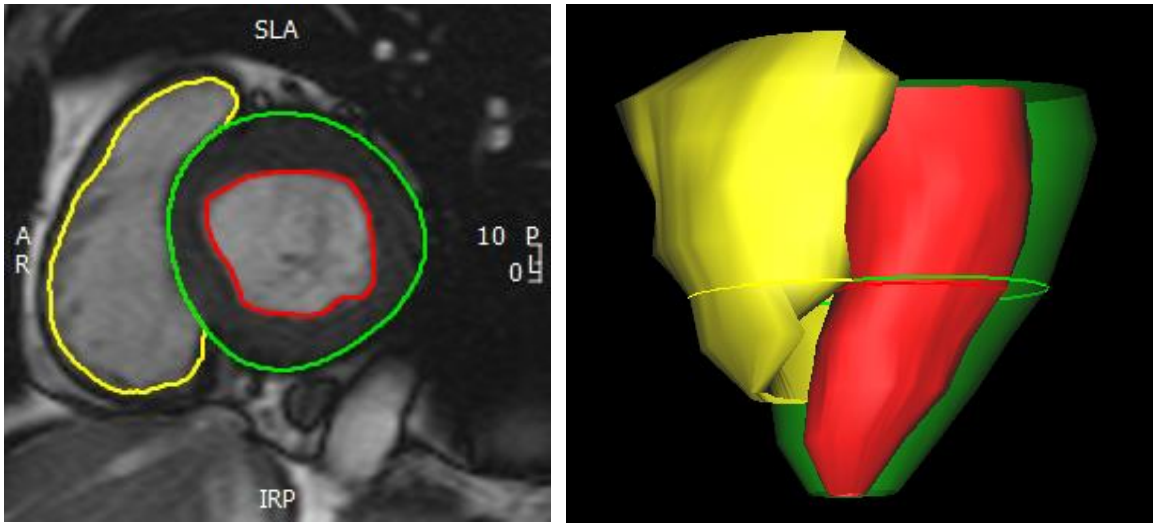


Figure 9. Left image: red colour marks the LV endocardium, green colour the LV epicardium and yellow colour the RV endocardium. Once completed in at all levels, a 3D model of the LV and RV can be obtained (right image: yellow RV, red LV) allowing volumetric measurements in diastole and systole. Biventricular stroke volume and ejection fraction are then automatically calculated.

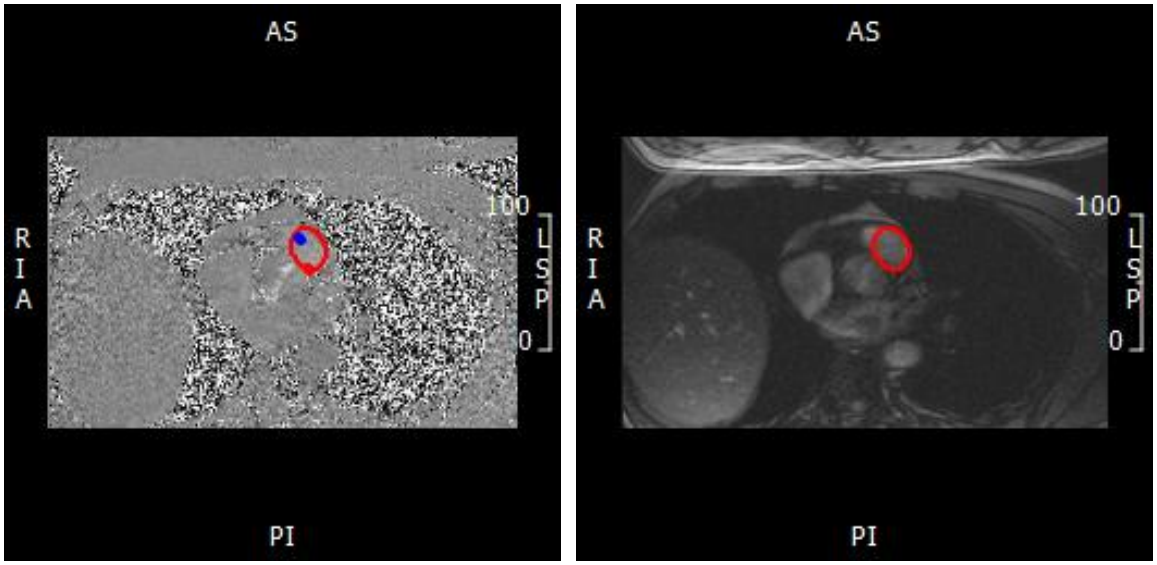


Figure 10: a region of interest is drawn on the pulmonary valve (red circle). This enables calculation of forward and backward flow, and the percentage of regurgitation (pulmonary regurgitant fraction).

4.3.3 Electrocardiogram

The most recent clinically indicated 12-lead electrocardiogram (ECG) was examined for rhythm, QRS morphology, and QRS duration. NNUH uses a Philips Page Writer TC50 electrocardiography system. The standard ECG recording protocol using 10mm/mV calibration recorded at 25mm/sec is used. The Page Writer TC50 uses the Philips ECG Algorithm that provides automated measurements of the various segmental intervals. The automated QRS duration calculation was used in this study. QRS duration was validated by manual calculation of the QRS duration, with results shown in section 5.6. An ECG calliper was used to measure the broadest QRS complex (excluding ectopic complexes) and an ECG ruler was used to measure this distance in milliseconds, figure 11.

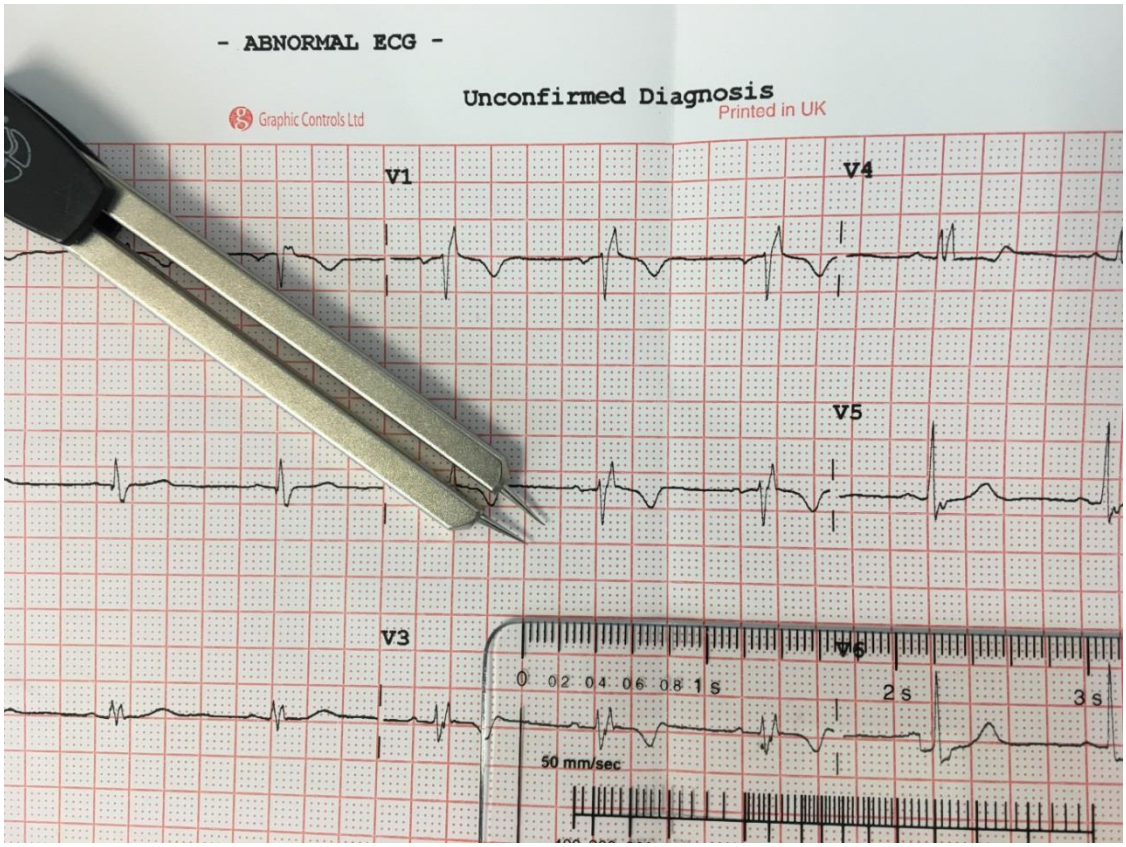


Figure 11: ECG calliper and ruler used in manual measurement of QRS duration.

4.3.4 Cardiopulmonary Exercise Test

Cardiopulmonary exercise testing (CPET) is used in adult congenital heart disease to objectify exercise intolerance. The results of routine clinical CPET were used in this study. A bicycle ergometer cardiopulmonary test was performed in participants at NNUH using a standard ramp protocol (Wasserman). NNUH uses the CareFusion Oxycon Pro system. A symptom limited exercise test was performed with simultaneous measurement of cardiac and respiratory function including workload, anaerobic threshold, MVO2 (maximum volume of oxygen consumed), VE/VCO2 slope (minute ventilation to carbon dioxide production ratio), heart rate and blood pressure response, oxygen pulse (surrogate for stroke volume), and electrocardiogram recording. The ten minute test uses an incremental increase in work rate so that the predicted MVO2 is

achieved at the end of the test. The predicted MVO2 is calculated using the subject's age, weight and height according to Wasserman's formula (Wasserman K 2005).

4.4 Other Investigations

4.4.1 Haematological and Biochemical Tests

All patients underwent blood tests at the NNUH phlebotomy department on their study day visit. The following tests were performed: Full Blood Count, Urea & Electrolytes, NT-Pro-BNP, total cholesterol, HbA1c, fasting glucose, alkaline phosphatase, total bilirubin, aspartate transaminase, alanine transaminase, gamma glutamyl transpeptidase (GGT), albumin, and coagulation screen including international normalised ratio (INR).

4.5 Data

4.5.1 Data Management

Data was collected on a paper case report form. The results of routine cardiological clinical tests were also recorded. Results were drawn from the various hospital web systems available at NNUH including ICE (Integrated Clinical Environment) and Cadran Image Platform. All data was then transferred to an excel spreadsheet and stored on the secure NNUH network. Data was anonymised to only include the study identification number. The trial master file contained the transfer sheet with study identification number and patient's full name, hospital number, age and date of birth.

4.5.2 Statistical Analysis

Statistical analysis was carried out using Microsoft Excel, and SAS (Statistical Analysis System) by the author and Mr. Ian Nunney (Statistician – University of East Anglia). Basic descriptive methods were used to present the data on study participants for all baseline clinical assessments. The three primary outcomes are the ELF score, Fibroscan result in kPa, and the MR Elastography result in kPa.

The hypothesis that there is no difference in the three primary outcomes between participants was tested using Analysis of Variance (ANOVA). All statistical tests use a significance level of $\alpha = 0.05$.

Univariate linear regression and Pearson's correlation analysis was performed for all participants (Groups 1,2 & 3) between the three primary outcomes and right ventricular systolic function (Ejection Fraction % on CMR), right atrial size, NT-Pro-BNP, body mass index, and Age.

In addition, univariate linear regression and Pearson's correlation analysis was then performed for participants in Groups 1 & 2 only (those with pulmonary regurgitation) between the hepatic primary outcomes and the following:

Cardiovascular Magnetic Resonance Imaging variables:

- Right ventricular end diastolic volume (RVEDV, ml/m^2)
- Right ventricular end systolic volume (ml/m^2)
- Right ventricular ejection fraction %
- Right ventricular stroke volume (ml)
- Left ventricular end diastolic volume (LVEDV, ml/m^2)

- Left ventricular end systolic volume (ml/m^2)
- Left ventricular ejection fraction %
- Left ventricular stroke volume (ml)
- Ratio of RVEDV:LVEDV
- Pulmonary regurgitant fraction %

Variables derived from Cardiopulmonary exercise test:

- Peak oxygen uptake MVO2 (ml/min/kg)
- Point at which anaerobic threshold reached as a percentage of predicted MVO2 - AT at VO2/VO2 %
- Minute ventilation to carbon dioxide production ratio - VE/VCO2
- O2 Pulse – VO2/Heart Rate (ml/beat)

Other relevant parameters:

- QRS durations (ms)
- N-terminal Prohormone of Brain Natriuretic Peptide (ng/L)
- Right ventricular systolic pressure as estimated on transthoracic echocardiography (mmHg)

Results of these statistical tests are presented in tables displaying Pearson's correlation coefficient (r), R^2 (coefficient of determination), linear regression coefficient, 95% confidence interval of the regression coefficient, and p-value. Significant

models/predictors are presented graphically with the regression equation displayed on the graph.

An attempt to fit multivariate linear regression models to predict the hepatic primary outcomes will be made.

5. Results I - Study Groups - Descriptive Statistics

5.1 Demographics

Demographic data was recorded on the study visit day, Table 11. Analysis of variance (ANOVA) was performed on the continuous variables to test for significant differences between groups. Only age of the participant was found to be significantly different between groups (p-value = 0.007), with Group 3 (Ebstein) having a significantly older population. The standard deviation for age in each group is relatively large denoting a large spread of values. Ranges for Group 1: 18-67 years; Group 2: 20-56 years; Group 3: 41-65 years. A reminder of the study groups is shown below:

- **Group 1 (Mod-Sev PR)**: Moderate to severe pulmonary regurgitation (n=16)
- **Group 2 (Mild PR)**: No more than mild pulmonary regurgitation (n=16)
- **Group 3 (Ebstein)**: At least mild tricuspid regurgitation associated with Ebstein's anomaly (n=8)

	Group 1 (Mod-Sev PR)	Group 2 (Mild PR)	Group 3 (Ebstein)	p-value
Number	**16**	**16**	**8**	
Age (years)	38.19 (16.17)	39.50(11.13)	56.50(8.26)	**0.007***
Height (m)	1.65(0.09)	1.71(0.09)	1.72(0.09)	0.065
Weight(kg)	70.50(11.98)	72.87(14.28)	84.00(24.73)	0.077
BMI	25.96(5.09)	24.75(4.01)	28.18(6.81)	0.476
F:M	1.66 : 1	1 : 1	3 : 1	N/A

Table 11: Demographic data expressed as mean (standard deviation). *m - metre, kg –kilograms, s BMI – body mass index in kg/m^2, F:M – Ratio of female to male participants.*

5.2 Underlying Cardiac and Surgical/Intervention History

Background information on participants' underlying condition was gathered from the NORPAP database and medical records (electronic and paper). Groups 1 & 2 are displayed in a comparative format in Table 12.

Transanunular patch repair is associated with obligatory pulmonary regurgitation in Fallot's Tetralogy, and in keeping with this, more participants in Group 1 have had this procedure compared to Group 2 (5 > 1).

Pulmonary valve replacement (PVR) had previously occurred in three participants within Group 2 but none in Group 1. It is worth noting that although the degree of pulmonary regurgitation was less than or equal to mild at the time of recruitment, those participants with PVR would have been exposed to severe chronic pulmonary

regurgitation before re-do surgery (none were performed for pulmonary stenosis in isolation). Consideration was given to the fact that duration of clinically significant pulmonary regurgitation, and therefore age of the participant, may significantly influence hepatic primary outcomes. Therefore, time interval between corrective surgery and study visit date are shown for Groups 1 (Mod-Sev PR) (Table 2 - App.E) and Groups 2 (Mild PR) (Table 3 - App.E) in Appendix E.

Presence and severity of tricuspid regurgitation was recognised as a factor that may significantly affect systemic venous pressure and may therefore lead to liver congestion. Significant (considered as at least moderate) tricuspid regurgitation was relatively uncommon in Groups 1 & 2. In each of Groups 1 & 2, only 3/16 (18.75%) participants had at least moderate tricuspid regurgitation.

Condition & Intervention	**Group 1 (Mod-Sev PR) n=16**		**Group 2 (Mild PR) n=16**	
	Frequency	**%**	**Frequency**	**%**
Fallot's Tetralogy	**12**	**75**	**9**	**56.25**
- Transannular Patch Repair	5	31.25	1	6.25
- Valvotomy/Valvuloplasty/Dilator	1	6.25	-	-
- RVOT Resection/Valvotomy	3	18.75	1	6.25
- RVOT Resection	2	12.5	5	31.25
- RVPA Homograft	1	6.25	1	6.25
- Unknown	-	-	1	6.25
Pulmonary Stenosis	**4**	**25**	**7**	**43.75**
Surgical valvotomy	2	12.5	3	18.75
- Percutaneous/balloon	2	12.5	4	25
Structural/haemodynamic abnormalities				
- ≤ Mild Tricuspid regurgitation	13	81.25	13	81.25
- > Mild Tricuspid regurgitation	3	18.75	3	18.75
- PVR	-	-	3	18.75
- Absent pulmonary valve	1	6.25	-	-
- Absent left PA	2	12.5	-	-
- Main & branch PA stenosis	2	12.5	1	6.25
- Atrial Septal Defect Closure	-	-	1	6.25
- Tricuspid Valve Repair	-	-	1	6.25
- Aortic Regurgitation	-	-	1	6.25

Table 12: Past medical / surgical history & structural / haemodynamic abnormalities.

RVOT –right ventricular outflow tract, RVPA homograft - right ventricular – pulmonary artery homograft, PVR - pulmonary valve replacement, PA – pulmonary artery.

Group 3 is shown in Table 13, with 2/8 participants (25%) having undergone Ebstein tricuspid valve repair. In contrast to Groups 1 & 2 (18.75% in each), 7/8 (87.5%) participants had at least moderate tricuspid regurgitation. The one participant with mild tricuspid regurgitation in this group had not had surgical repair.

Group 3 Ebstein	**n=8**	**%**
- Tricuspid valve repair	2	25
- No intervention	6	75
- ≤ Mild tricuspid regurgitation	1	12.5
- > Mild tricuspid regurgitation	7	87.5

Table 13: Surgical history and degree of tricuspid regurgitation in Group 3.

5.3 Relevant Past Medical and Drug History

Co-morbidities were assessed across study groups, Table 14. Given the small numbers, statistical analysis was not performed to assess for differences between groups. However, atrial arrhythmias and accessory pathways were more common in Group 3.

Condition	Group 1 (Mod-Sev PR) n=16	Group 2 (Mild PR) n=16	Group 3 (Ebstein) n=8
Hypertension	2(12.5)	3(18.75)	3(37.5)
Diabetes	1(6.25)	-	-
Cerebrovascular Accident	-	-	1(12.5)
Iron deficiency anaemia	2(12.5)	1(6.25)	-
Atrial Fibrillation	1(6.25)	2(12.5)	5(62.5)
Atrial Flutter	-	2(12.5)	3(37.5)
Atrial Tachycardia	1(6.25)	-	-
Accessory Pathways	-	-	4(50)
Aberrant coronary artery	1(6.25)	-	-
22q11 Deletion – DiGeorge Syndrome	1(6.25)	-	-
Noonan Syndrome	1(6.25)	-	-

Table 14: Co-morbidities and associated conditions. Denoted as frequency(%)

A complete medication history was taken on the study visit day. Table 15 shows common medications taken per study group. Diuretic use was more common in Group 3 Ebstein than in Groups 1 & 2 (12.5% in both). Given the propensity for atrial arrhythmias, a greater proportion of participants in Group 3 Ebstein were on Class I and Class III antiarrhythmic drugs (50%), compared with none in Groups 1 & 2. On a related note, anticoagulant use was more common in Group 3 (37.5%) than in Groups 1 (6.25%) & 2 (12.5%).

Medication	**Group 1 (Mod-Sev PR) n=16**	**Group 2 (Mild PR) n=16**	**Group 3 (Ebstein) n=8**
Diuretics (loop & thiazides)	2(12.5)	2(12.5)	3(37.5)
ACEi/ARBs	4(25)	4(25)	3(37.5)
B-Blockers/CCBs	4(25)	2(12.5)	2(25)
Class 1(A&C) Antiarrhythmics	-	-	4(50)
Class III Antiarrhythmics	1(6.25)	1(6.25)	2(25)
Anticoagulants (VKA and DOAC)	1(6.25)	2(12.5)	3(37.5)
Aspirin	3(18.75)	1(6.25)	1(12.5)
Statin	2(12.5)	1(6.25)	1(12.5)

Table 15: Medications across study groups. Denoted as frequency(percentage%).

ACEi: Angiotensin converting enzyme inhibitors. ARB – Angiotensin II receptor blockers. B-Blockers – Beta Blockers. CCB – Calcium channel blockers. VKA: Vitamin K Antagonists. DOAC: Direct Oral Anticoagulants.

5.4 Relevant Symptoms / Focussed History

A focussed history was taken during the study visit day and responses recorded on the paper case report form. Degree of breathlessness was assessed using the New York Heart Association (NYHA) functional classification (Dolgin 1994). 1. No limitation of physical activity, 2. Slight limitation of physical activity, 3. Marked limitation of physical activity. 4. Unable to carry out any physical activity without discomfort. The

majority of patients in Group 1 (62.5%) and Group 2 (68.8%) were NYHA 1. In Group 3, 75% of participants were at least NYHA 2 (Table 16).

NYHA	**Group 1 (Mod-Sev PR)**	**%**	**Group 2 (Mild PR)**	**%**	**Group 3 (Ebstein)**	**%**
1	10	62.5	11	68.8	2	25
2	5	31.3	5	31.3	5	62.5
3	1	6.3	-	-	1	12.5
4	-	-	-	-	-	-

Table 16: New York Heart Association score.

The subjective sensation of abdominal fullness (not related to eating large meals) was more common in Group 3 (37.5%) versus Groups 1 & 2 (6.3% in each), Table 17. This may relate to gastric/gut oedema as a consequence of systemic congestion and is perhaps in keeping with the greater need for diuretics in this group.

Abdominal Fullness	Group 1 (Mod-Sev PR)	%	Group 2 (Mild PR)	%	Group 3 (Ebstein)	%
Yes	1	6.3	1	6.3	3	37.5
No	15	93.8	15	93.8	5	62.5

Table 17: Response to interview question on abdominal fullness.

As alcohol excess is one of the most common causes of liver dysfunction and chronic liver disease, participants were asked how many units of alcohol they consumed per week, Table 18. This was documented and interpreted in the context of their other hepatic investigations. Only 1 patient from Group 1 consumed 31-40 units per week and of note all their hepatic investigations were normal. This was also the case for the 1 participant from both Group 2 & 3 who consumed 21-30 units per week.

ETOH	Group 1 (Mod-Sev PR)	%	Group 2 (Mild PR)	%	Group 3 (Ebstein)	%
0-10	14	87.5	12	75	7	87.5
11-20	1	6.25	3	18.75	-	-
21-30	-	-	1	6.25	1	12.5
31-40	1	6.25	-	-	-	-

Table 18: Alcohol intake in units/week. ETOH: Ethanol

5.5 Cardiovascular Magnetic Resonance Imaging

Participants with right and left ventricular systolic dysfunction were included in the study. Although Group 1 (Mod-Severe PR) contained three (18.75%) and Group 2 (Mild PR) contained five participants (31.25%) with right ventricular systolic dysfunction, no significant difference was found on ANOVA testing between all three study groups (Table 19). Furthermore, no significant difference was detected in right ventricular end systolic or diastolic volume, or stroke volume.

When the left heart was analysed, left ventricular ejection fraction (LVEF%) was significantly different between groups. Further t tests between individual study groups revealed that Group 1 (Mod-Sev PR) had significantly lower LVEF% than both Group 2 (Mild PR) ($p = 0.01$) and Group 3 (Ebstein) ($p < 0.01$). No difference was detected between Group 2 (Mild PR) and Group 3 (Ebstein).

Left ventricular stroke volume (LVSV) was also found to differ significantly between study groups. T testing revealed that Group 3 (Ebstein) had a lower LVSV than both Group 1 (Mod-Sev PR) ($p < 0.01$) and Group 2 (Mild PR) ($p < 0.01$). No difference was found between Group 1 (Mod-Sev PR) and Group 2 (Mild PR).

The ratio of right to left end diastolic volume (RVEDV:LVEDV) was significantly different between study groups. T testing revealed that Group 2 (Mild PR) had a lower RVEDV:LVEDV than Group 1 (Mod-Sev PR) ($p < 0.01$) and Group 3 (Ebstein) ($p = 0.01$).

In keeping with the inclusion criteria by which participants were assigned to study groups, the degree of pulmonary regurgitation was significantly different between study groups, $p <0.0001$.

Parameter	**Group 1 (Mod-Sev PR)**	**Group 2 (Mild PR)**	**Group 3 (Ebstein)**	**p-value**
RVEF%	54.64%(11.63%)	52.62%(10.61%)	51.37%(8.41%)	0.74
RVEDV ml/m^2	98.30(25.70)	83.02(21.56)	101.29(32.13)	0.15
RVESV ml/m^2	45.49(17.53)	39.83(14.29)	49.98(18.79)	0.34
RV SV ml	95.10(26.01)	79.04(20.03)	102.95(37.79)	0.87
LVEF%	50.68%(11.29%)	59.43(9.48%)	63.37(5.82%)	**0.007**
LVEDV ml/m^2	64.85(14.02)	70.19(17.86)	59.04(16.35)	0.27
LVESV ml/m^2	25.29(7.95)	28.68(10.43)	21.81(8.00)	0.21
LV SV ml	70.08(25.90)	76.26(18.59)	37.23(9.84)	**0.0003**
RVEDV:LVEDV	1.52(0.31)	1.20(0.23)	1.76(0.57)	**0.002**
PR%	30.69%(11.88%)	8.69%(7.45%)	0%(0%)	**<0.0001**

Table 19: Results from CMR. Denoted as mean(standard deviation)

RVEF%: Right ventricular ejection fraction in percentage, RVEDV: Right ventricular end diastolic volume ml/m^2, RVESV: Right ventricular end systolic volume ml/m^2, RV SV: Right ventricular stroke volume ml, LVEF%: Left ventricular ejection fraction in percentage, LVEDV: Left ventricular end diastolic volume ml/m^2, LVESV: Left ventricular end systolic volume ml/m^2, LV SV: left ventricular stroke volume ml, RVEDV: LVEDV: ratio of right to left end diastolic volumes, PR%: Pulmonary regurgitant fraction.

5.6 Electrocardiogram

The electrocardiogram (ECG) was examined for rhythm, QRS morphology and QRS duration. The QRS duration in milliseconds was taken from a resting 12-lead electrocardiogram on the most recent clinic attendance (<12 months) on all patients. The automated measurement from the Philips Page Writer TC50 ECG machine was used in all analyses. The author performed manual measurements (using callipers) of QRS duration on a 20% sample of participants' 12-lead electrocardiograms. Participants were selected to represent a range of QRS morphologies and durations. The mean difference between automated and manually calculated QRS durations was 2.60%. This is shown in table 20:

Study ID	Group	QRS Morphology	Automated ms	Manual ms	Δ%
1	1	RBBB	150	160	6.67%
2	1	pRBBB	116	120	3.45%
17	1	RBBB	173	180	4.05%
9	2	RBBB	154	160	3.75%
19	2	pRBBB	110	115	4.54%
22	2	Normal	93	100	7.53%
37	3	RBBB	169	160	-5.32%
43	3	LBBB	130	125	-3.85%
Mean Δ%	-	-	-	-	**2.60%**

Table 20: Automated and manually calculated qrs durations in a 20% sample.

RBBB – Right bundle branch block, LBBB – left bundle branch block, pRBBB – partial right bundle branch block (RSR' pattern in V1-V3 with QRS duration < 120ms). Automated ms – automated calculation of qrs duration in milliseconds. Manual ms – manual calculation of qrs duration in milliseconds. Δ% - delta/difference in percentage.

Although the mean QRS duration in Groups 1 (Mod-Sev PR) and 3 (Ebstein) were prolonged (>120ms), no significant difference was found on ANOVA testing between all groups with p-value 0.3314, Table 21. The majority of patients were in sinus rhythm, with one patient in Group 2 (Mild PR) in permanent atrial fibrillation. Right bundle branch block was more common in Group 1 (Mod-Sev PR), present in 68.75% of participants.

ECG Features	Group 1 (Mod-Sev PR)	Group 2 (Mild PR)	Group 3 (Ebstein)	p-value
QRS ms	134.94(29.11)	119.56(29.32)	125.63(31.62)	0.33
Sinus Rhythm	16/16 = 100%	15/16 =93.75%*	8/8 = 100%	n/a
QRS Morphology				
RBBB	11/16 = 68.75%	5/16 = 31.25%	3/8 = 37.5%	n/a
LBBB	-	-	1/8 = 12.5%	n/a
pRBBB/Normal	5/16 = 31.25%	11/16 = 68.75%	4/8 = 50%	n/a

Table 21: Electrocardiogram results.

QRS ms: QRS duration in milliseconds, expressed as mean(standard deviation).

*Sinus Rhythm: * One patient in Group 2 (Mild PR) was in atrial fibrillation.*

QRS Morphology: RBBB – Right bundle branch block, LBBB – left bundle branch block, pRBBB – partial right bundle branch block (RSR' pattern in V1-V3 with QRS duration < 120ms).

5.7 Cardiopulmonary Exercise Testing

Thirty six participants had cardiopulmonary exercise testing (CPET). One participant from Group 1 (Mod-Sev PR) refused the test on account of claustrophobia using the breathing mask. One participant each from Group 2 (Mild PR) and Group 3 (Ebstein) did not attend their appointments despite multiple attempts from the physiology laboratory and the chief investigator / author. One participant in Group 3 (Ebstein) had a relative contraindication on the basis of prior pre-excited atrial fibrillation. Results are shown in Table 22. Group 3 (Ebstein) has a significantly lower MVO_2 (maximum volume of oxygen consumed) than Groups 1 (Mod-Sev PR) and 2 (Mild PR). There is a significantly higher VE/VCO_2 slope in Group 3 (Ebstein), reflecting a relatively inefficient ventilatory response compared with Groups 1 (Mod-Sev PR) and 2 (Mild PR). Group 3 (Ebstein) had a significantly lower peak heart rate, heart rate response, and heart rate reserve, indicative of the older age and more prevalent use of antiarrhythmic and rate limiting drugs in this group.

Variable	Group 1 (Mod-Sev PR)	Group 2 (Mild PR)	Group 3 (Ebstein)	p-value
Duration min:secs	09:38(2:16)	09:34(02:13)	08:45(01:51)	0.83
Work Load watts	121.20(42.47)	145.33(46.74)	81.83(23.89)	**0.01**
Max work load %	84.80%(18.71%)	92.53%(26.14%)	86.50%(25.07%)	0.64
Heart rate peak bpm	161.53(21.17)	155.40(19.86)	117.33(20.66)	**0.0003**
Heart rate response %	89.20%(6.98%)	86.80%(11.41%)	72.16%(14.33%)	**0.006**
Heart rate reserve	19.60(11.37)	25.13(17.81)	40.50(20.60)	**0.03**
Peak Systolic BP	157.40(32.12)	151.53(24.31)	144.50(20.72)	0.61
Peak Diastolic BP	81.33(10.05)	85.13(15.30)	81.00(19.42)	0.71
RER	1.17(0.07)	1.20(0.09)	1.14(0.15)	0.39
MVO_2 ml/min/kg	26.77(9.02)	28.35(7.62)	17.48(0.84)	**0.02**
AT at VO_2/MVO_2 Predicted	53.93%(9.65%)	53.26(17.63%)	61%(15.38%)	0.56
VE/VCO_2	25.91(3.58)	24.38(3.17)	30.31(5.20)	**0.009**
O_2 Pulse	11.37(2.46)	13.64(3.96)	11.12(2.38)	0.10
Breathing Reserve	35.66(19.23)	30.33(12.89)	41.00(18.44)	0.39

Table 22: Select results from cardiopulmonary exercise tests. Mean(Standard Deviation).

bpm – beats per minute. BP – blood pressure. RER – respiratory exchange ratio. MVO_2 – Maximum volume of oxygen consumed ml/min/kg. AT at VO_2/VO_2 predicted – Point at which anaerobic threshold reached as a percentage of predicted MVO_2. VE/VCO_2 - minute ventilation to carbon dioxide production ratio. O_2 Pulse – VO_2/Heart Rate.

5.7 Liver Ultrasound

Liver Ultrasound was performed in all participants (Table 23). All scans were performed by the study Radiologist (PM). All abnormal results were discussed with the hepatologist supervising the study. Only one participant (Study ID 15, from Group 1) had an ultrasound with multiple abnormalities (consisting of irregular capsule, heterogenous parenchyma, and dilated hepatic vein) and was subsequently referred to the hepatology clinic.

Variable	Definition	Group 1 (Mod-Sev PR)	%	Group 2 (Mild PR)	%	Group 3 (Ebstein)	%
Morphology	Normal	15	93.8	16	100	8	100
	Abnormal	1	6.3	-	-	-	-
Capsule	Regular	14	87.5	13	81.3	8	100
	Irregular	2	12.5	3	18.8	-	-
Parenchyma	Homogenous	14	87.5	14	87.5	7	87.5
	Heterogenous	2	12.5	2	12.5	1	12.5
PV	Patent	16	100	16	100	8	100
	Obstructed	-	-	-	-	-	-
PV flow	Hepatopetal	16	100	16	100	8	100
	Hepatofugal	-	-	-	-	-	-
PV waveform	Normal	16	100	16	100	16	100
	Abnormal	-	-	-	-	-	-
HV	Normal	14	87.5	13	81.3	8	100
	Dilated	2	12.5	3	18.8	-	-
HV waveform	Triphasic	16	100	14	87.5	7	87.5
	Dampened	-	-	2	12.5	1	12.5
Varices	Absent	16	100	16	100	8	100
	Present	-	-	-	-	-	-
UA fluid	Absent	16	100	16	100	8	100
	Present	-	-	-	-	-	-

Table 23: Liver ultrasound results. Frequency and percentage %.

PV: portal vein (hepatopetal – normal flow of blood towards the liver. Hepatofugal – abnormal reversal of flow), HV: hepatic vein, UA fluid: upper abdominal fluid.

5.8 Splenic Size

The spleen is known to increase in size in portal hypertension related to chronic liver disease. Normal spleen size is considered < 13cm. The size of the spleen was measured in all participants during the liver ultrasound. Mean and standard deviation is shown in Table 24. There was no significant difference between study groups on ANOVA testing, p-value 0.2990.

	Group 1 (Mod-Sev PR)	**Group 2 (Mild PR)**	**Group 3 (Ebstein)**	**p-value**
Spleen Size	10.43(1.33)	10.36(1.74)	9.68(1.24)	0.29

Table 24: Spleen size in centimetres (cm) across all study groups. Denoted mean(standard deviation)

5.9 Haematological & Biochemical Tests

A panel of blood tests were performed on the study visit day. Table 25 displays results with mean and standard deviation. All mean values fall within the normal range. On ANOVA testing, only total serum cholesterol was significantly different between groups (p-value = 0.046). This is likely to reflect the older population in Group 3 (Ebstein). Mean NT-Pro-BNP was higher in Group 3 (Ebstein) but this was not found to be significantly different with a p-value = 0.066. Of note, mean values for liver enzymes, platelet count, and liver synthetic function, i.e. albumin & clotting profile were in the normal range and did not differ significantly between groups.

	Item/units	Group 1 (Mod-Sev PR)	Group 2 (Mild PR)	Group 3 (Ebstein)	p-value
FBC	Hb (g/L)	144.94(12.28)	148.00(11.39)	144.63(6.50)	0.89
	HCT (SI)	0.42(0.03)	0.43(0.03)	0.42(0.03)	0.73
	WCC (10^9/L)	6.53(1.70)	6.16(1.69)	7.59(1.42)	0.26
	Plts (10^9/L)	229.38(55.45)	231.44(54.55)	226.00(62.90)	0.92
U&Es	Na (mmol/L)	140.13(1.59)	140.06(1.81)	139.13(3.76)	0.35
	K (mmol/L)	4.40(0.36)	4.27(0.47)	4.24(0.18)	0.27
	Ur (mmol/L)	5.44(1.89)	4.36(1.02)	5.00(0.91)	0.27
	Creat (umol/L)	75.38(14.70)	77.50(16.57)	82.88(15.19)	0.28
LFTs	Albumin (g/L)	42.00(2.58)	41.81(2.54)	40.00(2.39)	0.10
	ALP (U/L)	66.00(15.16)	71.94(25.15)	70.75(12.73)	0.48
	ALT (U/L)	18.94(10.41)	21.25(9.02)	21.38(8.30)	0.49
	Bili (umol/L)	13.88(8.37)	14.00(8.17)	13.88(5.74)	0.99
	GGT (U/L)	24.75(17.13)	23.56(11.28)	32.75(22.79)	0.35
Clotting	PT (s)	14.98(3.33)	15.23(3.42)	15.91(4.32)	0.57
	APTT (s)	31.61(4.73)	31.76(2.78)	31.61(3.71)	0.97
	INR (SI)	1.18(0.38)	1.21(0.38)	1.31(0.45)	0.46
Other	Chol (mmol/L)	4.46(0.78)	4.86(1.03)	5.38(1.47)	**0.046**
	Glu (mmol/L)	5.12(0.80)	4.78(0.59)	4.74(0.35)	0.12
	HbA1c (mmol/mol)	38.31(5.80)	36.13(2.47)	37.38(3.02)	0.44
	NT-Pro-BNP (ng/L)	190.52(183.56)	152.03(126.28)	383.50(271.14)	0.06

Table 25: Results of all haematological and biochemical tests. Denoted as Mean(standard deviation).

FBC: full blood count, Hb: haemoglobin, HCT: haematocrit, WCC: white cell count, Plts: Platelets, Na: Sodium, K: Potassium, U&Es: Urea & Electrolytes, Ur: Urea, Creat: Creatinine, LFTs: Liver function tests, ALP: Alkaline phosphatase, ALT: Alanine transaminase, Bili: total bilirubin, GGT Gamma-glutamyl transpeptidase, PT: Prothrombin time, APTT: Activated partial thromboplastin time, INR: international normalised ratio, Chol: total fasting cholesterol, Glu: total fasting glucose, HbA1c: Glycated haemoglobin, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide.

5.10 Summary

The study groups were designed to separate clinically distinct groups of patients within the broad category of right sided congenital valvular abnormalities.

Demographically, Group 3 (Ebstein) represented an older population (p-value 0.0072) who were on average taller, heavier, and with higher body mass indexes. In keeping with their older age in the Ebstein group, total serum cholesterol was significantly higher (p-value 0.046) than Groups 1 & 2. Other cardiovascular risk factors were fairly evenly spread across all study groups. The only case of stroke was in Group 3, although this was thought to have arisen from an arrhythmic / cardio-embolic source. This age difference also likely influenced performance on CPET.

Participants in Group 3 (Ebstein) were more likely to experience abdominal bloating. In keeping with this, diuretic use was more common in this group. Given the propensity for atrial arrhythmias, a greater proportion of participants in Group 3 (Ebstein) were on Class I and Class III antiarrhythmic drugs than those with pulmonary regurgitation. On a related theme, anticoagulant use was more common in those with Ebstein's anomaly than in those with pulmonary regurgitation.

All right ventricular parameters including end diastolic and systolic volumes, ejection fraction, and stroke volume were not significantly different between study groups. The left ventricular measurements painted a different picture. Group 1 (Mod-Sev PR) had lower LVEF% than both Group 2 (Mild PR) and Group 3 (Ebstein). Group 3 (Ebstein) had significantly lower LVSV than the other two groups.

Group 2 (Mild PR) had lower RVEDV:LVEDV than both Group 1 (Mod-Sev PR) and Group 3 (Ebstein). This is in keeping with the criteria used to determine study groups;

both Group 1 (Mod-Sev PR) and 3 (Ebstein) were expected to have more significant right ventricular dilatation.

The majority in Group 1 (Mod-Sev PR) (62.5%) and Group 2 (Mild PR) (68.8%) were NYHA 1. By contrast Group 3 (Ebstein) contained 75% of participants who were at least NYHA 2. This participant selection represents a relatively stable group of those with pulmonary regurgitation. In keeping with this, significant tricuspid regurgitation (a poor prognostic marker in pulmonary regurgitation) was uncommon in Groups 1 & 2. Note is therefore made of this particularly clinically stable participant selection (particularly in Group 1), and the impact this may have when testing the main study hypotheses.

6. Results II – Enhanced Liver Fibrosis Score

6.1 ELF Score - Overview

Blood samples collected on the study visit day were stored in the NNUH laboratory. All samples were processed at the end of the study by the Wolfson Laboratory (Royal Free Hospital, London). The mean and median ELF scores fell within an abnormal range. As described in section 4.2.4, in chronic liver disease, an ELF score of < 7.7 is predictive of none/mild fibrosis, ≥ 7.7 to < 9.8 moderate fibrosis, and ≥ 9.8 severe fibrosis. The mean and median ELF scores in all study groups fell in the moderate fibrosis category (Table 26). In addition, there were outliers in each group that fell into the severely fibrotic category. However caution should be applied when interpreting values for an untested population against reference ranges derived from a population with chronic liver disease.

6.2 ELF Score – ANOVA Testing

Although the mean and median values for all study groups fell into an abnormal range, no significant difference was seen between study groups on ANOVA testing with p-value 0.98, Table 26. This is illustrated in Figure 12. Mean and standard deviation for all participants was 8.58 ± 0.92.

Group	Mean	SD	Min	Max	Med	CI	IQ	p-value
1 (Mod-Sev PR)	8.6	0.831	7.32	10.42	8.59	(8.19,9.01)	(7.95,9.27)	0.98
2 (Mild PR)	8.57	0.694	7.45	10.18	8.42	(8.23,8.91)	(8.14,8.99)	0.98
3 (Ebstein)	8.6	1.51	5.46	10.45	8.81	(7.55,9.65)	(8.17,9.47)	0.98

Table 26: ELF scores across all study groups.

N = number, SD = standard deviation, Min= Minimum, Max = Maximum, Med = Median, CI = confidence interval, IQ = interquartile range.

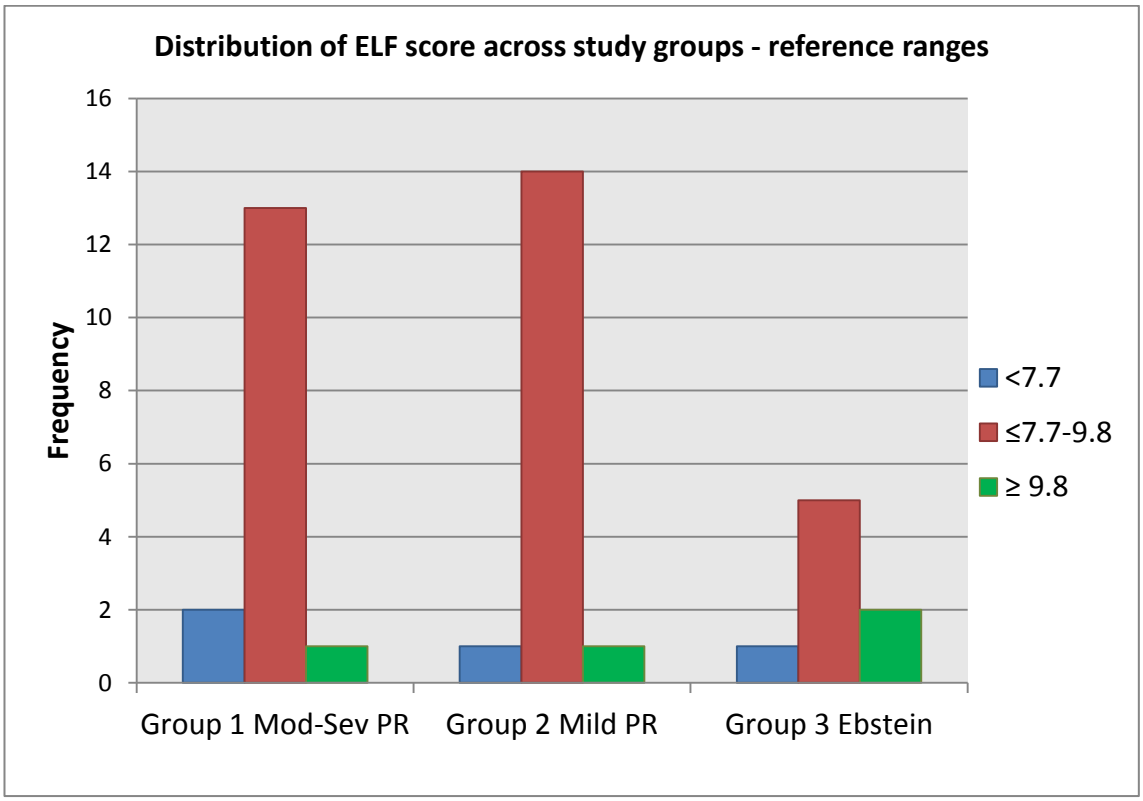


Figure 12: ELF score across study groups.

Blue: <7.7 – Mild Fibrosis. Red: ≤7.7 – 9.8 – Moderate Fibrosis. Green: ≥ 9.8 Severe Fibrosis

6.3 ELF Score – Correlations and Linear Regression

Assuming a linear relationship, linear regression was performed to calculate correlation coefficients for the ELF score (as the dependent variable) against explanatory variables in Table 27. A non-significant negative correlation was seen with right ventricular ejection fraction (RVEF%) on CMR. A scatter plot showing this association is shown in Figure 13. Body mass index had a significant correlation with the ELF score, Figure 14. This finding is consistent with other studies involving normal subjects (Yoo EJ 2013). The other variables also only showed weak or moderate correlation, and no significant models were detected.

ELF Score	**r**	**R^2**	**Regression Coefficient**	**95% CI**	**P-Value**
RVEF%	-0.27	0.07	-2.46	(-5.28,0.36)	0.08
RA	0.08	0.006	0.01	(-0.03,0.05)	0.61
NT-Pro-BNP	0.05	0.002	0.0002	(-0.0017,-0.0012)	0.74
Age	0.26	0.07	0.01	(-0.002,0.03)	0.09
BMI	0.39	0.15	0.07	(0.01,0.12)	**0.01**

Table 27: Univariate linear regression for ELF score versus postulated associated variables in all study groups. r = pearson's correlation coefficient . R^2 = coefficient of determination. 95% CI – 95% confidence interval. P-value: $\alpha = 0.05$.

RVEF% : Right ventricular ejection fraction in percentage, RA: Right atrial size cm^2, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide (ng/L) . Age in years. BMI – body mass index kg/m^2.

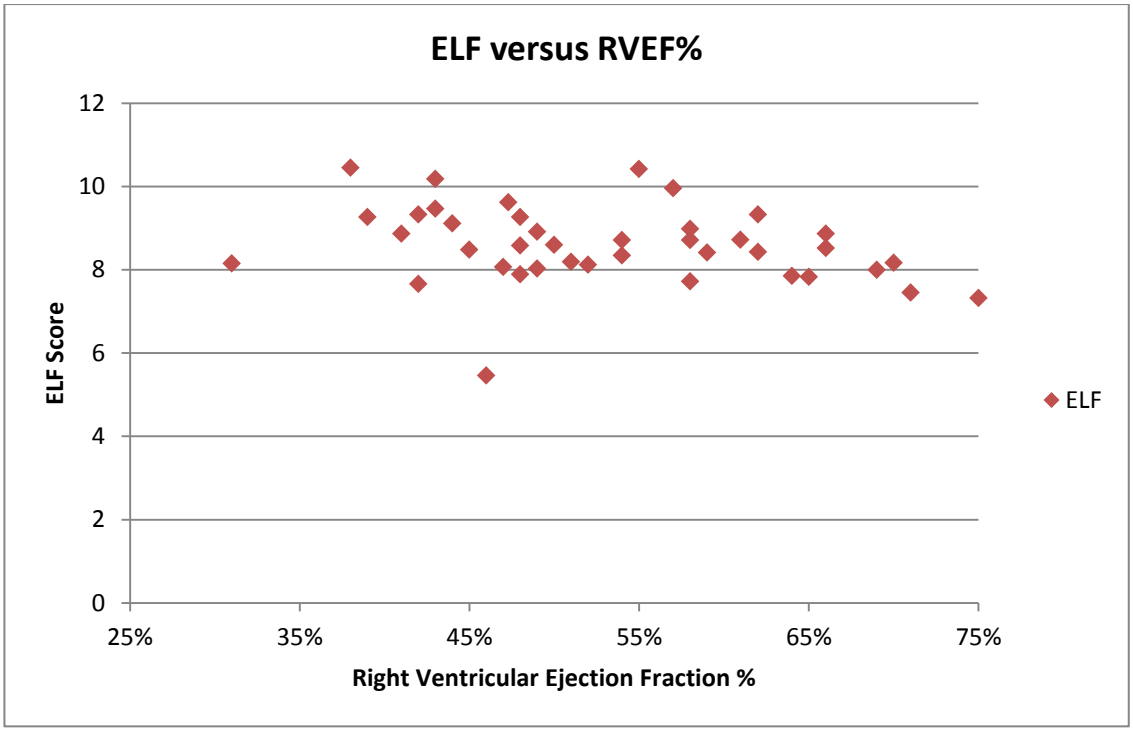


Figure 13 – Scatter plot of right ventricular ejection fraction % versus ELF score in all participants

RVEF% - Right ventricular ejection fraction %. ELF – enhanced liver fibrosis score

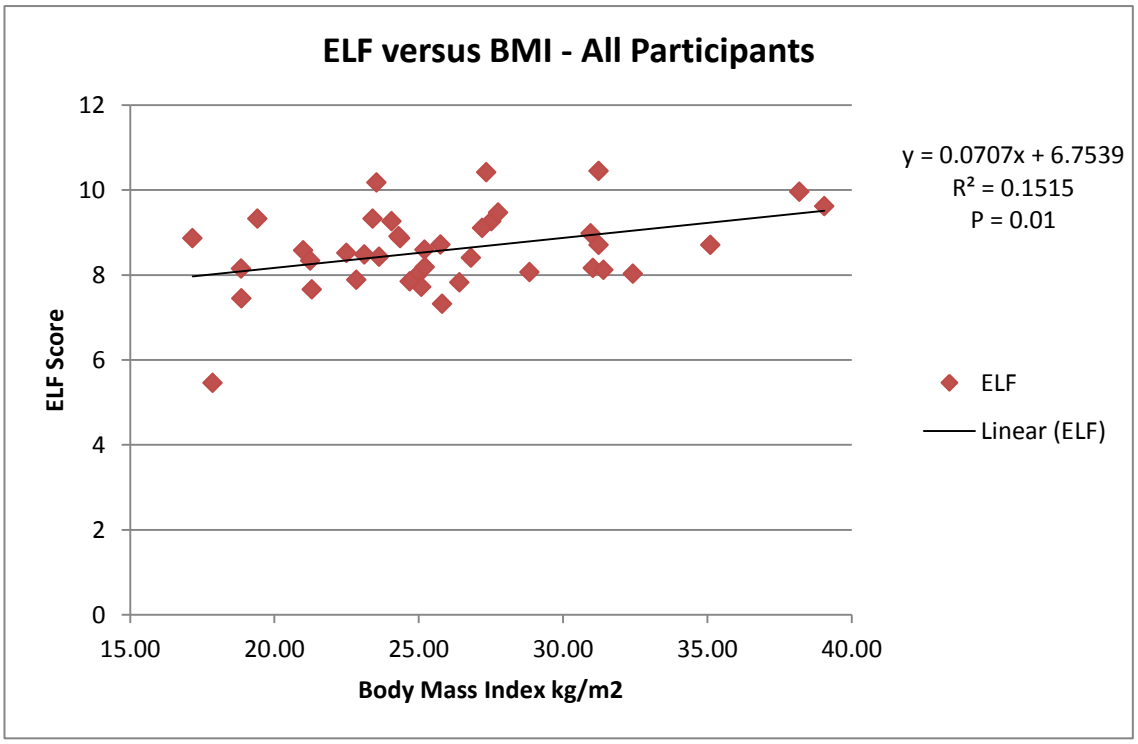


Figure 14: Linear regression model between ELF score (y axis) and BMI (x axis) with corresponding equation – All Participants

ELF – enhanced liver fibrosis score, BMI – body mass index kg/m^2

Univariate linear regression was next performed using the ELF score as the dependent variable in Groups 1 & 2 (those with pulmonary regurgitation). Independent variables tested include prognostic markers in pulmonary regurgitation, CMR data, right ventricular systolic pressure (RVSP), NT-Pro-BNP, and CPET data, Table 28. RVSP is estimated using the Bernoulli equation on transthoracic echocardiography. The echocardiography technician's measurement from the most recent clinically indicated study is used in this analysis. The measurement is reliant on a sufficient tricuspid regurgitation jet, and was only measurable in 24/32 participants from Groups 1 (Mod-Sev PR) and 2 (Mild PR).

The strongest statistically significant correlation was seen with QRS duration, with a correlation coefficient of 0.51, p-value 0.0025. The R^2 (coefficient of determination) of 0.26 shows that the independent variable QRS duration explains 26% of the variation in the ELF score; this relationship is shown in Figure 15. A significant linear model was also found for NT-Pro-BNP as a predictor of the ELF score. This had a moderate correlation of 0.39 and was statistically significant with $p = 0.02$. The R^2 of 15% (relatively poor fit) is partly explained by the presence of outliers, as shown in Figure 16.

In contrast to the prior analysis involving RVEF% in all participants, a significant negative correlation was found when the analysis was limited to those with pulmonary regurgitation only, Figure 17.

ELF Score	r	R^2	Regression Coefficient	95% CI	P-value
QRS	0.51	0.26	0.01	(0.004,0.021)	**0.002**
NT-Pro-BNP	0.39	0.15	0.001	(0.0002,0.003)	**0.02**
RVEDV	0.20	0.04	0.006	(-0.005,0.01)	0.26
RVSEV	0.34	0.12	0.01	(-0.0001,0.03)	0.05
RVEF%	-0.37	0.14	-2.62	(-5.01,-0.23)	**0.03**
RV SV	0.11	0.01	0.003	(-0.008,0.01)	0.54
LVEDV	0.08	0.007	0.004	(-0.01,0.02)	0.63
LVESV	0.19	0.03	0.01	(-0.01,0.04)	0.29
LVEF%	0.06	0.004	0.43	(-2.07,2.94)	0.72
LV SV	0.34	0.11	0.01	(-0.0003,0.02)	0.05
PR%	0.01	0.0001	0.0005	(-0.01,0.01)	0.95
RVEDV:LVEDV	0.16	0.02	0.39	(-0.47,1.26)	0.35
MVO_2	-0.06	0.003	-0.005	(-0.04,0.02)	0.75
AT at VO_2/VO_2 %	0.11	0.01	0.59	(-1.46,2.66)	0.55
VE/VCO_2	-0.18	0.03	-0.04	(-0.12,0.04)	0.32
O_2 Pulse	0.11	0.01	0.02	(-0.05,0.10)	0.55
RVSP	0.19	0.03	0.02	(-0.02,0.06)	0.39

Table 28: Linear regression models & correlation coefficients for ELF Score in Groups 1 & 2.

r = pearson's correlation coefficient. R^2 = coefficient of determination. 95% CI – 95% confidence interval. P-value: $\alpha = 0.05$.

QRS: QRS duration on electrocardiogram in milliseconds, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide (ng/L), RVEDV: right ventricular end diastolic volume ml/m^2, RVESV: right ventricular end systolic volume ml/m^2, RVEF%: right ventricular ejection fraction %, RV SV: right ventricular stroke volume ml, LVEDV: left ventricular end diastolic volume ml/m^2, LVESV: left ventricular end systolic volume ml/m^2, LVEF%: left ventricular ejection fraction %, LV SV: left ventricular stroke volume, PR%: pulmonary regurgitant fraction %, RVEDV:LVEDV: ratio of right ventricular end diastolic volume to left ventricular end diastolic volume, MVO_2: peak oxygen uptake ml/min/kg, AT at VO_2/VO_2 %: Point at which anaerobic threshold reached as a percentage of predicted MVO_2, VE/VCO_2 - minute ventilation to carbon dioxide production ratio. O_2 Pulse – VO_2/Heart Rate in ml/beat. RVSP: Right ventricular systolic pressure mmHg.

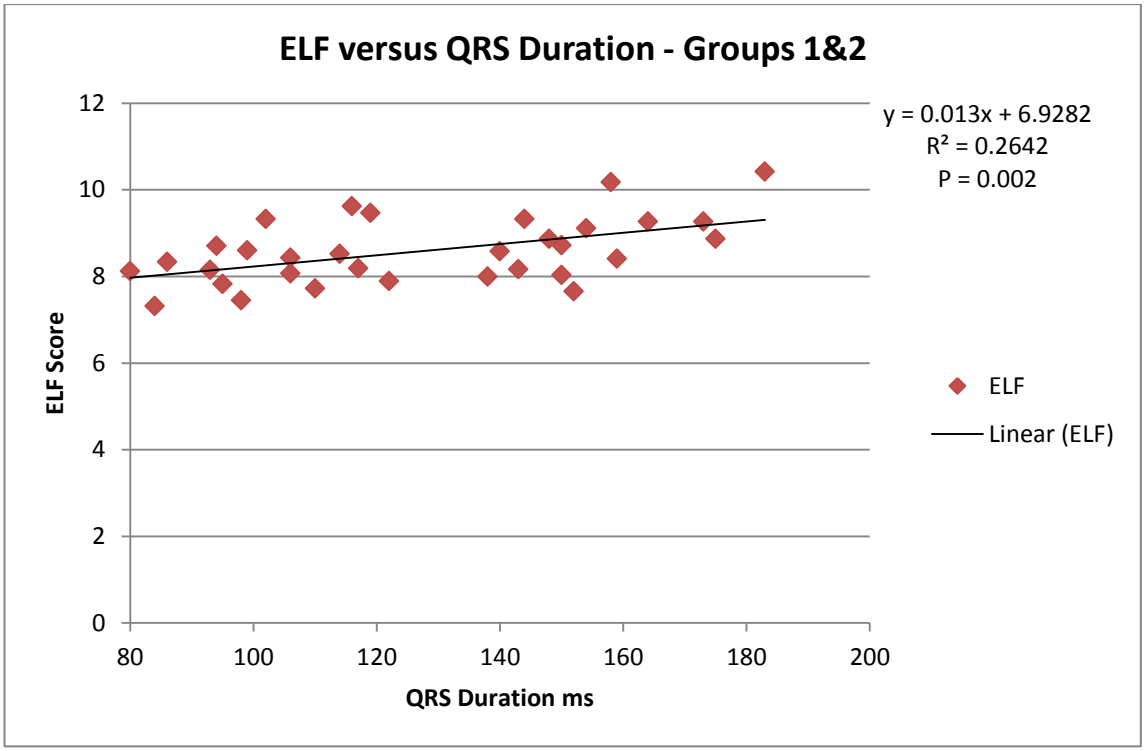


Figure 15: Linear regression model between ELF score (y axis) and QRS duration (x axis) with corresponding equation – Groups 1&2.

ELF – Enhanced liver fibrosis score. QRS: qrs duration in milliseconds

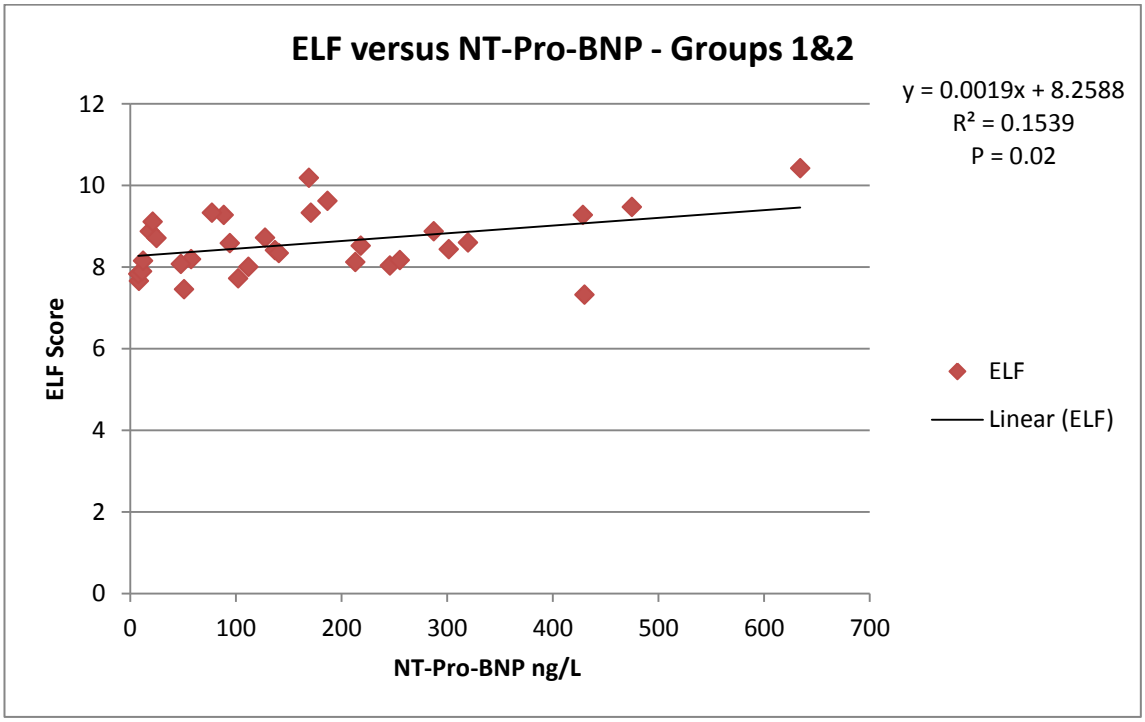


Figure 16: Linear regression model between ELF score (y axis) and NT-Pro-BNP (x axis) with corresponding equation – Groups 1&2.

ELF – Enhanced liver fibrosis score. NT-Pro-BNP – N-terminal prohormone of brain natriuretic peptide (ng/L)

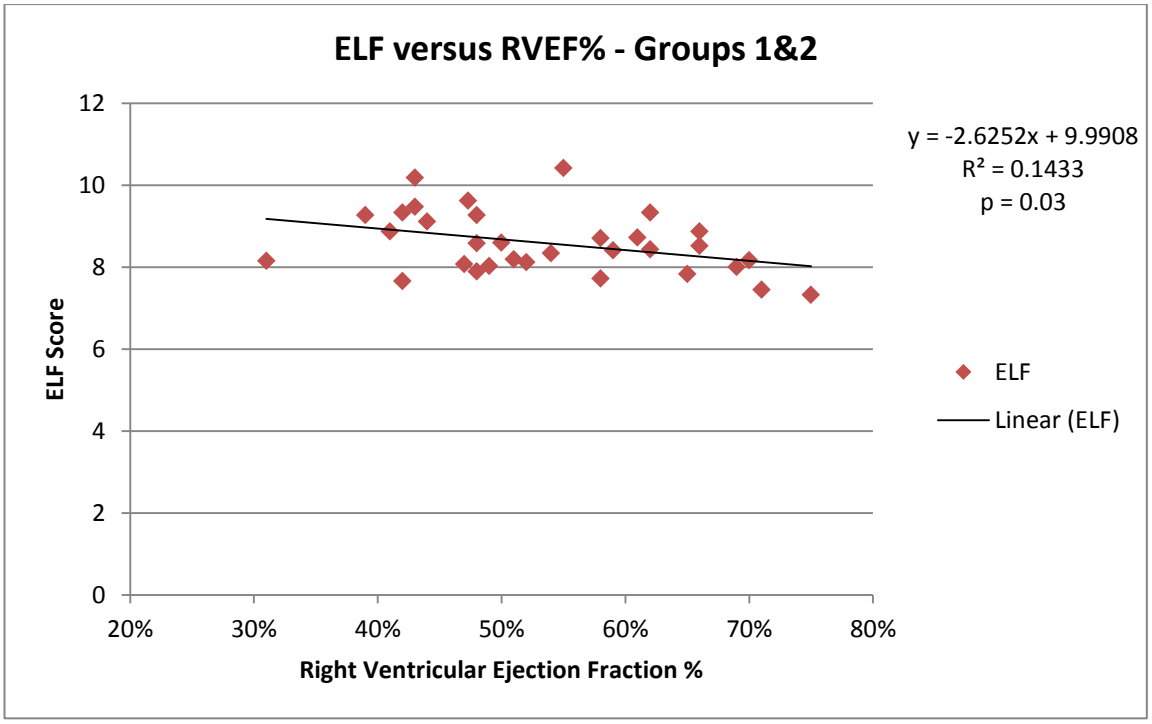


Figure 17: Linear regression model between ELF score (y axis) and RVEF% (x axis) with corresponding equation – Groups 1&2.

ELF – Enhanced liver fibrosis score. RVEF% - right ventricular ejection fraction %.

The relationship between the ELF score and QRS duration was further examined by performing linear regression on all participants (rather than just Groups 1 & 2). This was also statistically significant with correlation coefficient 0.46, p = 0.002, shown in Figure 18.

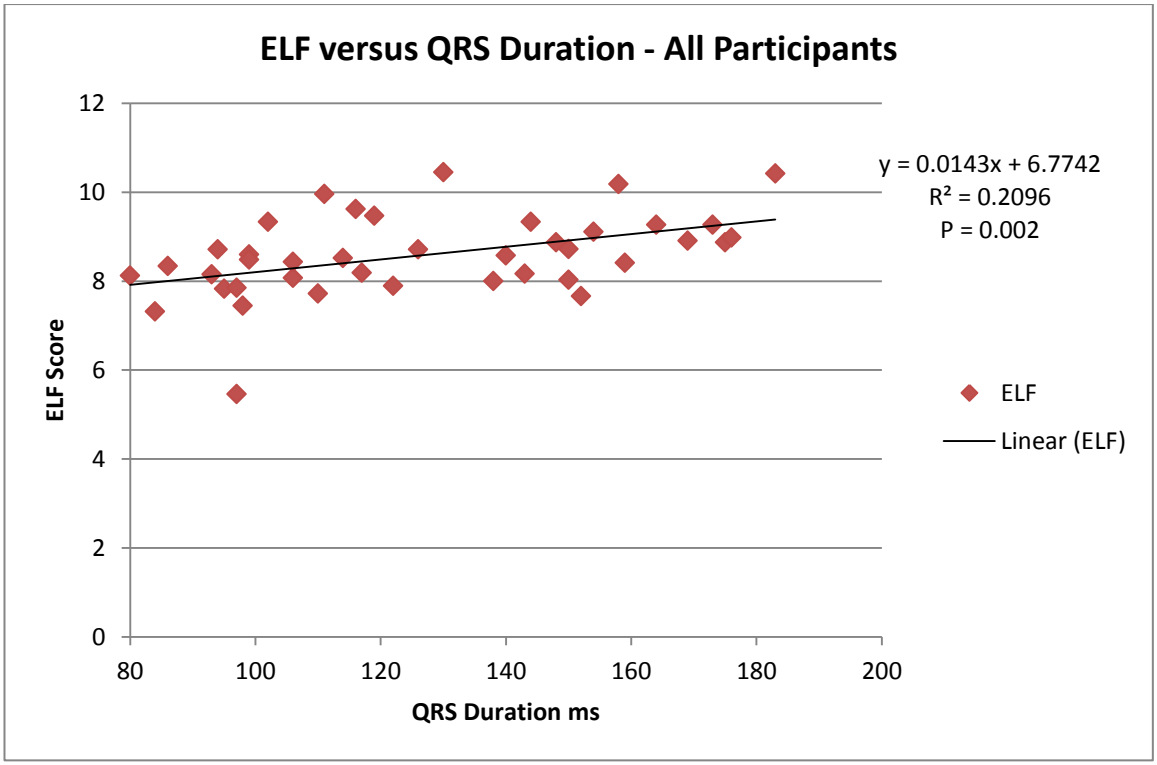


Figure 18: Linear regression model between ELF score (y axis) and QRS Duration (x axis) with corresponding equation – All Participants

ELF – Enhanced liver fibrosis score. QRS: qrs duration in milliseconds

6.4 Components of the ELF Score

6.4.1 Components of the ELF Score – ANOVA Testing

As described previously, the ELF score is a single number generated from an algorithm using measurements of hyaluronic acid (HA), metalloproteinase 1 (TIMP-1), and amino-terminal propeptide of type III procollagen (PIIINP). Further ANOVA tests were performed to determine if there were any differences between the means of the study groups, Table 29. This showed that the mean of TIMP-1 was significantly different between groups with p = 0.003. T-testing between individual study groups showed that Group 3 (Ebstein) had a significantly higher mean than Group 1 (Mod-Sev PR) (p = 0.016) and Group 2 (Mild 2) (p = 0.008).

Components	**Group 1 (Mod-Sev PR)**	**Group 2 (Mild PR)**	**Group 3 (Ebstein)**	**p-value**
HA	35.10(35.44)	30.08(32.43)	48.28(37.61)	0.48
PIIINP	8.07(2.57)	8.49(2.87)	6.66(3.37)	0.34
TIMP-1	202.80(54.82)	152.02(126.28)	383.50(271.14)	**0.003**

Table 29: Components of the ELF score: mean (standard deviation)

HA - hyaluronic acid, TIMP-1 - metalloproteinase 1, PIIINP - amino-terminal propeptide of type III procollagen

6.4.2 Hyaluronic Acid (HA)

Linear regression was performed against the variables tested previously against the ELF score (Table 30). QRS duration maintained a significant relationship with HA (figure 19), showing an increase in the significance of the correlation and linear model when the analysis was limited to those in Groups 1 & 2 (pulmonary regurgitation) (figure 20). Right ventricular ejection fraction had a negative correlation with HA (figure 21), however this relationship was not significant when tested in only Groups 1 & 2 (pulmonary regurgitation). Right ventricular end systolic volume significantly correlated with HA in Groups 1 & 2 (pulmonary regurgitation), figure 22. NT-Pro-BNP also significantly correlated with HA in Groups 1 & 2, figure 23.

Hyaluronic Acid	r	R^2	Regression Coefficient	95% CI	p-value
QRS Duration All Participants	0.38	0.14	0.44	(0.09,0.79)	**0.01**
QRS Duration Groups 1 & 2	0.47	0.22	0.53	(0.16,0.90)	**0.006**
RVEF% All Participants	- 0.32	0.10	-107.78	(-211.07,-4.49)	**0.04**
RVEF% Groups 1&2	- 0.30	0.09	-92.94	(-202.61, 16.72)	0.09
RVESV All Participants	0.24	0.05	0.50	(-0.15, 1.16)	0.12
RVESV Groups 1&2	0.36	0.13	0.76	(0.04, 1.49)	**0.03**
NT-Pro-BNP All Participants	0.26	0.06	0.04	(-0.009, 0.09)	0.10
NT-Pro-BNP Groups 1&2	0.44	0.19	0.09	(0.02, 0.16)	**0.01**

Table 30: Linear regression models & correlation coefficients for HA.

r = pearson's correlation coefficient . R^2 = coefficient of determination. 95% CI – 95% confidence interval. P-value: $\alpha = 0.05$.

HA – hyaluronic acid, QRS – qrs duration in milliseconds, RVEF% - right ventricular ejection fraction in percentage, RVESV: right ventricular end systolic volume ml/m^2, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide (ng/L).

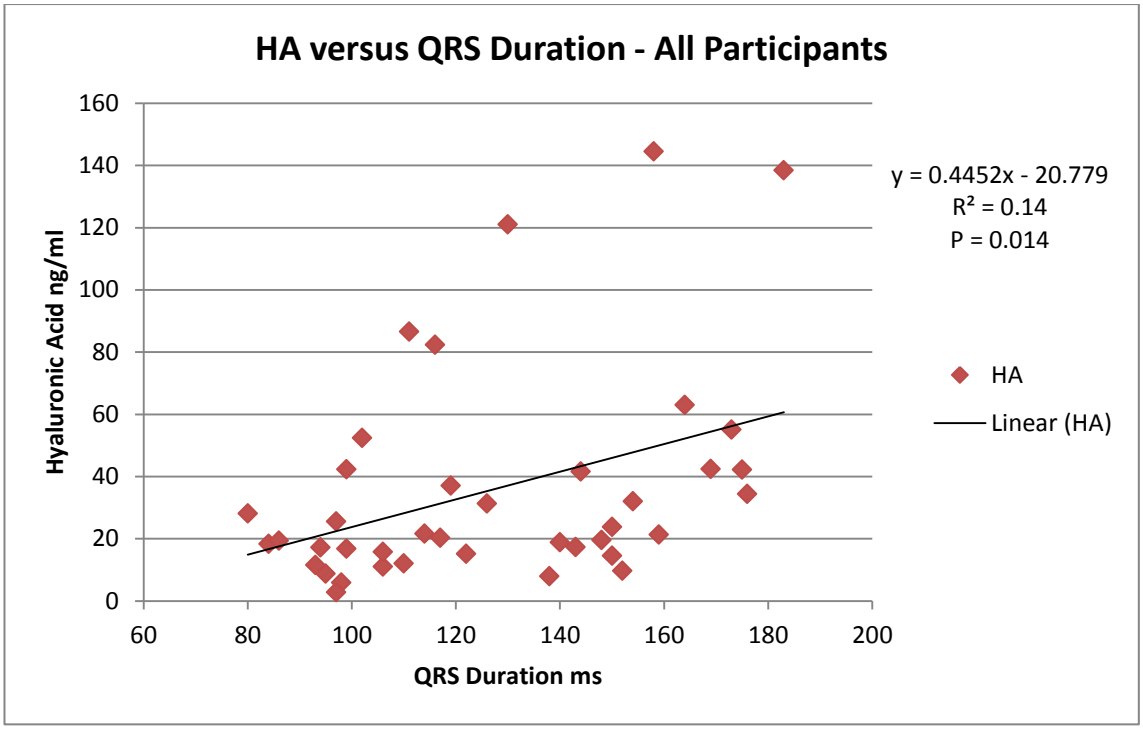


Figure 19: Linear regression model between Hyaluronic acid (y axis) and QRS Duration (x axis) with corresponding equation – All Participants

HA – hyaluronic acid, QRS – QRS duration in ms

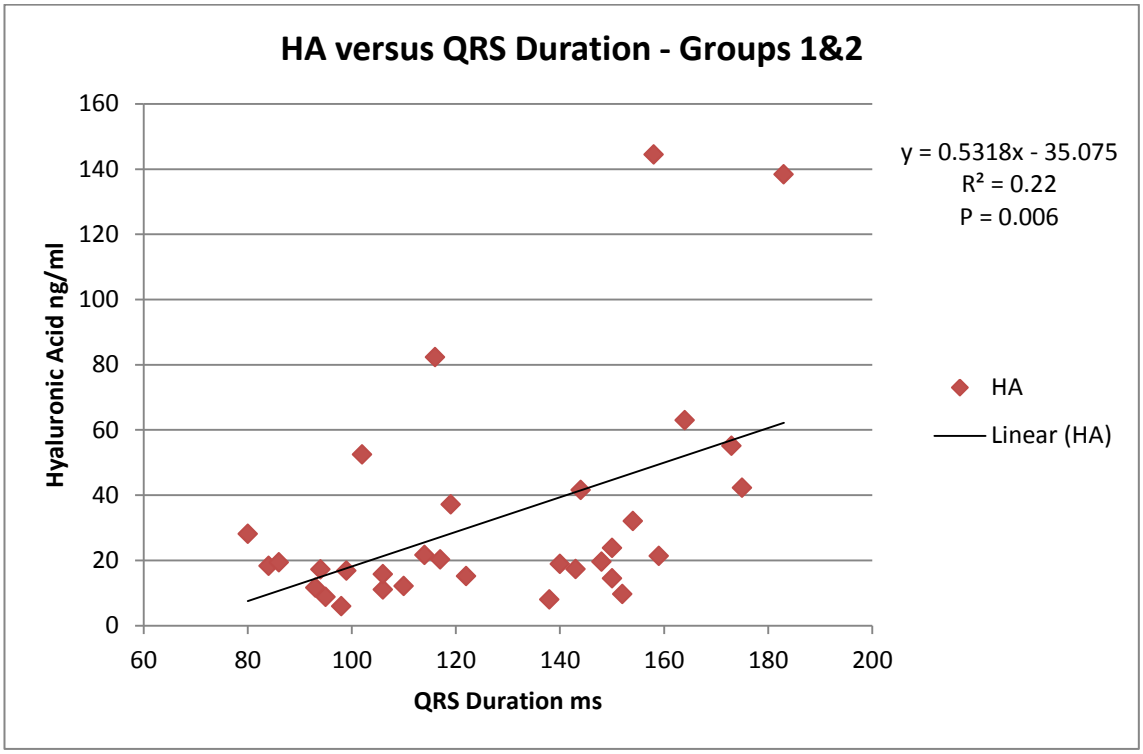


Figure 20: Linear regression model between Hyaluronic acid (y axis) and QRS Duration (x axis) with corresponding equation – Groups 1 & 2

HA – hyaluronic acid, QRS – QRS duration in ms

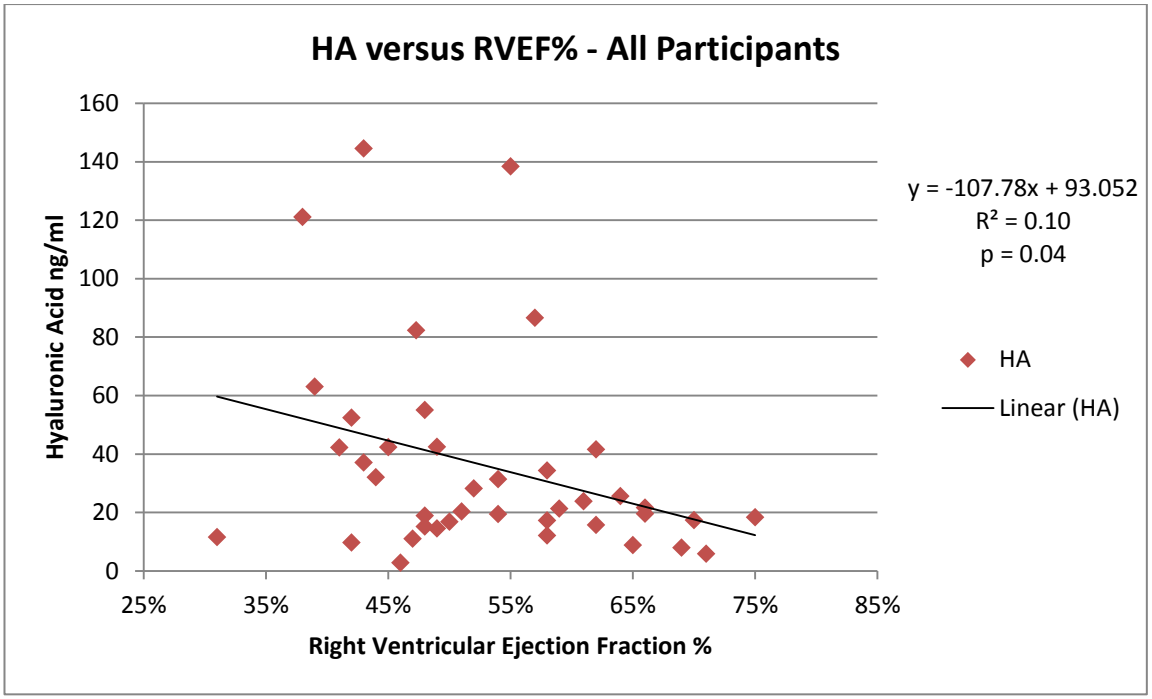


Figure 21: Linear regression model between Hyaluronic acid (y axis) and RVEF% (x axis) with corresponding equation – All Participants.

HA – hyaluronic acid, RVEF% - right ventricular ejection fraction in percentage

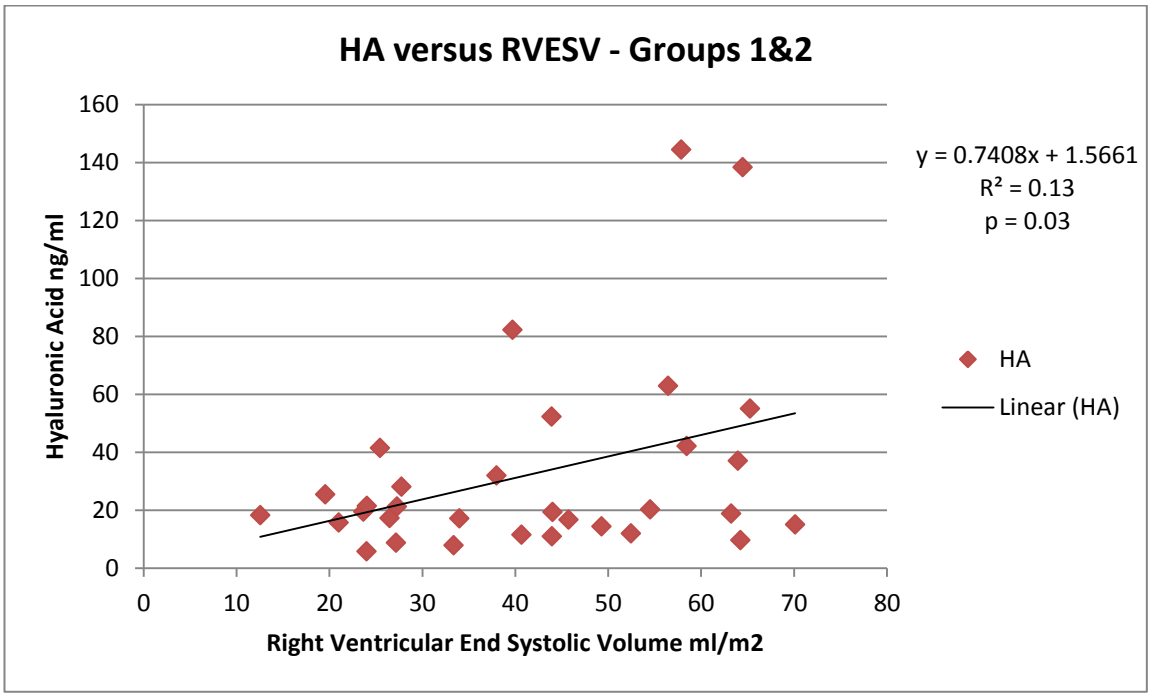


Figure 22: Linear regression model between Hyaluronic acid (y axis) and RVESV (x axis) with corresponding equation – Groups 1&2

HA – hyaluronic acid, RVESV – right ventricular end systolic volume.

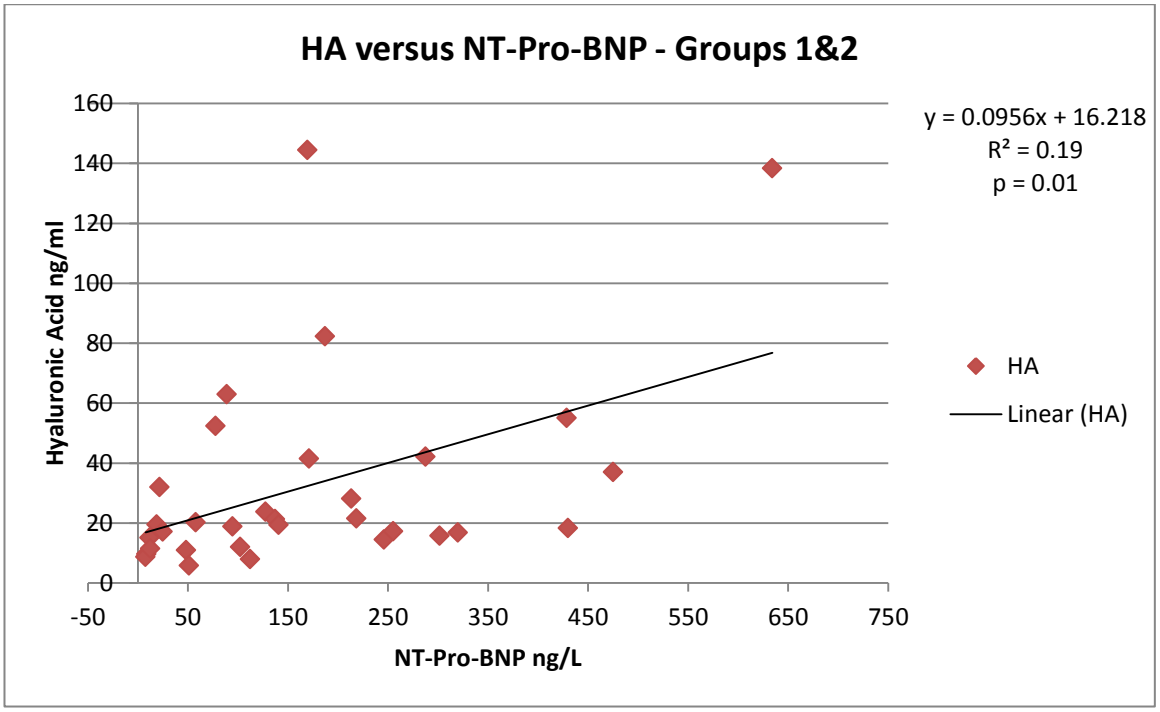


Figure 23: Linear regression model between Hyaluronic acid (y axis) and NT-Pro-BNP (x axis) with corresponding equation – Groups 1&2

HA – hyaluronic acid, NT-Pro-BNP – N-terminal prohormone of brain natriuretic peptide.

6.4.3 Metalloproteinase-1 (TIMP-1)

In section 6.4.1, the mean of TIMP-1 was shown to be significantly different between the study groups (Table 29), with Group 3 (Ebstein) having a higher mean than Groups 1 and 2 (PR). Further linear regression analysis was performed against the variables previously described. Only QRS duration was seen to have a significant correlation with TIMP-1 (Table 31). This was significant in the analysis for all participants and for those with pulmonary regurgitation in Groups 1 & 2, figures 24 & 25.

TIMP-1	r	R^2	Regression Coefficient	95% CI	p-value
QRS Duration All Participants	0.51	0.26	0.88	(1.37,0.40)	**0.0007**
QRS Duration Groups 1&2	0.44	0.19	0.65	(0.15,1.14)	**0.01**

Table 31: Linear regression models & significant correlation coefficients for TIMP-1.

r = pearson's correlation coefficient . R^2 = coefficient of determination. 95% CI – 95% confidence interval. P-value: α = 0.05.

TIMP-1 - Metalloproteinase-1, QRS – qrs duration in milliseconds.

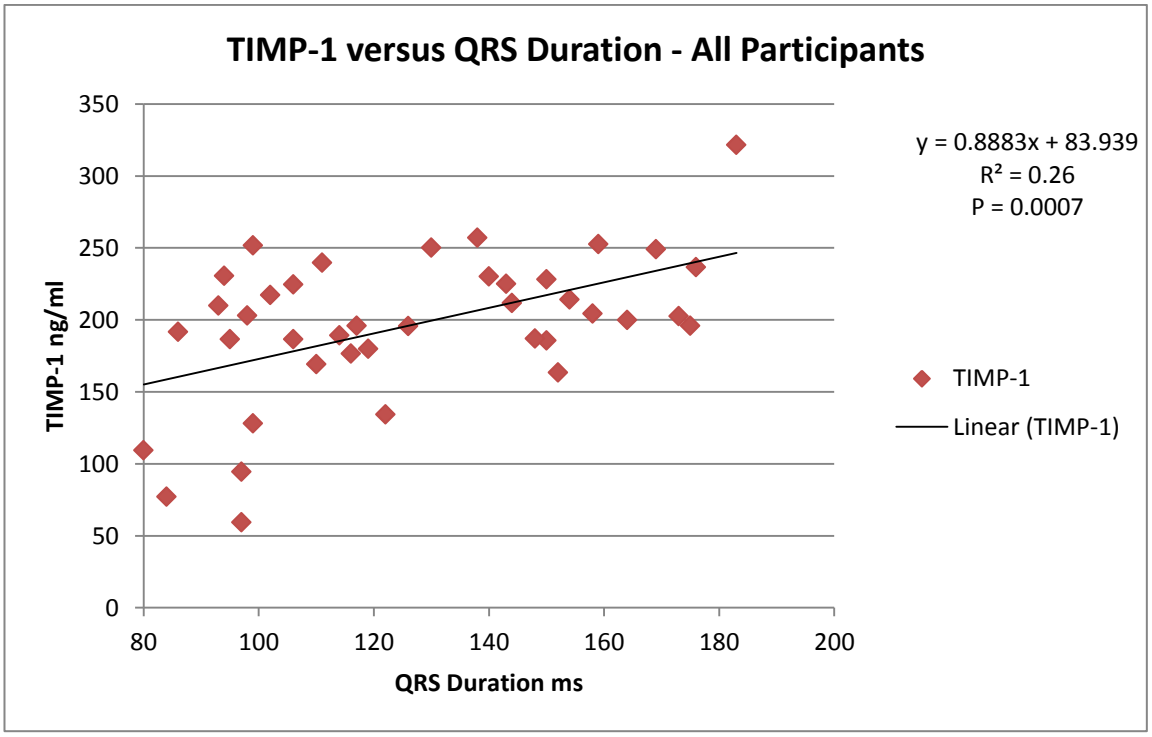


Figure 24: Linear regression model between TIMP-1 (y axis) and QRS Duration (x axis) with corresponding equation – All Participants.

TIMP-1 - Metalloproteinase-1, QRS – QRS duration in ms.

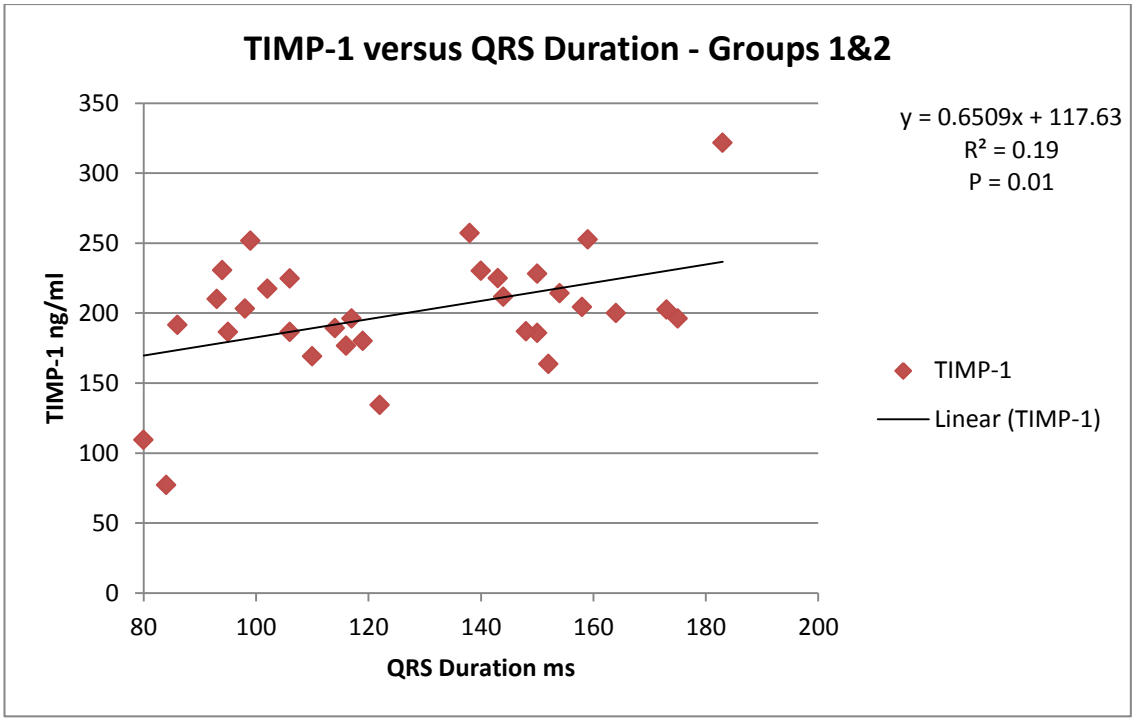


Figure 25: Linear regression model between TIMP-1 (y axis) and QRS Duration (x axis) with corresponding equation – Groups 1 & 2.

TIMP-1 - Metalloproteinase-1, QRS – qrs duration in milliseconds.

6.4.4 Amino-Terminal Propeptide of Type III Procollagen (PIIINP)

No significant difference was found on ANOVA testing between groups (p = 0.34). In contrast to HA and TIMP-1, no significant correlations were seen with the explanatory variables of interest on linear regression analysis, either when examined against all participants or for those with pulmonary regurgitation only.

6.5 Summary

The mean and median values of the ELF score were in the moderate fibrosis category in all study groups. No significant difference was found between study groups.

Linear regression in all participants showed that right ventricular ejection fraction was negatively correlated with the ELF score however this correlation was weak and not significant. The correlation strengthened and became significant when the analysis was limited to those in Groups 1 & 2 (pulmonary regurgitation).

The strongest correlation seen was with QRS duration – this relationship strengthened when the analysis was limited to just Groups 1 & 2 (pulmonary regurgitation). NT-Pro-BNP was also significantly correlated with the ELF score in Groups 1 & 2 only.

The components of the ELF score - hyaluronic acid (HA), metalloproteinase 1 (TIMP-1), and amino-terminal propeptide of type III procollagen (PIIINP) were also examined. HA was the dominant component of the three factors, showing complete consistency with the correlators of the ELF score. TIMP-1 only correlated significantly with QRS duration, whereas PIIINP did not correlate with any independent variable. The significant correlations of the components of the ELF score are shown in the Venn diagram (figure 26).

All models were associated with a low R^2 value (the highest being 26%) implying that despite identifying a significant predictor, the ‘fit’ of the regression equations were relatively poor.

Predictor Variables – Components of the ELF Score

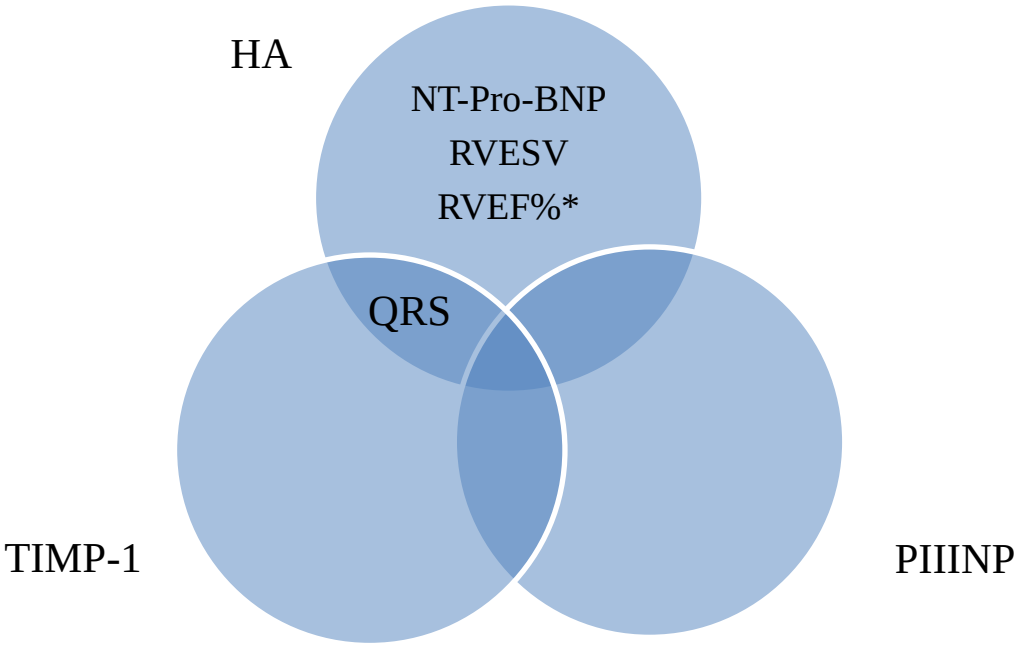


Figure 26: Venn diagram displaying significant predictor variables of components of ELF score (HA, TIMP-1, PIIINP) in Groups 1 & 2.

* Represents analysis of all participants rather than only those with pulmonary regurgitation.

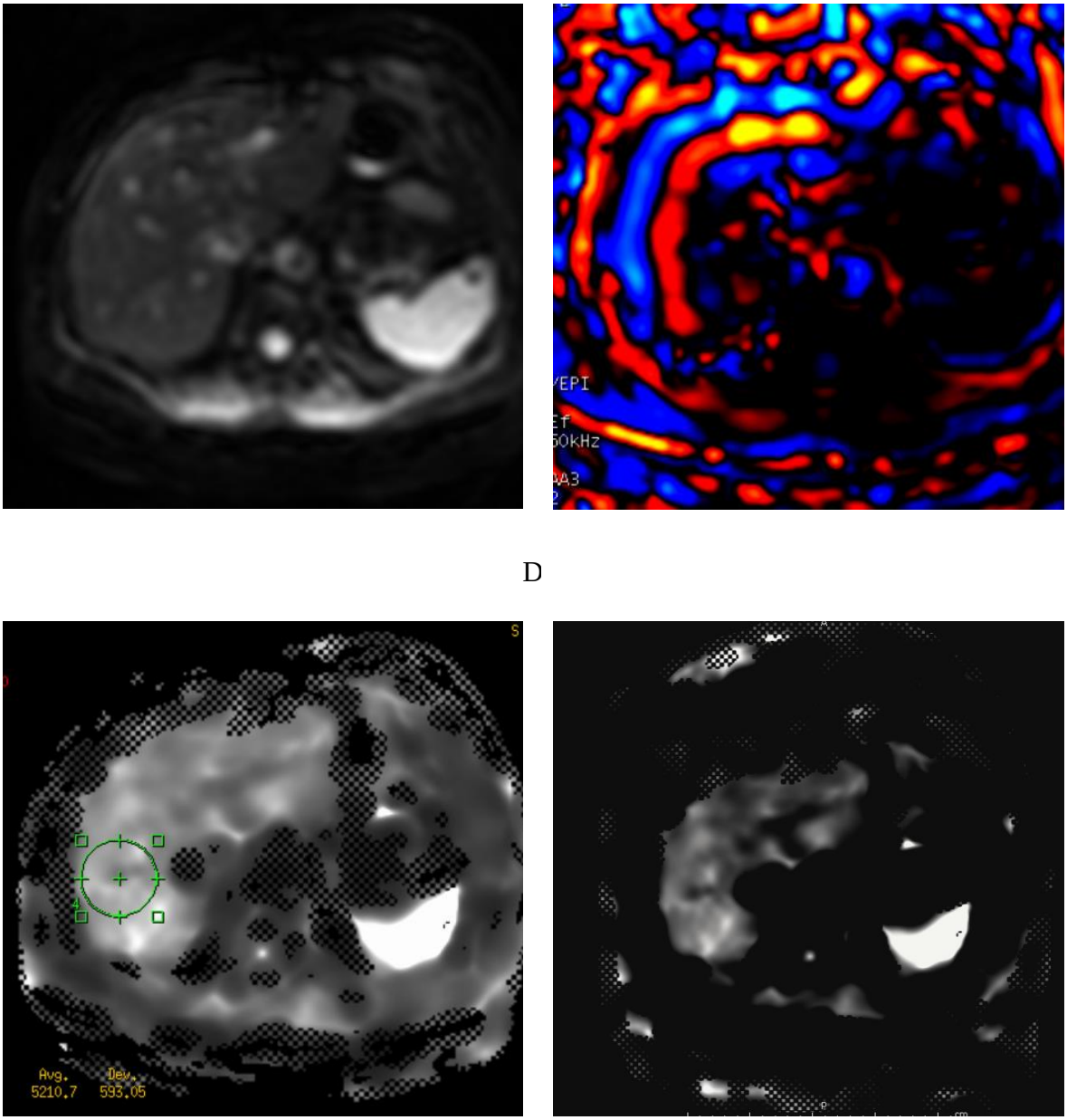
ELF – Enhanced liver fibrosis score, HA – Hyaluronic acid, TIMP-1 – Metalloproteinase-1, PIIINP - Amino-Terminal Propeptide of Type III Procollagen, QRS – qrs duration in milliseconds, NT-Pro-BNP – N-terminal prohormone of brain natriuretic peptide, RVESV – right ventricular end systolic volume ml/m^2. RVEF% - right ventricular ejection fraction.

7. Results III - Magnetic Resonance Elastography

7.1 Magnetic Resonance Elastography – Overview

One participant each from Group 1 and 2 were unable to have magnetic resonance elastography (MRE) due to safety concerns. One participant had a pulmonary artery stent, and another had an intrauterine contraceptive device. Both were contraindicated on the 3 tesla MRI scanner. This information came to light after having undergone all other investigations in the study protocol and therefore their inclusion in the study was maintained. The MRE value used for all other participants is the mean value of 4 elastogram slices through the liver, as reported by the study radiologist (PM). Normal values for MRE are considered to be 1.54 to 2.87 kPa. Although there were a few outliers, particularly in Groups 1 & 2 (pulmonary regurgitation), the mean and median values for all Groups lie within the normal range (Table 32). Figure 27 shows a series of images (A-D) of study ID 15, who had the highest reading (in the cirrhotic range) of 4.81 kPa.

A B



C D

Figure 27: Magnetic Resonance Elastography of the Liver of study ID 15 with high reading of 4.81 kPa. **A** – Source data and anatomical image. **B** – Wave propagation image. **C** – Elastogram plus mask image showing region of interest where measurement is taken (green crosshair). **D** – Elastogram screen save image.

7.2 Magnetic Resonance Elastography – ANOVA Testing

38 participants underwent magnetic resonance elastography (MRE) of the liver – 15 each from Groups 1 & 2 (pulmonary regurgitation), and 8 from Group 3 (Ebstein). No significant difference was found on ANOVA testing between groups with a p-value of 0.17, Table 32.

Group	Mean	SD	Min	Max	Med	CI	IQ	p-value
1 (Mod-Sev PR)	2.64	0.86	1.2	4.81	2.58	(2.20,3.07)	(2.17,2.89)	0.17
2 (Mild PR)	2.34	0.60	1.42	3.53	2.18	(2.03,2.65)	(1.94,2.73)	0.17
3 (Ebstein)	2.25	0.46	1.57	2.82	2.2	(1.93,2.57)	(1.92,2.68)	0.17

Table 32: MRE results across all study groups.

N = number, SD = standard deviation, Min = Minimum, Max = Maximum, Med = Median, CI = confidence interval, IQ = interquartile range.

7.3 Magnetic Resonance Elastography – Correlations and Linear Regression

Magnetic Resonance Elastography (MRE) values were used as the dependent variable in univariate linear regression, performed in all participants (Groups 1, 2 & 3) against variables shown in Table 33. A weak non-significant correlation was seen against right ventricular ejection fraction %. Figure 28 shows a scatter plot of right ventricular ejection fraction (%) against MRE.

MRE	r	R^2	Regression coefficient	95% CI	P-value
RVEF%	0.06	0.003	0.42	(-1.85,2.70)	0.70
RA	-0.02	0.0005	-0.002	(-0.03,0.03)	0.88
NT-Pro-BNP	0.19	0.03	0.006	(-0.0004,0.001)	0.24
Age	0.16	0.02	0.008	(-0.008,0.02)	0.31
BMI	0.003	0.00001	0.0004	(-0.04,0.04)	0.98

Table 33 – correlation coefficients for MRE versus postulated associated variables in all study groups.

r = pearson's correlation coefficient . R^2 = coefficient of determination. 95% CI – 95% confidence interval. P-value: $\alpha = 0.05$.

RVEF%: Right ventricular ejection fraction in percentage, RA: Right atrial size cm^2, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide (ng/L). Age in years. BMI – body mass index kg/m^2

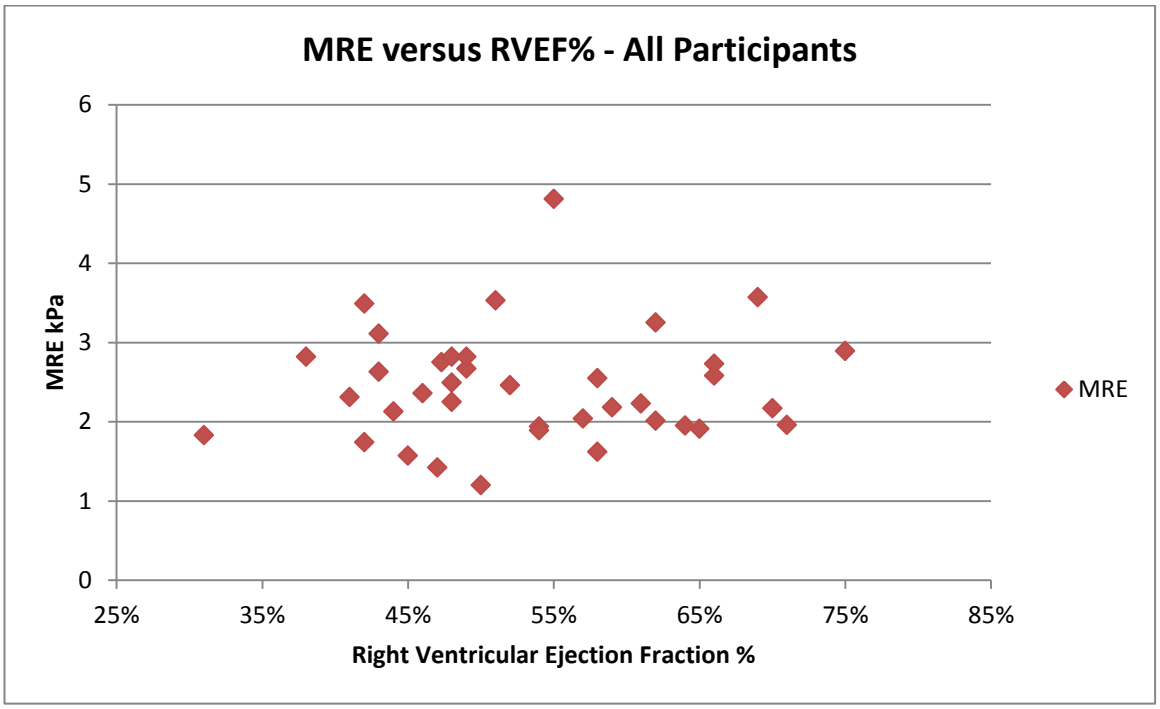


Figure 28: Scatter plot of right ventricular ejection fraction % versus MRE kPa.

MRE – Magnetic resonance elastography of the liver. RVEF% - right ventricular ejection fraction in percentage.

Linear regression was then performed with MRE as the dependent variable, and using prognostic markers in chronic pulmonary regurgitation, other CMR data, right ventricular systolic pressure (RVSP, estimated using the Bernoulli equation on transthoracic echocardiography), NT-Pro-BNP, and CPET data, for Groups 1 (Mod-Sev PR) and 2 (Mild PR) Table 34. As previously stated, RVSP could only be measured in 24/32 participants.

In keeping with the analysis of the ELF score, QRS duration also displayed a moderate and statistically significant correlation with MRE, shown in Figure 29. If all participants (rather than just Groups 1&2) are considered in the analysis, a correlation coefficient of 0.45 is seen with QRS duration, also statistically significant with p-value = 0.004, displayed in figure 30.

NT-Pro-BNP was also a significant predictor of MRE in Groups 1 & 2 (pulmonary regurgitation), correlation coefficient of 0.48 and p = 0.006, figure 31.

A moderate correlation (r = 0.42, p-value = 0.01) was also found against right ventricular end diastolic volume (figure 32). RVEDV is perhaps the most well established prognostic marker in patients with pulmonary regurgitation, and this is therefore a significant finding.

Right (figure 33) and left (figure 34) ventricular stroke volume measured on CMR were also significant correlates. In the analysis of CPET data, the O_2 Pulse had a significant negative correlation with MRE, r = -0.39, p = 0.03, figure 35. Oxygen pulse is the ratio of oxygen uptake to heart rate and is a surrogate for stroke volume.

MRE	r	R^2	Regression Coefficient	95% CI	P-Value
QRS	0.43	0.18	0.01	(0.002,0.01)	**0.017**
NT-Pro-BNP	0.48	0.23	0.002	(0.0007,0.003)	**0.006**
RVEDV	0.42	0.18	0.01	(0.002,0.02)	**0.01**
RVESV	0.26	0.07	0.01	(-0.004,0.02)	0.15
RVEF%	0.09	0.008	0.64	(-2.01,3.29)	0.62
RV SV	0.50	0.25	0.01	(0.005,0.02)	**0.004**
LVEDV	0.26	0.07	0.01	(-0.005,0.03)	0.15
LVESV	0.006	0.00004	0.0005	(-0.03,0.03)	0.97
LVEF%	0.11	0.01	0.79	(-1.77,3.37)	0.52
LV SV	0.38	0.14	0.01	(0.0009,0.02)	**0.03**
RVEDV:LVEDV	0.15	0.02	0.37	(-0.52,1.27)	0.39
PR%	0.13	0.01	0.006	(-0.01,0.02)	0.39
MVO2	-0.20	0.04	-0.01	(-0.05,0.01)	0.28
AT at VO_2/VO_2 %	-0.09	0.009	-0.50	(-2.56,1.54)	0.61
VE/VCO_2	0.06	0.004	0.04	(-0.07,0.10)	0.74
O_2 Pulse	-0.39	0.15	-0.08	(-0.15,-0.004)	**0.03**
RVSP	-0.41	0.17	-0.04	(-0.09,0.001)	0.06

Table 34 - Linear regression models & correlation coefficients for MRE in Groups 1 & 2.

r = pearson's correlation coefficient. R^2 = coefficient of determination. 95% CI – 95% confidence interval. P-value: $\alpha = 0.05$.

QRS: QRS duration on electrocardiogram in milliseconds, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide, RVEDV: right ventricular end diastolic volume ml/m^2, RVESV: right ventricular end systolic volume ml/m^2, RVEF%: right ventricular ejection fraction %, RV SV: right ventricular stroke volume ml, LVEDV: left ventricular end diastolic volume ml/m^2, LVESV: left ventricular end systolic volume ml/m^2, LVEF%: left ventricular ejection fraction %, LV SV: left ventricular stroke volume, PR%: pulmonary regurgitant fraction %, RVEDV:LVEDV: ratio of right ventricular end diastolic volume to left ventricular end diastolic volume, MVO_2: peak oxygen uptake ml/min/kg, AT at VO_2/VO_2 %: Point at which anaerobic threshold reached as a percentage of predicted MVO_2, VE/VCO_2 - minute ventilation to carbon dioxide production ratio. O_2 Pulse – VO_2/Heart Rate in ml/beat. RVSP: Right ventricular systolic pressure mmHg

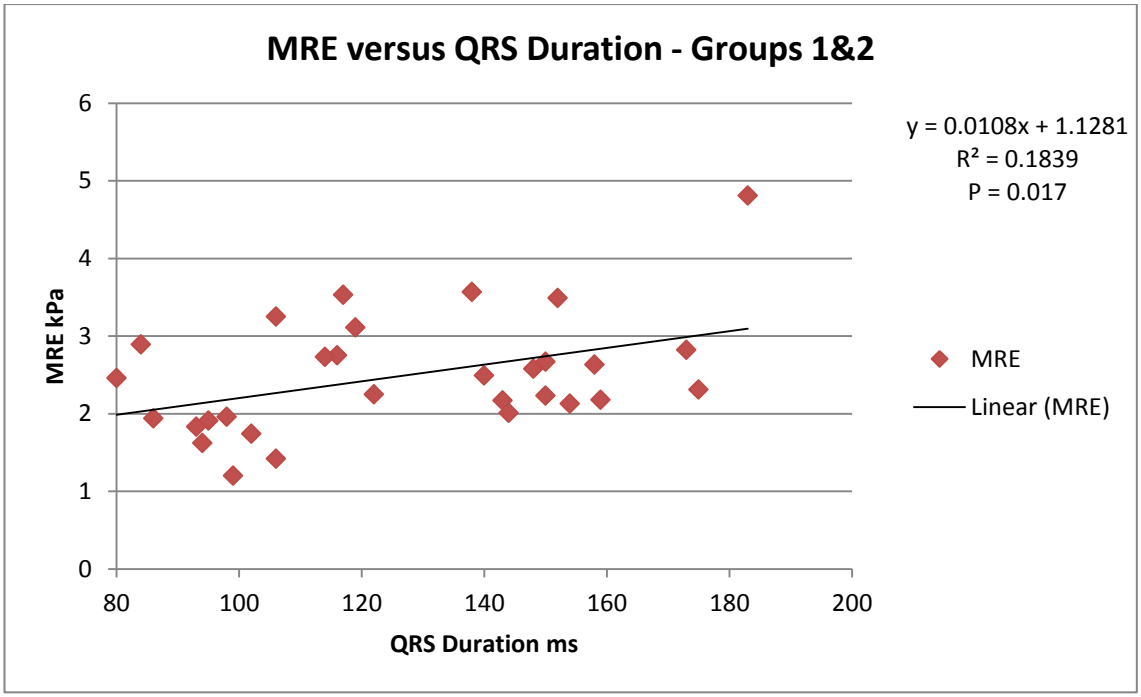


Figure 29: Linear regression model between MRE (y axis) and QRS duration (x axis) with corresponding equation – Groups 1&2.

MRE – Magnetic resonance elastography of liver in kPa. QRS – QRS duration in milliseconds.

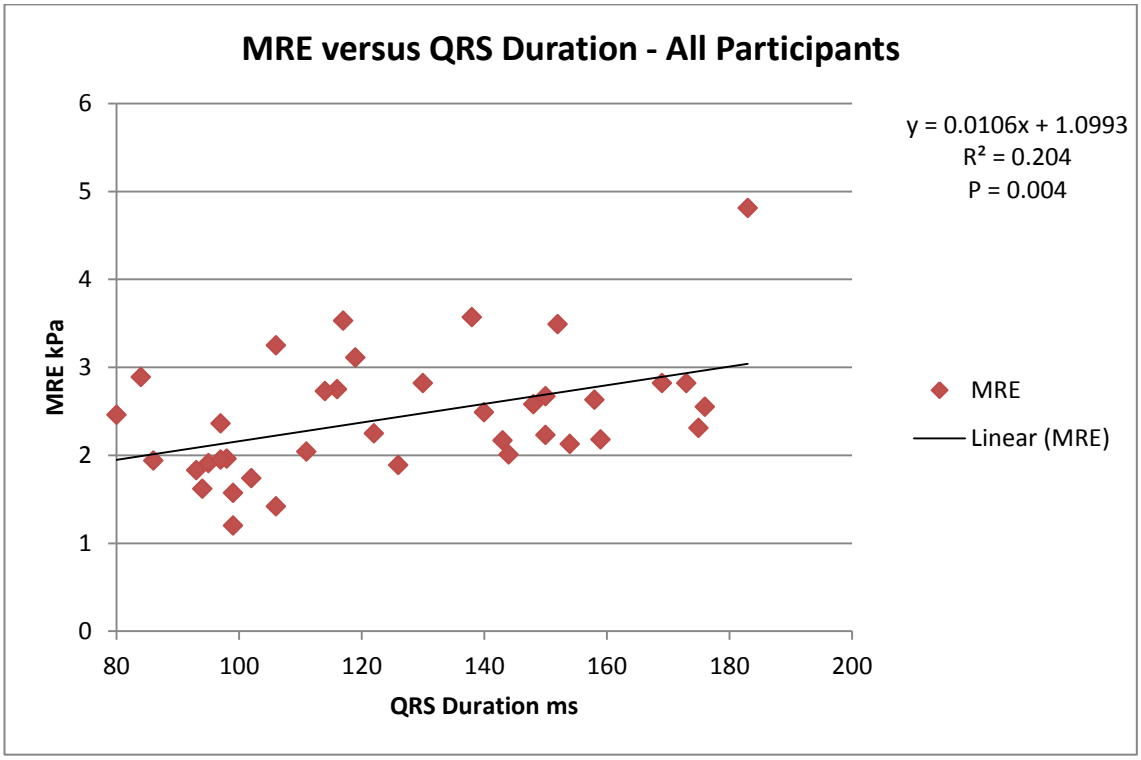


Figure 30: Linear regression model between MRE (y axis) and QRS duration (x axis) with corresponding equation – All Participants.

MRE – Magnetic resonance elastography of liver in kPa. QRS – QRS duration in milliseconds.

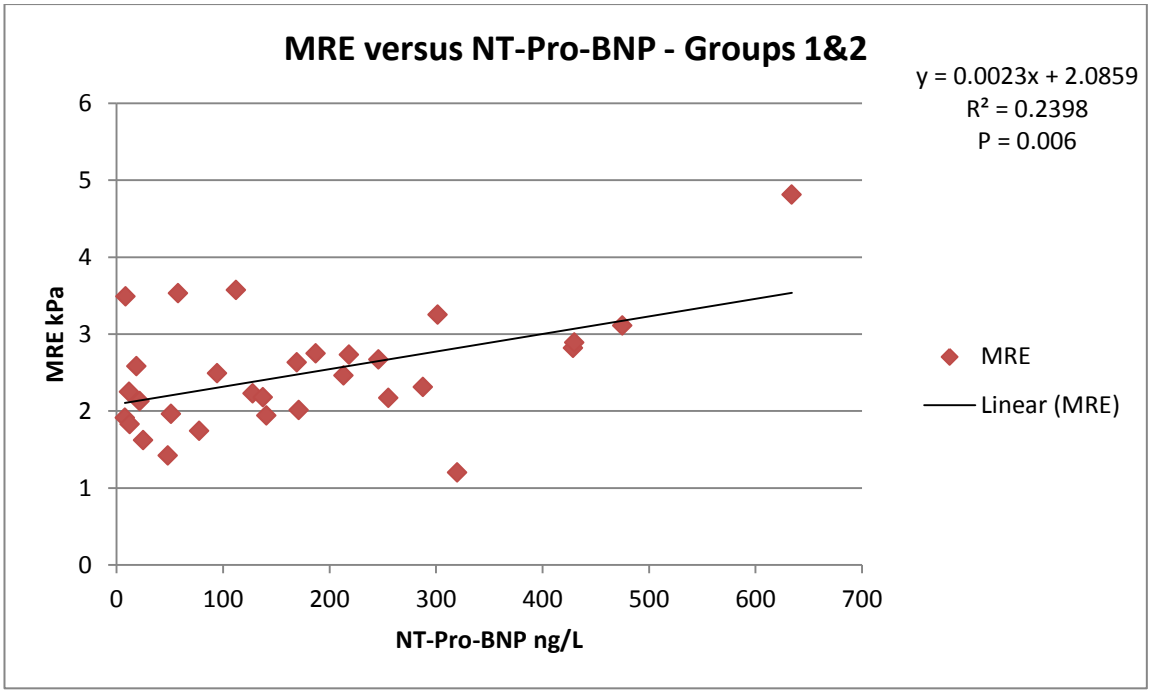


Figure 31: Linear regression model between MRE (y axis) and NT-Pro-BNP (x axis) with corresponding equation – Groups 1&2

MRE – Magnetic resonance elastography of liver in kPa, NT-Pro-BNP – N-terminal prohormone of brain natriuretic peptide ng/L.

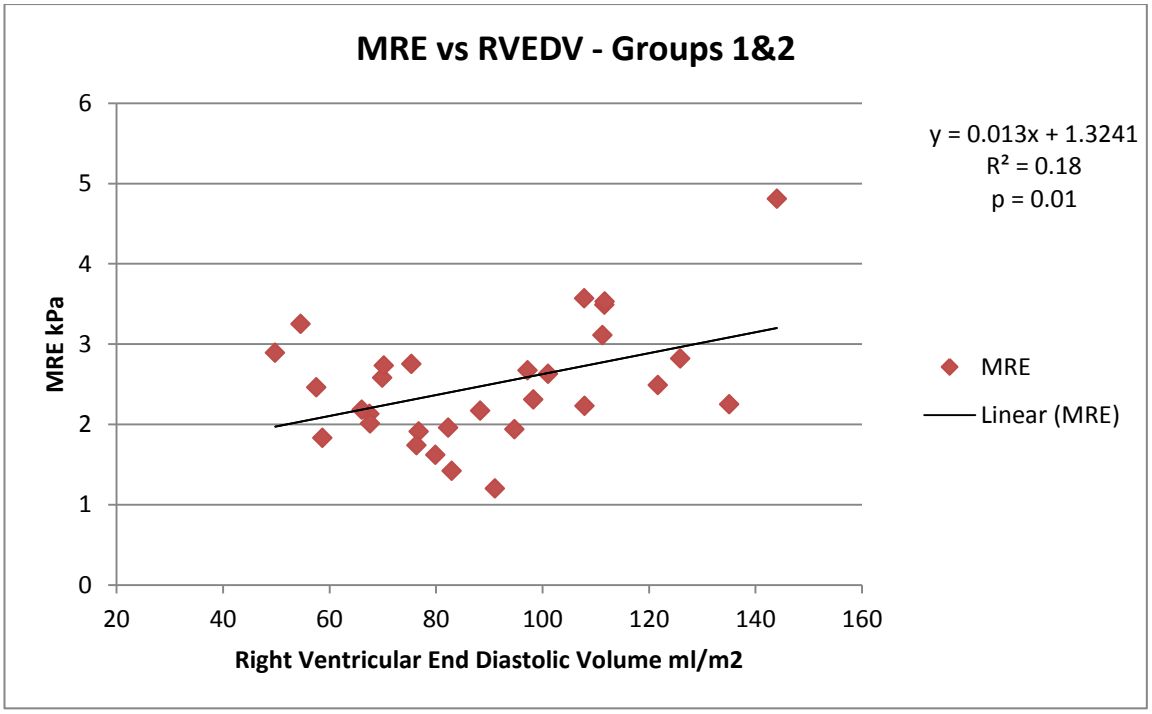


Figure 32: Linear regression model between MRE (y axis) and RVEDV (x axis) with corresponding equation – Groups 1&2

MRE – Magnetic resonance elastography of liver in kPa. RVEDV – Right ventricular end diastolic volume ml/m^2.

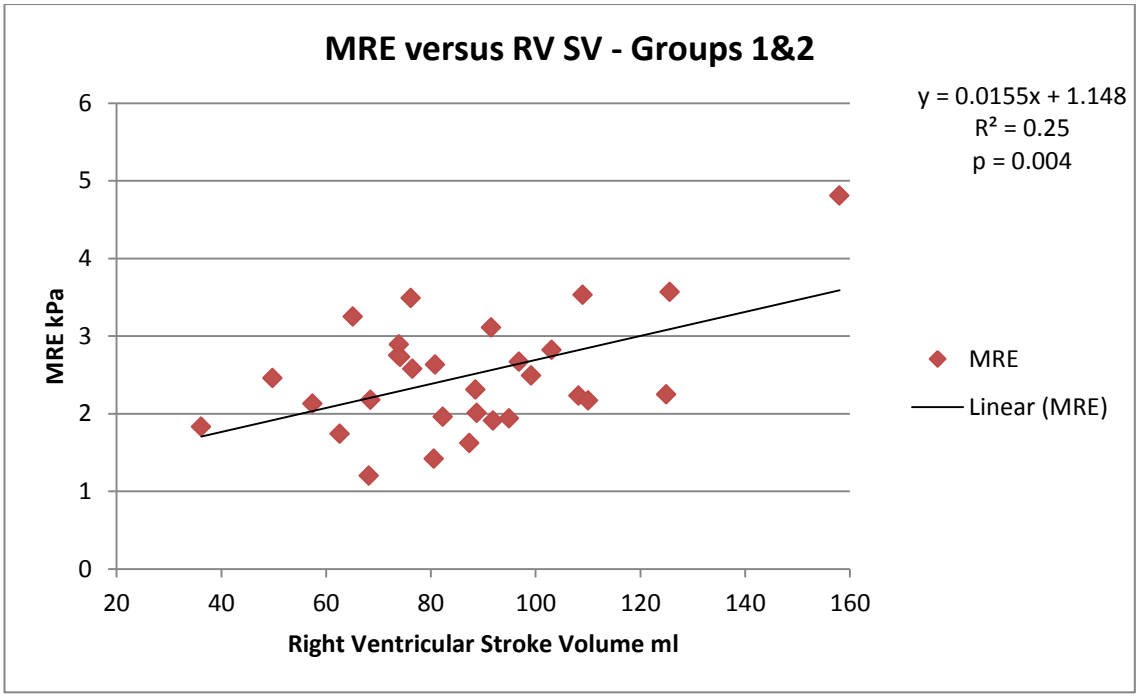


Figure 33: Linear regression model between MRE (y axis) and RV SV (x axis) with corresponding equation – Groups 1&2

MRE – Magnetic resonance elastography of liver in kPa. RV SV – right ventricular stroke volume ml.

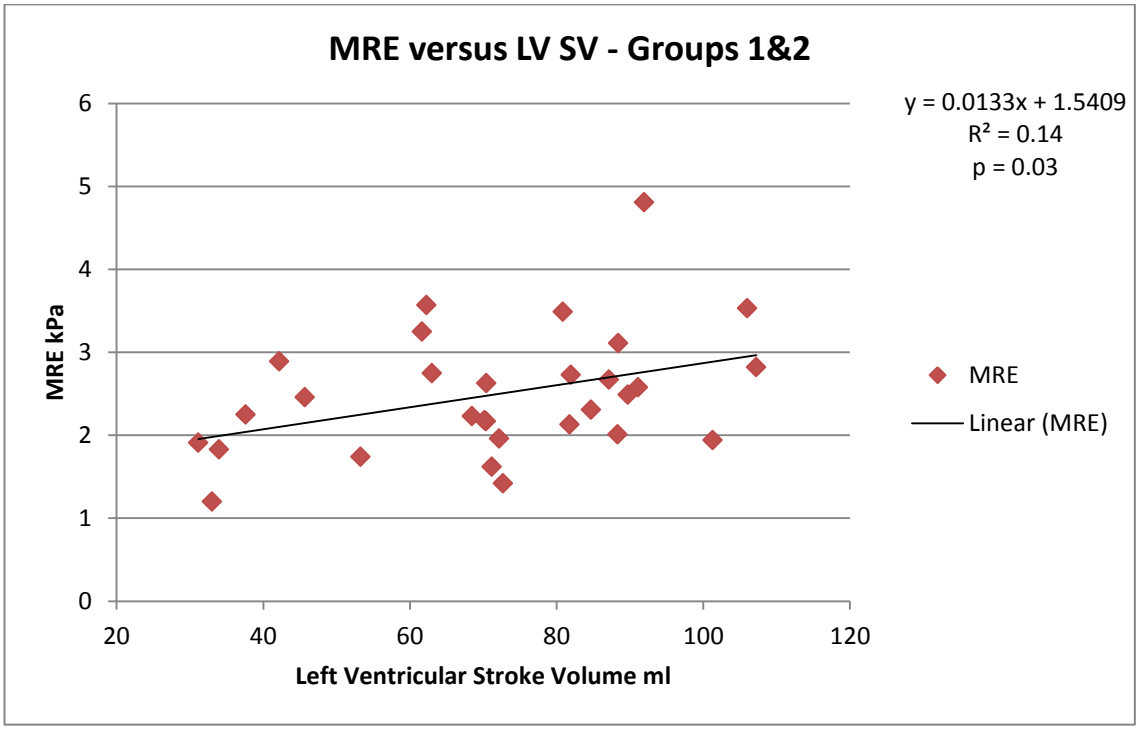


Figure 34: Linear regression model between MRE (y axis) and LV SV (x axis) with corresponding equation – Groups 1&2

MRE – Magnetic resonance elastography of liver in kPa. LV SV – left ventricular stroke volume ml.

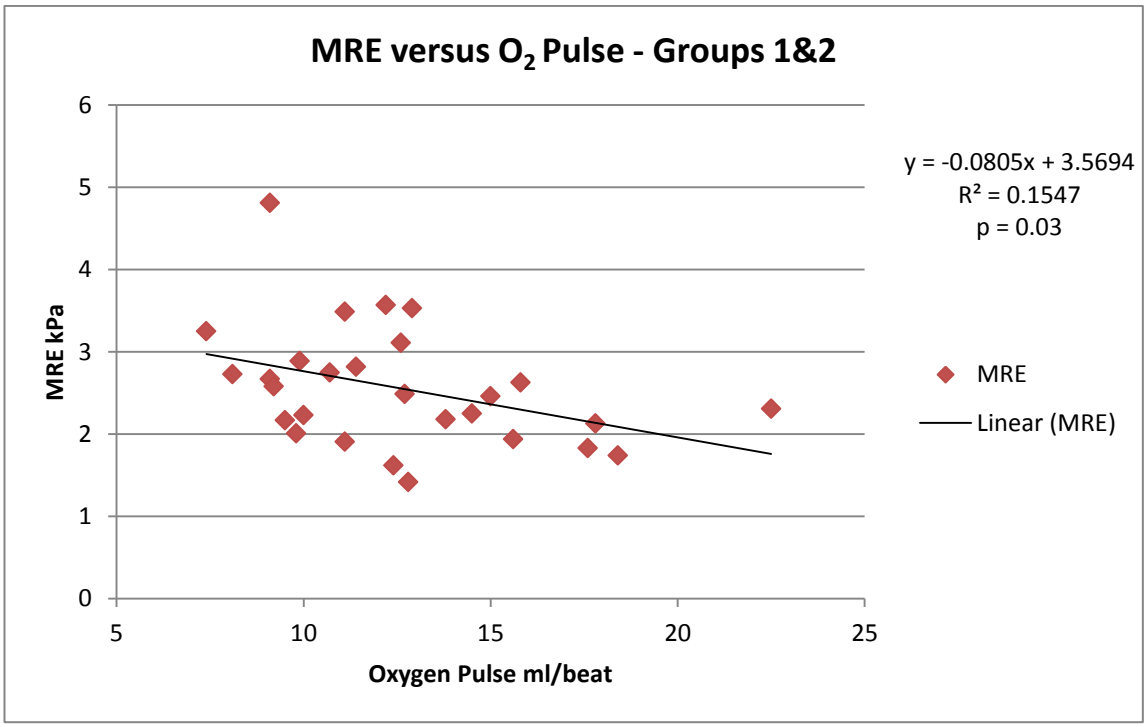


Figure 35: Linear regression model between MRE (y axis) and O_2 Pulse (x axis) with corresponding equation – Groups 1&2

MRE – Magnetic resonance elastography of liver in kPa. O_2 Pulse – Oxygen Pulse in ml/beat.

7.4 Summary

The mean and median values for Magnetic Resonance Elastography (MRE) lay within the normal range. There was no significant difference between study groups.

There was a negative but insignificant correlation between MRE and right ventricular ejection fraction on linear regression analysis. In the analysis of MRE versus volume measurements of the right ventricle, RVEDV was a significant correlate. Right and left ventricular stroke volumes were also both significantly correlated. The O_2 pulse was the only significant CPET parameter associated with MRE, displaying a negative correlation.

QRS duration was a significant predictor in the analysis involving all participants, and in those with pulmonary regurgitation only (a similar pattern seen in the ELF score).

Another correlator shared with the ELF score, was NT-Pro-BNP, displaying a significant relationship with MRE.

8. Results IV – Fibroscan

8.1 Fibroscan – Overview

The Fibroscan value used in the analysis is the median of 10 readings taken during the investigation. A Fibroscan session and its subsequent result is considered valid if all of the following criteria are met: 1) a number of valid 'shots' of at least 10, 2) a success rate (the ratio of valid shots to the total number of shots) above 60%; and 3) an interquartile range (IQR, reflecting the variability of measurements) less than 30% of the median measurements value (IQR/M ≤ 30%). Three Participants (two from Group 1, one from Group 2) failed the above criteria and therefore had invalid readings. They have been excluded from the following analysis.

8.2 Fibroscan - ANOVA Testing

Considering all study groups, the mean and median values for Fibroscan lie within the normal range (table 35). In people without liver disease, a Fibroscan result of <7kPa is considered normal.

ANOVA testing showed no significant difference between study groups, $p = 0.97$.

Group	Mean	SD	Min	Max	Median	CI	IQ	p-value
1 (Mod-Sev PR)	5.81	2.748	2.9	13.1	4.85	(4.46,7.15)	(3.85,6.90)	0.97
2 (Mild PR)	4.75	1.265	2.4	6.6	4.85	(4.13,5.37)	(3.85,5.65)	0.97
3 (Ebstein)	6.19	2.691	3.6	11.6	5.75	(4.32,8.05)	(4.00,7.40)	0.97

Table 35: Fibroscan results across all study groups.

N = number, SD = standard deviation, Min = Minimum, Max = Maximum, CI = confidence interval, IQ = interquartile range.

8.3 Fibroscan – Correlations and Linear Regression

Linear regression was performed in two phases. Initially, correlation coefficients were calculated between Fibroscan values across all Groups (1, 2 & 3) and pre-determined variables of interest, Table 36. Right atrial size (cm^2) measured on echocardiography had a moderate significant correlation with Fibroscan, p = 0.04, figure 36. Age was also a significant predictor of Fibroscan with p= 0.03, figure 37.

Fibroscan	r	R^2	Regression Coefficient	95% CI	p-value
RVEF%	0.29	0.08	6.52	(-0.85,13.90)	0.08
RA	0.33	0.10	0.11	(0.002,0.221)	**0.04**
NT-Pro-BNP	0.16	0.02	0.001	(-0.002,0.005)	0.33
Age	0.34	0.11	0.05	(0.003,0.106)	**0.03**
BMI	-0.005	0.00002	-0.002	(-0.16,0.16)	0.97

Table 36: Linear regression models & correlation coefficients for Fibroscan in All Participants

r = pearson's correlation coefficient . R^2 = coefficient of determination. 95% CI – 95% confidence interval. P-value: α = 0.05.

RVEF% : Right ventricular ejection fraction in percentage, RA: Right atrial size cm^2, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide. Age in years. BMI – body mass index kg/m^2

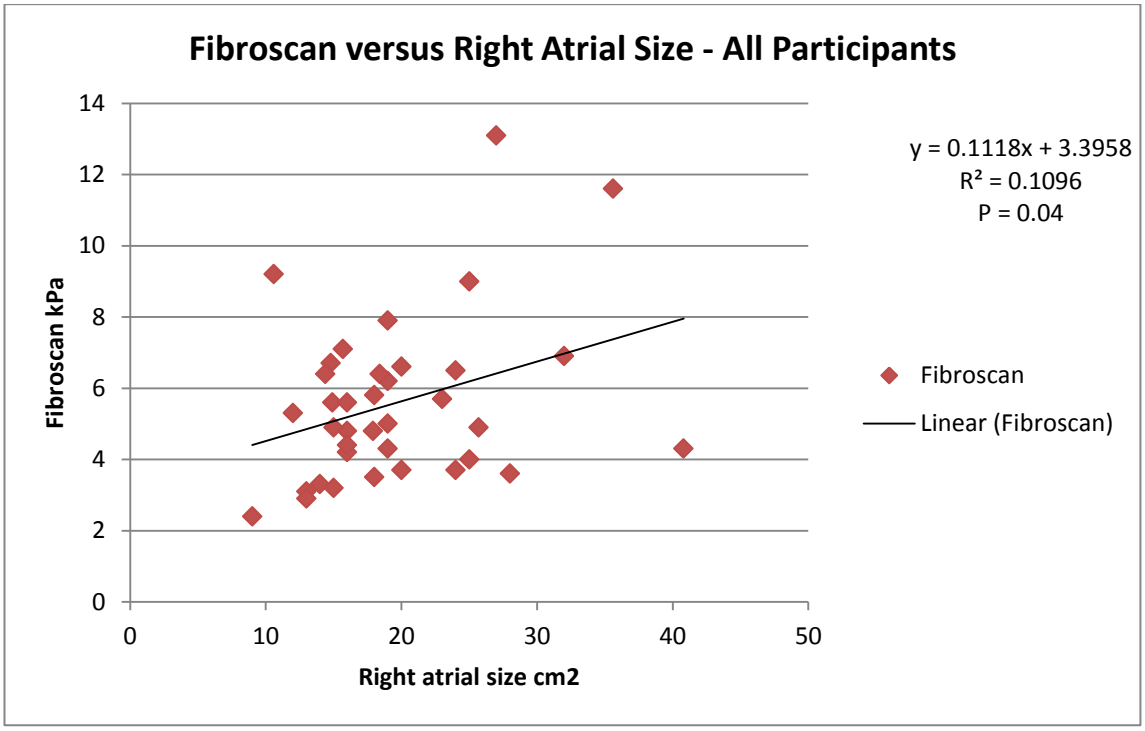


Figure 36 – Linear regression model between Fibroscan (y axis) and right atrial size (x axis) with corresponding equation – All Participants.

RA – right atrial size in cm^2.

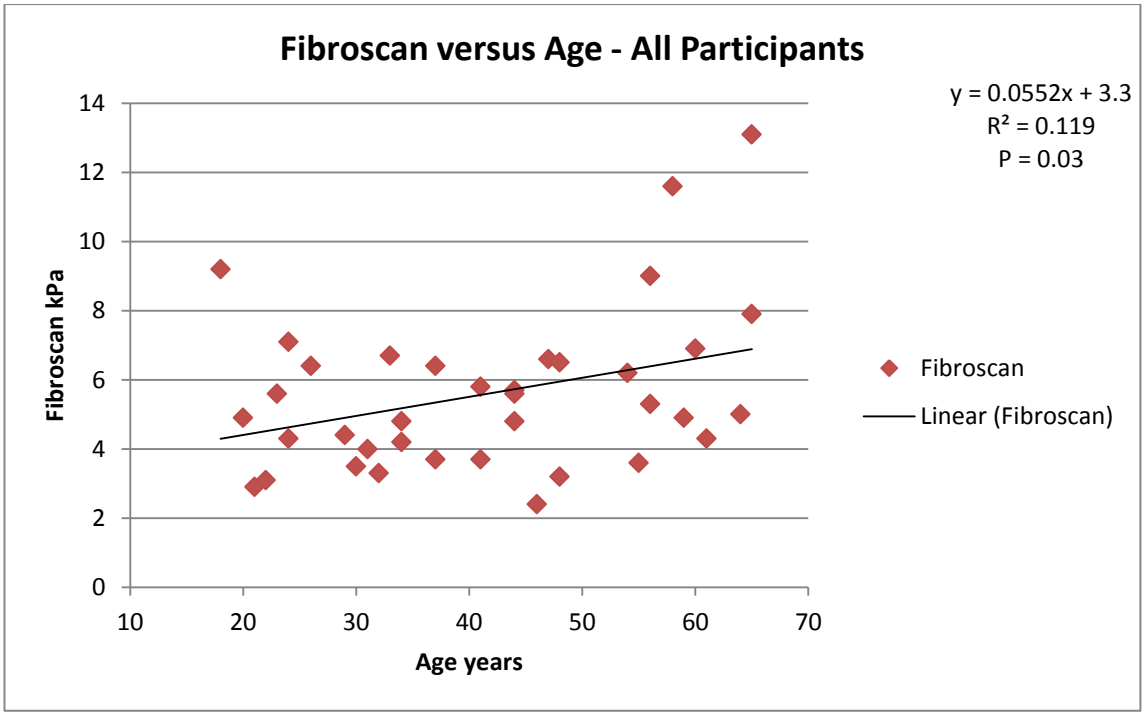


Figure 37: Linear regression model between Fibroscan (y axis) and Age (x axis) with corresponding equation – All Participants.

Age in years.

Univariate linear regression was next performed for participants from Groups 1 & 2 using prognostic markers in pulmonary regurgitation, other CMR data, right ventricular systolic pressure (RVSP, estimated using the Bernoulli equation on transthoracic echocardiography), NT-Pro-BNP, and CPET data, Table 37. As previously stated, only 24/32 participants had measurable RVSP.

Only NT-Pro-BNP was a significant predictor with a moderate positive correlation of 0.46 and p-value of 0.01, figure 38.

Fibroscan	**r**	**R^2**	**Regression Coefficient**	**95% CI**	**p-value**
QRS	0.08	0.007	0.006	(-0.02,0.03)	0.65
NT-Pro-BNP	0.46	0.22	0.006	(0.001,0.011)	**0.01**
RVEDV	-0.08	0.007	-0.007	(-0.04,0.02)	0.64
RVESV	-0.16	0.02	-0.02	(-0.07,0.03)	0.38
RVEF%	0.30	0.09	6.21	(-1.55,13.99)	0.11
RV SV	0.11	0.01	0.01	(-0.02,0.04)	0.55
LVEDV	-0.11	0.01	-0.01	(-0.07,0.03)	0.54
LVESV	-0.15	0.02	-0.03	(-0.12,0.05)	0.41
LVEF%	0.002	0.00008	0.05	(-7.61,7.73)	0.98
LV SV	0.08	0.007	-0.008	(-0.03,0.04)	0.65
RVEDV:LVEDV	-0.04	0.001	-0.28	(-2.98,2.42)	0.83
PR%	0.04	0.002	0.006	(-0.04,0.06)	0.81
MVO2	-0.21	0.04	-0.05	(-0.17,0.05)	0.28
AT at VO_2/VO_2 %	0.03	0.001	0.54	(-5.98,7.07)	0.86
VE/VCO_2	0.10	0.01	0.07	(-0.21,0.35)	0.61
O_2 Pulse	-0.25	0.06	-0.16	(-0.41,0.08)	0.19
RVSP	0.35	0.12	0.10	(-0.02,0.23)	0.10

Table 37: Linear regression models & correlation coefficients for Fibroscan in Groups 1 & 2.

r = pearson's correlation coefficient. R^2 = coefficient of determination. 95% CI = 95% confidence interval. P-value: α = 0.05.

QRS: QRS duration on electrocardiogram in milliseconds, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide, RVEDV: right ventricular end diastolic volume ml/m^2, RVESV: right ventricular end systolic volume ml/m^2, RVEF%: right ventricular ejection fraction %, RV SV: right ventricular stroke volume ml, LVEDV: left ventricular end diastolic volume ml/m^2, LVESV: left ventricular end systolic volume ml/m^2, LVEF%: left ventricular ejection fraction %, LV SV: left ventricular stroke volume, PR%: pulmonary regurgitant fraction %, RVEDV:LVEDV: ratio of right ventricular end diastolic volume to left ventricular end diastolic volume, MVO_2: peak oxygen uptake ml/min/kg, AT at VO_2/VO_2 %: Point at which anaerobic threshold reached as a percentage of predicted MVO_2, VE/VCO_2 - minute ventilation to carbon dioxide production ratio. O_2 Pulse – VO_2/Heart Rate in ml/beat. RVSP: Right ventricular systolic pressure mmHg.

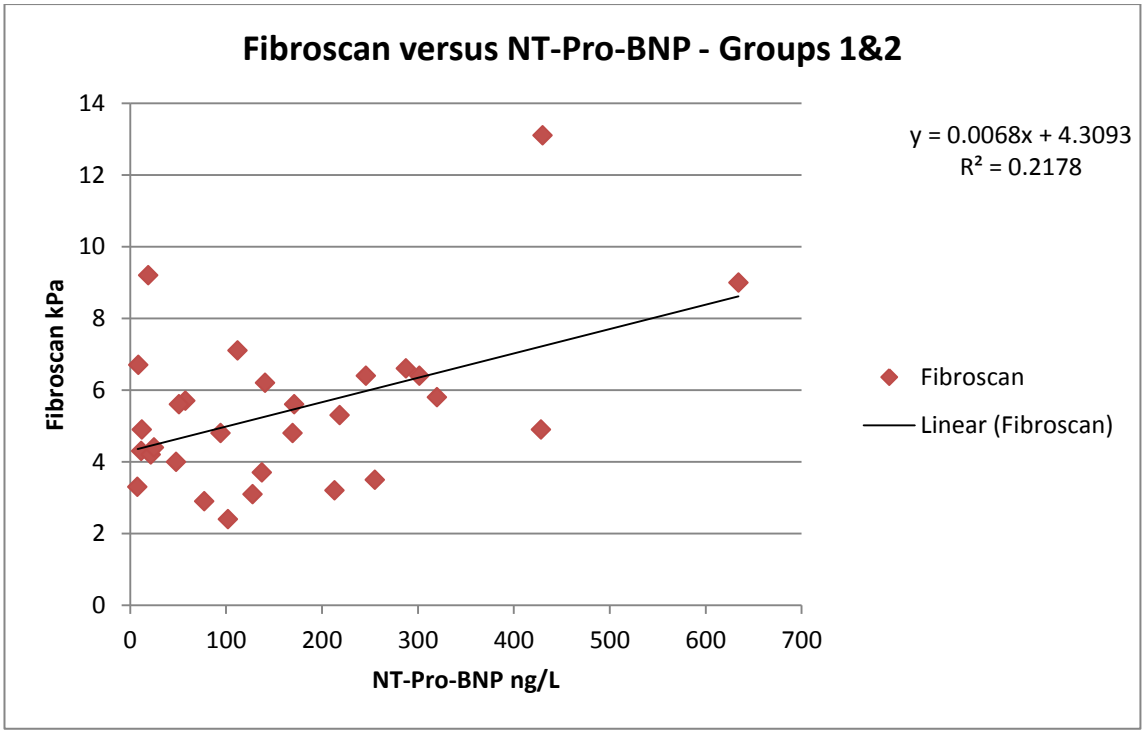


Figure 38: Linear regression model between Fibroscan (y axis) and NT-Pro-BNP (x axis) with corresponding equation – Groups 1&2.

NT-Pro-BNP – N-terminal prohormone of brain natriuretic peptide.

8.4 Summary

Fibroscan mean and median values fell within the normal range with no significant difference seen between study groups. Right atrial size and Age were significantly correlated with Fibroscan values in all study groups.

NT-Pro-BNP was a significant predictor for Fibroscan - the only common predictor between all three measures of hepatic stiffness.

The significant predictor variables associated with all three modalities are displayed as Venn diagrams for all participants (figure 39) and for participants in Groups 1 & 2 (figure 40).

All Participants

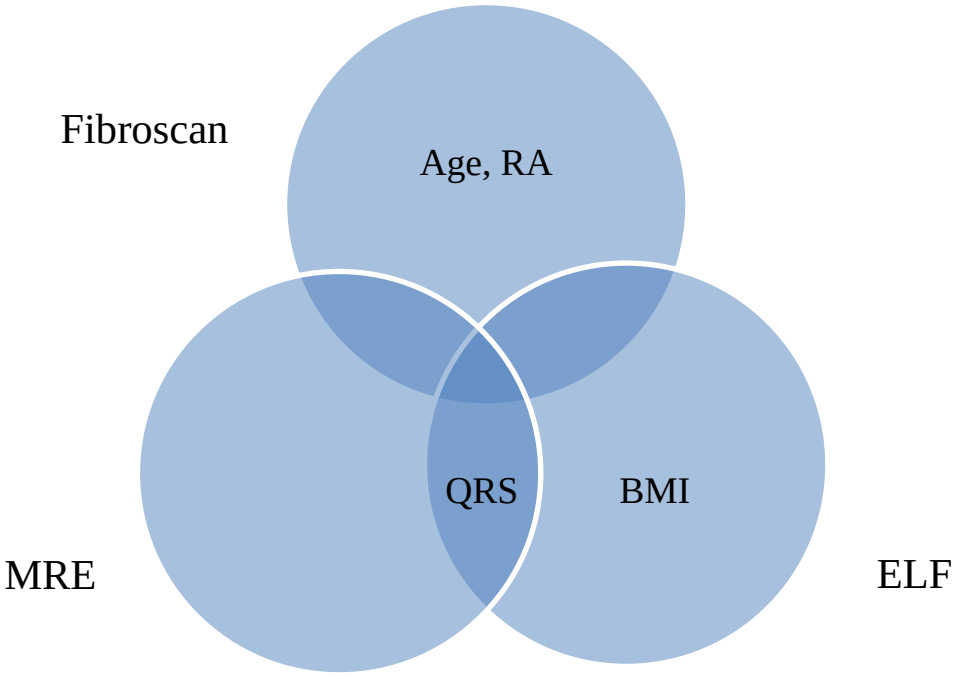


Figure 39: Venn diagram displaying significant predictor variables for all modalities (Fibroscan, MRE and ELF) in all participants.

MRE – Magnetic resonance elastography of liver, ELF – enhanced liver fibrosis score, Age in years, RA – right atrial size on echocardiography cm^2, QRS – qrs duration in milliseconds, BMI – Body mass index kg/m^2

Groups 1 & 2

Pulmonary Regurgitation

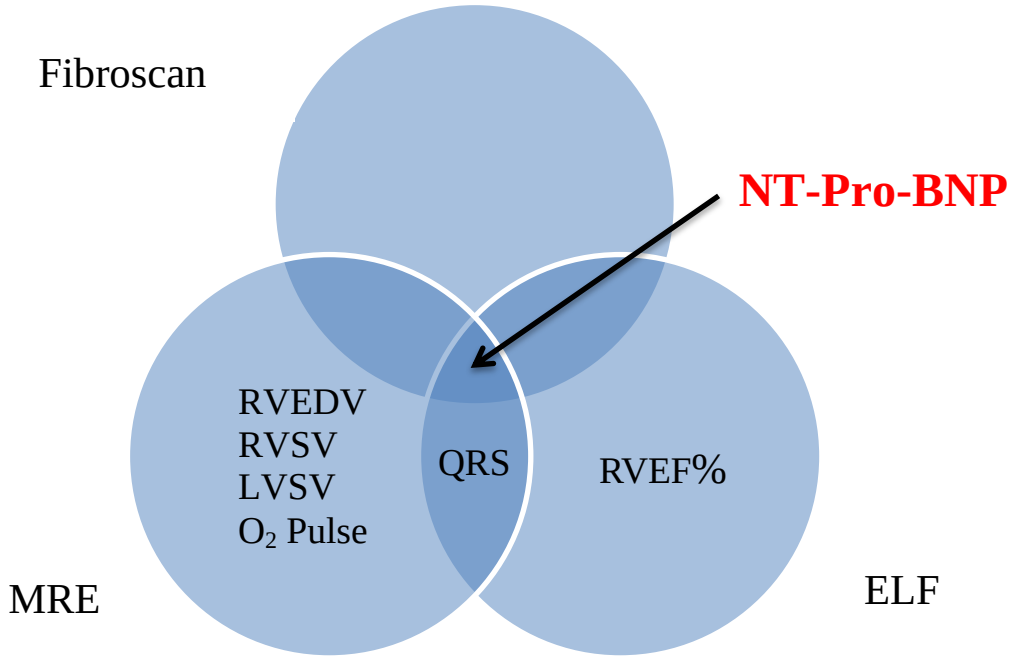


Figure 40: Venn diagram displaying significant predictor variables for all modalities (Fibroscan, MRE and ELF) in Groups 1 & 2.

MRE – Magnetic resonance elastography of liver, ELF – enhanced liver fibrosis score, RVEDV – right ventricular end diastolic volume ml/m^2, RVSV – right ventricular stroke volume ml, LVSV – left ventricular stroke volume ml, O_2 Pulse – VO_2/Heart Rate in ml/beat, QRS – qrs duration in milliseconds, RVEF%- right ventricular ejection fraction %, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide.

9. Results V – Additional Analyses

9.1 Correlation Between Primary Hepatic Outcomes

The correlation between the three primary hepatic outcomes is shown in Table 38. Only Fibroscan and MRE were significantly correlated (linear regression) with a correlation coefficient of 0.37, p-value 0.02.

Correlation Coefficients	**Fibroscan**	**MRE**	**ELF**
Fibroscan		r = 0.37 **p-value = 0.02**	r = - 0.01 p-value = 0.92
MRE	r = 0.37 **p-value = 0.02**		r = 0.16 p-value = 0.32
ELF	r = - 0.01 p-value = 0.92	r = 0.16 p-value = 0.32	

Table 38: Correlation coefficients between primary hepatic outcomes

MRE – Magnetic resonance elastography, ELF – enhanced liver fibrosis score, r – pearson's correlation coefficient.

9.2 N-Terminal Prohormone of Brain Natriuretic Peptide

In this study, N-terminal prohormone of brain natriuretic peptide (NT-Pro-BNP) was performed as part of a panel of tests in an exploratory manner. In those with pulmonary regurgitation, it was found to be the only common significant predictor of all primary hepatic outcomes. Further exploratory analysis was therefore performed between NT-Pro-BNP and other cardiovascular parameters in Groups 1 (Mod-Sev PR) and 2 (Mild PR), table 39. One participant in Group 2 (Mild PR) was in atrial fibrillation and has been excluded from the following analysis. The rationale for including this participant in all prior, was that arrhythmia did not form part of the exclusion criteria.

NT-Pro-BNP had a significant negative correlation with MVO2 (peak oxygen uptake, figure 41). This relationship is in keeping with a patient whose clinical condition is deteriorating. Age is also likely to play a contributory role.

Right ventricular systolic pressure (RVSP, measurable in 23/31 participants – excluding the omitted participant with atrial fibrillation) was a significant predictor of NT-Pro-BNP (figure 42).

NT-Pro-BNP	r	R^2	Regression Coefficient	95% CI	p-value
QRS	0.27	0.07	1.30	(-0.49,3.10)	0.15
RVEDV	0.15	0.02	0.92	(-1.33,3.17)	0.40
RVESV	0.04	0.001	0.40	(-3.16,3.98)	0.81
RVEF%	0.18	0.03	249.58	(-260.59,759.77)	0.32
RV SV	0.31	0.09	1.88	(-0.28,4.06)	0.08
LVEDV	0.04	0.001	0.38	(-3.12,3.90)	0.82
LVESV	-0.10	0.01	-1.62	(-7.70,4.46)	0.58
LVEF%	-0.03	0.001	-46.14	(-542.05,449.76)	0.85
LV SV	0.09	0.009	0.63	(-1.85,3.12)	0.60
RVEDV:LVEDV	0.05	0.003	25.92	(-147.51,199.36)	0.76
PR%	0.05	0.003	56.36	(-316.93,429.66)	0.75
MVO2	-0.55	0.30	-9.86	(-15.74,-3.97)	**0.002**
AT at VO_2/VO_2 %	0.05	0.002	56.80	(-357.61,471.22)	0.78
VE/VCO_2	0.17	0.03	7.68	(-8.99,24.36)	0.35
O_2 Pulse	-0.27	0.07	-11.88	(-28.04,4.28)	0.14
RVSP	0.42	0.17	9.17	(0.08,18.26)	**0.04**

Table 39: Linear regression models & correlation coefficients for NT-Pro-BNP in Groups 1 & 2.

QRS: QRS duration on electrocardiogram in milliseconds, NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide, RVEDV: right ventricular end diastolic volume ml/m^2, RVESV: right ventricular end systolic volume ml/m^2, RVEF%: right ventricular ejection fraction %, RV SV: right ventricular stroke volume ml, LVEDV: left ventricular end diastolic volume ml/m^2, LVESV: left ventricular end systolic volume ml/m^2, LVEF%: left ventricular ejection fraction %, LV SV: left ventricular stroke volume, PR%: pulmonary regurgitant fraction %, RVEDV:LVEDV: ratio of right ventricular end diastolic volume to left ventricular end diastolic volume, MVO_2: peak oxygen uptake ml/min/kg, AT at VO_2/VO_2 %: Point at which anaerobic threshold reached as a percentage of predicted MVO_2, VE/VCO_2 - minute ventilation to carbon dioxide production ratio. O_2 Pulse – VO_2/Heart Rate in ml/beat. RVSP: Right ventricular systolic pressure mmHg.

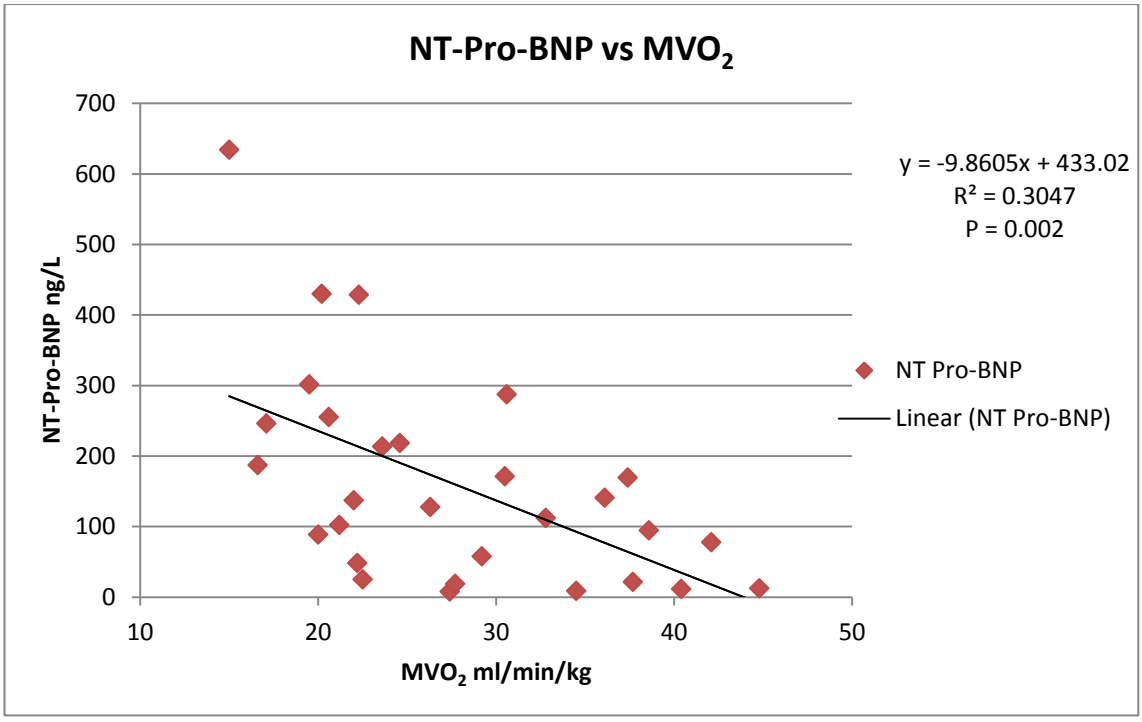


Figure 41 - Linear regression model between NT-Pro-BNP (y axis) and MVO_2 (x axis) with corresponding equation – Groups 1&2

NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide. MVO_2: peak oxygen uptake ml/min/kg.

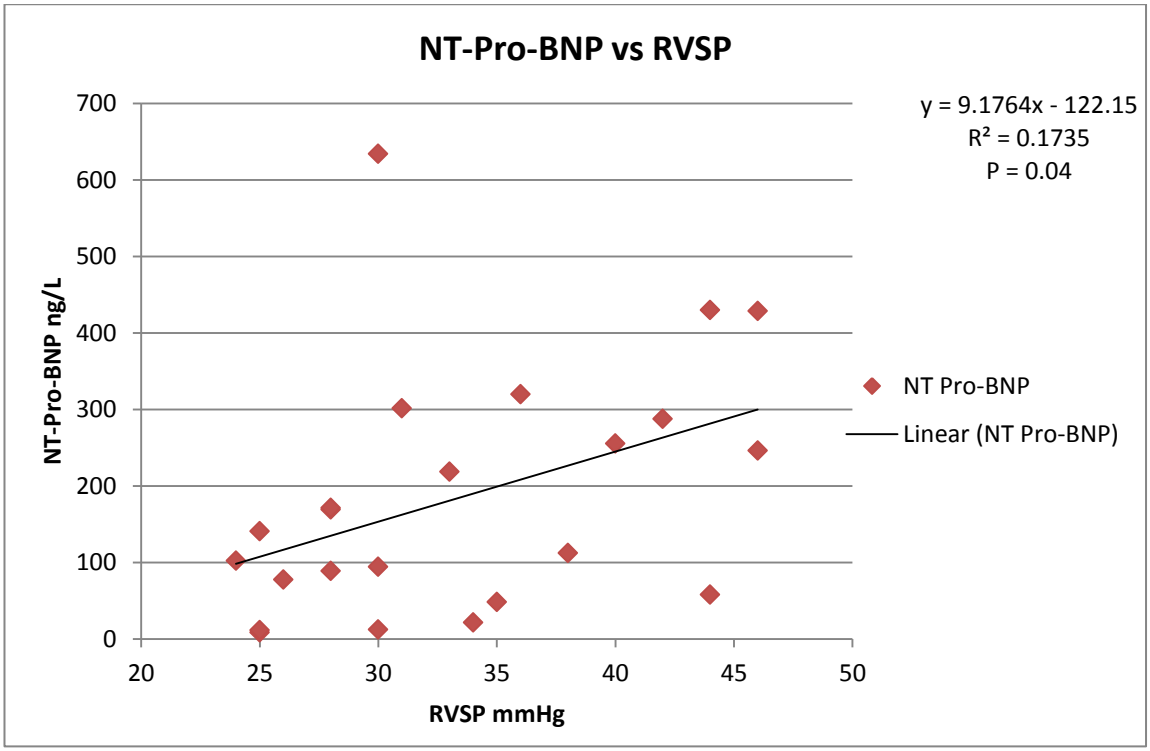


Figure 42 - Linear regression model between NT-Pro-BNP (y axis) and RVSP (x axis) with corresponding equation – Groups 1&2

NT-Pro-BNP: N-terminal prohormone of brain natriuretic peptide. RVSP: Right ventricular systolic pressure mmHg

9.3 Summary

In the analysis comparing the hepatic outcome modalities to each other, in all study participants, only Fibroscan and MRE were significantly correlated. Correlation coefficient of 0.37, p-value 0.02.

In the additional analysis exploring explanatory variables associated with NT-Pro-BNP, two significant relationships were detected. Peak oxygen uptake (MVO2) had a negative correlation, and right ventricular systolic pressure had a positive correlation.

10. Discussion

10.1 Study Overview

This study was designed as an observational cross-sectional study. The primary study population were those with chronic pulmonary regurgitation secondary to repair of Fallot's Tetralogy or intervention to treat pulmonary stenosis. Participants with tricuspid regurgitation secondary to Ebstein's anomaly were chosen as a comparator group. Timing of pulmonary valve replacement in adults with pulmonary regurgitation is a dynamic field with clinical practice and guidelines in constant flux. Currently, consideration is given to the presence of symptoms, arrhythmias, QRS duration, progressive tricuspid regurgitation, right ventricular systolic dysfunction, right ventricular volume measurements on cardiac MRI, and performance on exercise testing. Other factors that are considered include the size and function of the left heart. The ratio of right ventricular to left ventricular end diastolic volume has been of interest for some time. It is now recognised that this rising ratio is not just due to increasing right ventricular size, but rather an underfilled left ventricle. In addition there has been a move towards pulmonary valve replacement before right ventricular ejection fraction deteriorates. Liver involvement in right heart failure is well documented. The association between advanced liver diseases seen in the Fontan circulation directed the study objective of whether examining the liver would provide any additional information in patients with pulmonary regurgitation. The recent progress in both imaging and serum markers of liver stiffness provided a novel approach to exploring the hepatic effects of right sided valvular regurgitation in adult congenital heart disease.

10.2 Main Findings – Study Objectives

1. Are right heart lesions associated with subclinical abnormality of hepatic structure and/or function?

Subclinical abnormalities of hepatic stiffness on the two imaging modalities were found infrequently and inconsistently in our study population. In particular, mean and median values for Fibroscan and MRE were in the normal range. Outliers were present in both imaging modalities. Considering the ELF score, mean and median values were in the moderate fibrosis category, when using reference ranges derived from data on patients with chronic liver disease. Liver ultrasound abnormalities were reported in several patients. These were discussed with the hepatology supervisor on the study – one patient (study ID 15, see section 10.4) has been diagnosed with cardiac cirrhosis and entered onto the hepatocellular carcinoma surveillance programme with 6-monthly measurements of alpha-fetoprotein (AFP) and liver ultrasound.

2. If so, whether the presence of hepatic abnormality correlates with right ventricular function (as assessed by cardiovascular magnetic resonance imaging).

A key consideration in study design and the inclusion/exclusion criteria was whether to include patients with documented reduced right ventricular ejection fraction (RVEF%). The decision to include these patients was based on the potential to collect a wider spectrum of data that may enable a definition of normal ranges in this population. This study questioned whether measurements of hepatic stiffness may correlate with right ventricular function.

The ELF score had a significant negative correlation with RVEF% when tested in those with pulmonary regurgitation, although this was a relatively weak correlation with correlation coefficient $r = -0.37$. In the sub-analysis of the components of the ELF score, hyaluronic acid (HA) was the dominant component of this relationship. This negative

correlation is conceptually elegant; as the right ventricle fails, systemic venous pressure rises and this is reflected on to the liver. Over time this causes hepatic congestion, which if left untreated may progress to hepatic fibrosis and cirrhosis. Neither Fibroscan nor MRE had a significant relationship with RVEF%.

3. For participants with Pulmonary Regurgitation, whether any hepatic abnormality correlates with established prognostic markers such as degree of RV dilatation and peak oxygen uptake on exercise. As such may this contribute to definition of the optimal timing of pulmonary valve replacement?

Significant relationships were found between prognostic markers and the hepatic primary outcomes. The ELF score and MRE were found to significantly correlate with QRS duration. This relationship was not only seen in those with pulmonary regurgitation where there is clear prognostic evidence, but also in the analysis of all study participants.

MRE significantly correlated with right ventricular end diastolic volume, a prequel to failure of the right ventricle. There were further relationships with right and left ventricular stroke volume. Conversely, there was a negative correlation with Oxygen Pulse which is commonly used as a surrogate for stroke volume. The significance of these isolated relationships are unclear and caution should be applied to their interpretation.

Although not a known prognostic marker in pulmonary regurgitation, NT-Pro-BNP had a significant correlation with all three measures of liver stiffness.

10.3 Study Groups

The study groups were designed to separate clinically distinct groups of patients within the broad category of right sided congenital valvar abnormalities. By applying the same hepatic investigations to all participants, it was postulated that significant differences would be seen between study groups. This was not seen for any of the primary hepatic outcomes. Given the small sample size it is plausible the study was simply not adequately powered to detect a difference between study groups (Type 2 error).

The Ebstein group represented an older population, who were taller, heavier and with higher body mass indexes. Clinically significant tricuspid regurgitation on its own did not seem to exert an effect on liver stiffness. Given the older age of participants in the Ebstein group, and the potential for duration of tricuspid regurgitation to cause systemic congestion, it was unusual that markers of hepatic fibrosis were not elevated compared with other groups. Consideration was given to the specific phenotype of tricuspid regurgitation in Ebstein's anomaly - the capacious right atrium may serve as a reservoir and provide relative protection to the central venous system.

When assessing participants' symptoms, the majority in Group 1 (62.5%) and Group 2 (68.8%) were NYHA 1. Overall, this participant selection represented a relatively 'stable' population. This may have influenced the lack of any significant difference in hepatic outcomes between groups.

The initial analysis comparing all study groups showed that the hepatic parameters were not significantly different between all groups. We then examined those with pulmonary regurgitation only by combining groups 1 (Mod-Sev PR) and 2 (Mild PR). This further analysis revealed that several cardiovascular variables were exclusively associated with participants with pulmonary regurgitation only.

10.4 Case Study – Study ID 15

A 57 year old lady 'Study ID 15' with a history of Fallot's Tetralogy with absent left pulmonary artery and four associated thoracotomies is discussed. Her initial surgery at the age of six was a pulmonary valvotomy (1965), followed a year later by further pulmonary valvotomy, right ventricular infundibular resection and ventricular septal defect patch closure (1966). In 1972 she had redo surgery and total correction, followed by redo surgery with a further ventricular septal defect patch in 1974. She also had a history of atrial arrhythmias (previous focal atrial tachycardia and typical atrial flutter ablations).

Her most recent 12-lead electrocardiogram showed sinus rhythm with 1st degree heart block, pr interval 240ms, and QRS duration of 183ms. Echocardiogram showed moderate pulmonary regurgitation, mild pulmonary stenosis, moderate to severe eccentric tricuspid regurgitation, a dilated impaired right ventricle with severely dilated atria and a normal sized and functioning left ventricle. Cardiac MRI 2016 showed sub-pulmonary stenosis with a gradient of 26mmHg and moderate pulmonary regurgitation (PR fraction 26%). The right ventricle was dilated with an end diastolic volume of 159ml/m^2 and end systolic volume of 81ml/m^2 with an ejection fraction of 51% (reduced). The left ventricular size and function were normal. Cardiopulmonary exercise testing in 2016: exercised for 7.4mins, achieved 82% of the target workload, MVO_2 15ml/kg/min, VE/VCO_2 slope of 24, VO_2 at anaerobic threshold as a percentage of predicted 44%, normal heart rate and blood pressure response.

She agreed to take part in the study and her results were: Fibroscan 9 kPa (raised), MRE: 4.81 kPa (severe fibrosis/cirrhosis range), ELF: 10.42 (severe range). Images from MRE are shown in Figure 11, section 7.1. In addition her liver ultrasound showed an irregular capsule with heterogeneous parenchyma and dilated hepatic veins. Spleen

size was within the normal range. Although her Gamma-glutamyltransferase was mildly raised at 76 U/L, her other liver enzymes, renal function, full blood count and clotting screen were normal. The NT-Pro-BNP was raised at 634 ng/L.

The results attained during the research study prompted a referral to the hepatology clinic, where a diagnosis of 'cardiac cirrhosis' has been made and she is now on their surveillance programme for hepatocellular carcinoma. She is relatively asymptomatic (NYHA 1-2) with a reasonable exercise tolerance however has been discussed twice for possible re-intervention (once in 2009 and again in 2016). The tertiary adult congenital surgical multi-disciplinary team felt that re-intervention would be at high-risk and since her symptoms were only mild, that medical management would suffice for now.

Her case is highlighted as she had the most advanced liver stiffness parameters in the study. Firstly it must be questioned whether the cardiac cirrhosis would have occurred had she had intervention to restore pulmonary and tricuspid comptetence as an adult. Secondly, concerning the raised indices of liver stiffness – at what point did these change/advance? The lack of serial measurements and liver histological data in our study leaves this question unanswered. Would intervention at this stage cause a reversal or any attenuation to these parameters?

10.5 Enhanced Liver Fibrosis Score

The ELF score has been tested in several populations. In our study of patients with pulmonary regurgitation and Ebstein's anomaly, the mean and standard deviation for all participants was 8.58 ± 0.92. This is similar to the values published in the study comparing Fontan patients to viral cirrhotics (Guha IN 2013), where the Fontan group had a mean and standard deviation of 7.97 ± 1.16. This is an unexpected result as one

may expect the Fontan group, where there is high systemic venous pressure (and a known association with advanced liver diseases), to have higher measures of liver stiffness.

The range of ELF values in this study is similar to that reported in published studies of normal subjects. Our study had a range of 5.46 - 10.45 (mean 8.58), compared with 5.88 - 10.30 (mean 8.06) in the study by Lichtinghagen and colleagues, and 5.37 - 9.10 (mean 7.75) in the study by Yoo and colleagues (Lichtinghagen R 2013, Yoo EJ 2013).

Factors that influence the ELF score have been studied with univariate linear regression in other studies containing normal subjects. Whilst Lichtinghagen and colleagues reported that age was a significant predictor, this relationship was not seen in our study (Lichtinghagen R 2013). Yoo and colleages showed that age, body mass index and gender were significant predictors of the ELF score. In this study, only body mass index was a significant predictor.

The QRS duration has been shown to correlate with right ventricular dilatation and dysfunction. Furthermore a prolonged QRS duration is known to confer a risk of arrhythmias and sudden death (Gatzoulis MA 1995, Gatzoulis MA 2000). It is therefore a key marker in the surveillance of patients with pulmonary regurgitation. The QRS duration has not been previously studied as a correlator or predictor of markers of hepatic stiffness. In this study, it was shown to have a significant correlation with the ELF score in all participants. The ELF score was also negatively associated with right ventricular ejection fraction measured on CMR in those with pulmonary regurgitation. Both QRS duration and right ventricular ejection fraction are prognostic in the long term follow up of pulmonary regurgitation, and therefore these findings are worthy of future exploration. It is suggestive that hepatic stiffness is influenced by right

ventricular dilatation and dysfunction. However we acknowledge that actual measurements of right ventricular dimensions (RVEDV, RVESV) did not correlate with the ELF score, which may be a function of the small sample size in this study.

Whilst both imaging modalities (Fibroscan and MRE) measure liver stiffness as a surrogate marker for liver fibrosis, the ELF score uses 3 direct measures of liver fibrosis turnover. The components of the ELF score (HA, PIIINP, TIMP-1) were also examined. Interpretation of the results are challenging in the absence of histological data. Furthermore the behaviour of the components of the ELF score has not been defined in congestive hepatopathy, or this population. TIMP-1 levels were significantly higher in Group 3 (Ebstein) - it is not clear whether this implies that the livers in Group 3 were more fibrosed, particularly as there was no significant difference seen in either HA or PIIINP.

Analysis of cardiovascular associations of the various components of the ELF score revealed that Hyaluronic Acid was the dominant predictor, with complete consistency. TIMP-1 also significantly correlated with QRS duration, whilst PIIINP did not correlate with any cardiovascular parameter. All three components are direct measures of liver fibrosis, and these findings are perhaps suggestive of the pattern seen in congested livers (a prequel to fibrosis).

The moderate correlation coefficient and low R^2 suggests that 1) more data is needed in order to test the strength of the correlations, and 2) further multivariate analysis is required to determine the other factors causing the majority of the variation in the models presented. This is true of all variables detected as significant predictors in this study.

10.6 Magnetic Resonance Elastography

Mean and median values across all study groups were in the normal range. A few outliers were encountered – three participants in the mild fibrosis category, and one in the severe category. This is in keeping with results seen in studies of normal subjects (Rouviere O 2006, Yin M 2007). Magnetic resonance elastography has not been studied exclusively in patients with pulmonary regurgitation or tricuspid regurgitation related to Ebstein's anomaly. The values generated in this study are expectedly lower than those published in studies on those with a Fontan circulation (Serai SD 2014, Poterucha JT 2015, Sugimoto M 2016). Sugimoto and colleagues' study contained only 4 participants with repaired Tetralogy of Fallot in one of the study groups but specific data on these participants were not published.

Despite the majority of values lying in the normal range, significant predictors of liver stiffness measured by MRE were discovered**.** Similar to the ELF score, QRS duration was also a significant predictor of MRE. This has not been studied before and represents a new finding.

As previously described, NT-Pro-BNP was also a significant correlator of MRE in those with pulmonary regurgitation. Liver stiffness measured by any means is known to be influenced by other mechanisms other than liver parenchymal fibrosis, such as fluid congestion (Mueller S 2014). This correlation seen between NT-Pro-BNP (a marker of fluid status) and MRE is therefore supportive of this causative mechanism. This finding is out of keeping with the non-significant correlation between NT-Pro-BNP and MRE reported by Sugimoto and colleagues (Sugimoto M 2016) on linear regression analysis.

Several cardiovascular parameters detected on CMR were found to significantly predict the MRE result. One of the most well established prognostic markers - right ventricular

end diastolic volume, was significantly associated (Vliegen HW 2002, Therrien J 2005, Oosterhof T 2007, Burchill L 2011, Lee C 2012, Bokma JP 2016, Ferraz Cavalcanti PE 2013). Although this study examined a relatively stable population (i.e. with only a few being actively considered for pulmonary valve replacement), this association suggests that MRE measurements of liver stiffness may prove to be clinically worthwhile if further studies can confirm this relationship. In keeping with this relationship, right ventricular stroke volume was also associated. This is mechanistically elegant – one would expect to see the right ventricular stroke volume increase as the right ventricular end diastolic volume increases. The left ventricular stroke volume was also positively correlated with MRE. This is out of keeping with current clinical thinking, where the left ventricular dimensions and stroke volume are thought to reduce in the face of chronic pulmonary regurgitation. Certainly the evidence suggests that left ventricular volumes and systolic function increase following pulmonary valve replacement ([1]Frigiola A 2008, [2]Frigiola A 2008). This finding concerning a positive association between left ventricular stroke volume and MRE is therefore likely to be inaccurate, and may have occurred by chance. Furthermore, the Oxygen Pulse (O2 Pulse = Peak oxygen uptake MVO2 / heart rate in ml/beat) which is a surrogate for left ventricular stroke volume, in fact had a negative association with MRE. This has not been previously studied, and is more in keeping with current understanding regarding the left ventricle in chronic pulmonary regurgitation.

10.7 Fibroscan

The mean and median Fibroscan values lay in the normal range. Significant predictor variables included right atrial size (RA) and age in all participants. The correlation between RA and Fibroscan is challenging to interpret. This study contains participants

with Ebstein's anomaly and associated large right atriums – the relationship between right atrial size and pressure is therefore not consistent across all study participants.

As with the other two modalities, NT-Pro-BNP was significantly correlated with Fibroscan in those with pulmonary regurgitation. This was not seen in the only other study comparing the two modalities in patients with acquired left sided heart failure, although the correlation approached statistical significance ($p = 0.056$) (Colli A 2010). Liver stiffness as measured by Fibroscan has previously been shown to correlate with invasively measured central venous pressure (Millonig G 2010, Jalal Z 2015, Taniguchi T 2014). Elevated levels of central venous pressure and NT-Pro-BNP are expected in states of fluid congestion, and therefore this finding (association between NT-Pro-BNP and Fibroscan) is supportive that Fibroscan readings are influenced by fluid status.

10.8 N-Terminal Prohormone of Brain Natriuretic Peptide

The consistent relationship seen between NT-Pro-BNP and all hepatic primary outcomes in those with pulmonary regurgitation was an unexpected finding. Compared with other studies, correlations between NT-Pro-BNP and other cardiovascular parameters were not consistent. We were unable to demonstrate a relationship with right ventricular volumes or pulmonary regurgitant fraction as in the work by Paulino and colleagues (Paulino A 2017), and Valderde and colleagues (Valderde I 2015). These studies were, however, in paediatric participants.

Significant associations were found between NT-Pro-BNP and right ventricular systolic pressure & peak oxygen uptake (inverse relationship). This is consistent with the findings reported by Norozi and colleagues in 50 adult participants with repaired Tetralogy of Fallot. NT-Pro-BNP does not form part of the routine assessment in

patients with Tetralogy of Fallot, however this study has suggested it does hold some promise.

10.9 Limitations

This study had only 40 participants divided into 3 unequal clinically relevant groups. A power calculation to set the sample size was not possible due to a lack of literature in this field and limitations in feasibility. This has increased the probability of both Type 1 and Type 2 errors in the statistical analysis. In addition there were missing values in both MRE (contraindications at 3 Tesla) and Fibroscan (invalid results) which may have influenced the statistical analysis. Furthermore right ventricular systolic pressure was only measurable in a proportion of participants (75%). Therefore although this study has detected significant correlators of the hepatic outcomes, and NT-Pro-BNP, this needs validation in a larger study.

An attempt to model multivariate linear regression models was made for all three hepatic outcomes. This generated several equations which contained counterintuitive models, for example a positive and significant regression coefficient for left ventricular ejection fraction to predict MRE. These were abandoned as they do not fit with standard clinical understanding and most likely occurred by chance.

Participants were selected against the inclusion and exclusion criteria. As previously described, patients tolerate significant pulmonary regurgitation over many years. Participants with abnormal liver stiffness (for example Study ID 15) were perhaps further along the natural course of this condition. Inclusion of more patients from this category may have resulted in the detection of more subclinical abnormalities.

A degree of selection bias in an observational study of this size is perhaps unavoidable. Participants were selected from a clinical database which is updated on a weekly basis. After the inclusion and exclusion criteria were applied, only 83 patients were eligible for a target sample size of 40. The recruitment strategy was applied evenly to all eligible participants, On the whole, clinical background and demographics were comparable between those recruited and eligible patients not recruited (Appendix D).

Data from clinically indicated cardiovascular investigations was used in the analysis. The aim was to utilise contemporaneous data, ideally less than 12 months interval between investigations and study visit date. Table 1 – App.E in Appendix E show the intervals for all participants between CMR and study visit date. 34/40 participants had an interval of less than 12 months. In the remaining 6, the responsible clinician did not feel there were clinical indicators to organise up to date investigations. Although the mean interval between CMR and hepatic investigations was 6 months (and median 4 months), some participants had much longer intervals with a maximum of 25 months. Caution is therefore warranted when interpreting results from this study that has used partly non-contemporaneous data.

Time interval between corrective surgery and study visit date for those with pulmonary regurgitation (Groups 1 & 2) is provided in Appendix E (Table 2 – App.E & Table 3 – App.E). Participants in Group 3 with Ebstein's Anomaly are excluded as only 2 out of 8 had a history of cardiac surgery. On the whole, intervals in Group 1 (mean 34 years, median 32 years) and Group 2 (mean 28 years, median 33 years) were comparable.

Right ventricular restrictive physiology is associated with poor short-term outcomes after repair of Fallot's Tetralogy, but positive outcomes at mid-term follow-up (Norgard G 1996). Table 1 – App.F shows the frequency of right ventricular restrictive

physiology in Groups 1 & 2. Only 3/16 in Group 1, and 1/16 in Group 2 had evidence of right ventricular restrictive physiology on echocardiography. Although restrictive physiology may influence right atrial size, and therefore hepatic congestion, the small numbers encountered are not thought to significantly alter the results of this study.

The author trained in Fibroscan in the hepatology clinic, and completed official training / certification run by the manufacturer. Invalid results (total of 3) were excluded from the analysis. One criteria that defines a valid Fibroscan result = interquartile range/median (IQR/M) ≤ 30%. Despite the satisfactory IQR/M in this study (17%), it has been suggested that this represents a relatively high variation in readings compared with what is seen in clinical practice. A future study may therefore consider the benefit of choosing a more experienced operator.

The interpretation of measurements of liver stiffness is difficult in a population where normal ranges have not been defined. This is compounded by the lack of histological data where we are left with unanswered questions as to the cause of the increased stiffness in select individuals. It is difficult to ethically justify the need for liver biopsy in this population and therefore further studies must focus on the behaviour of these measurements over time to truly understand what constitutes normal. Enrolling patients from the more severe/pre-operative end of the spectrum in a longitudinal study may contribute to this understanding.

The correlation between modalities was only significant between Fibroscan and MRE, and the strength of this relationship was only moderate. This is out of keeping with most published studies in chronic liver disease which suggest there is a much closer correlation between all three modalities. It is plausible that the relationship between

these methods does not exist within the normal reference ranges, i.e. in those without liver disease.

10.10 Conclusions and Future Study

This study has shown that liver stiffness can be measured reliably in patients with chronic pulmonary regurgitation and Ebstein's anomaly using a variety of methods. The results generated would be of interest to investigators of hepatic abnormalities in the Fontan circulation, as the optimum hepatic screening protocol is yet to be defined.

This study has shown that subclinical abnormalities of liver stiffness occur in only some patients with repaired Tetralogy of Fallot and Ebstein's anomaly. Distinguishing between normal and abnormal values has been a particular challenge in this study since reference ranges for the population in question are not available. Significantly correlated independent variables (QRS duration, NT-Pro-BNP and right ventricular volume and function) suggest that measurements of liver fibrosis and stiffness are influenced by right ventricular size, its function, and the fluid status of the subject. Perhaps the most elegant and satisfying of all findings in this study was the discovery of a relationship between the ELF Score & MRE values with the QRS duration - one of the earliest proposed prognostic markers in pulmonary regurgitation.

This study has examined the clinical utility of established markers of liver stiffness in those with pulmonary regurgitation. Significant correlations with established prognostic markers are interesting as it suggests that these hepatic parameters may add to the surveillance in this population. In particular clinicians may find these hepatic investigations useful when certain cardiovascular investigations are contraindicated (commonly CMR, or CPET). More data would be required to confirm this proposal. A

future multicentre longitudinal study is suggested that would select patients going forward for pulmonary valve replacement, i.e. at more advanced stages during the natural course of this condition. Serial measurements of liver stiffness pre and post-operatively would enable the definition of normal ranges and to determine the dynamic nature of these parameters. Outcomes from this study would include 1) Definition of normal liver stiffness measured by ELF score, MRE and Fibroscan, 2) Examine delta change in hepatic parameters post pulmonary valve replacement, 3) Associations established in this study including the QRS duration, NT-Pro-BNP and right ventricular dimensions and function would be validated. These measures of liver stiffness may then contribute to the routine surveillance of patients with pulmonary regurgitation.

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Appendix A - Study Protocol

The hepatic effects of right heart lesions in adults with congenital heart disease

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Professor Marcus Flather - Professor Clinical Research UEA, Honorary Consultant Cardiologist NNUH – academic supervisor
Ian Nunney - Statistician University of East Anglia

Sponsor: The University of East Anglia (UEA)

MD Registration: The University of East Anglia (UEA)

Protocol code number:
Protocol version number and date: Version 9: 4th January 2016

Version 1: 18th June 2015
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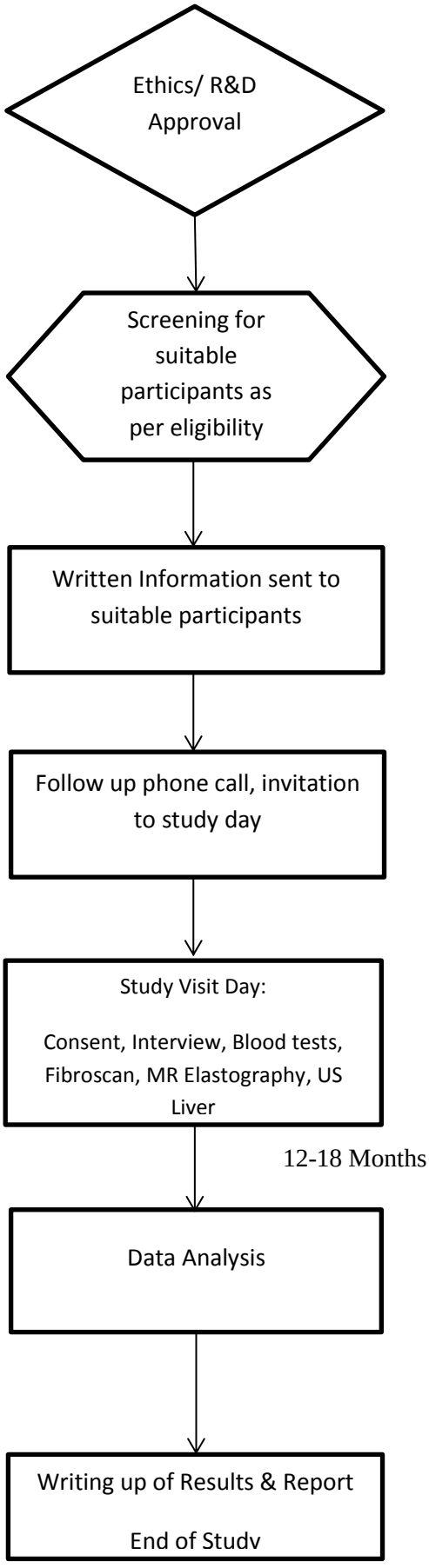
Version 4: 4th September 2015
Version 5: 18th September 2015
Version 6: 25th September 2015
Version 7: 14th October 2015
Version 8: 29th October 2015

Chief Investigator: Dr. Dilip Abraham

Signature: _____ Date 4th January 2016___

Flow Chart


Ethics/ R&D Approval
Screening for suitable participants as per eligibility
Written Information sent to suitable participants
Follow up phone call, invitation to study day
Study Visit Day:
Consent, Interview, Blood tests, Fibroscan, MR Elastography, US Liver
12-18 Months
Data Analysis
Writing up of Results & Report
End of Studv

Protocol Summary

Title	The hepatic effects of right heart lesions in adults with congenital heart disease
Study Aims	1. Whether right heart lesions are associated with subclinical abnormality of hepatic structure and/or function 2. If so, whether the presence of hepatic abnormality correlates with right ventricular function (as assessed by echocardiography and cardiac MRI) 3. For participants with PR, whether any hepatic abnormality correlates with established prognostic markers such as degree of RV dilatation and peak oxygen uptake on exercise. As such may this contribute to definition of the optimal timing of pulmonary valve replacement?
Study Design	Cross-sectional Observational Study
Eligibility Criteria	**Inclusion Criteria**: 1) All patients will have a prior diagnosis of tetralogy of Fallot, or Ebsteins anomaly of the tricuspid valve (associated with tricuspid regurgitation), or prior pulmonary stenosis treated with valvotomy/valvuloplasty with subsequent pulmonary regurgitation **Exclusion criteria:** 1) Any other haemodynamically significant lesion including RV outflow tract obstruction with gradient >3m/s, with the exception of tricuspid regurgitation 2) Contraindications to MRI 3) Pre-existing chronic liver disease 4) Currently Pregnant 5) Unable to complete the study protocol for non cardiac reason 6) Age <17 or >70 7) Lack of capacity to consent
Study Investigations	**Cardiology/General:** • History and Examination + BMI • FBC, UE, NT Pro BNP, Cholesterol, HbA1c • ECG • Transthoracic Echocardiography • Cardiac MRI • Cardiopulmonary Exercise Testing

	Hepatology: • LFTs, Clotting Profile, AST, GGT, Albumin • Enhanced Liver Fibrosis Score • Fibroscan • MR Elastography • Ultrasound of Liver
Sample Size	Target sample size of 40-60 participants.
Study Investigators	**Chief Investigator**: Dr. Dilip Abraham – MD Student & Clinical Research Fellow in Cardiology Supervisors: Dr. Leisa Freeman - Consultant Cardiologist Dr. Catherine Head - Consultant Cardiologist Dr. Simon Rushbrook - Consultant Hepatologist Dr. Paul Malcolm - Consultant Radiologist Professor Marcus Flather – Professor Clinical Research UEA, Honorary Consultant Cardiologist NNUH Ian Nunney – Statistician University of East Anglia
Study Site	Norfolk and Norwich University Hospital
Funding	Charitable Fund Cardiac F019. Held by Dr. Leisa Freeman
Sponsor	University of East Anglia
Student Project	Dr. Dilip Abraham MD Registration at University of East Anglia

Abbreviations: MRI – Magnetic Resonance Imaging; PR – Pulmonary Regurgitation; RV – Right Ventricle; BMI – Body Mass Index; FBC – Full Blood Count; UE – Urea and Electrolytes; NT Pro-BNP - N-terminal of the prohormone brain natriuretic peptide; HbA1c – Glycated Haemoglobin; ECG – Electrocardiogram; LFTs – Liver Function Tests; AST – Aspartate Transaminase; GGT – Gamma-glutamyl transferase; MR – Magnetic Resonance

Background

Clinical Need

The incidence of congenital heart disease is 6.9 – 8 per 1000 live births[1,2]. Of those born with congenital heart disease, at least 85% now survive into adulthood[3]. There are currently more than 150, 000 adults with congenital heart disease in England[4], of whom 2,800 are under the care of Norfolk and Norwich University Hospitals (NNUH = 2000) and Papworth Hospital collectively.

Adults with congenital heart disease are a growing population thanks to improvements in initial diagnosis and management. As adults they are sometimes faced with progression of disease and/or the sequelae of their initial surgery. We are particularly interested in congenital conditions of the right heart, particularly those that involve the pulmonary and tricuspid valve. Since the liver is directly connected to the right atrium (via the inferior vena cava) and thus the right ventricle, markers of liver dysfunction may provide early evidence for a compromised right heart. Therefore this study will address if novel biomarkers of liver dysfunction and fibrosis (which may not be reflected on standard liver function tests) will add to the overall assessment of patients with significant pulmonary and/or tricuspid valve regurgitation in the adult congenital heart disease population. This could then contribute to the decision making around valve replacement or repair.

Pulmonary regurgitation in adults with congenital heart disease

Some congenital cardiac lesions are associated with the development of severe pulmonary regurgitation (PR), either as a primary pathology or, more commonly, as a consequence of initial surgical treatment of Tetralogy of Fallot or pulmonary stenosis. Over time this may ultimately lead to progressive right ventricular (RV) dilatation and impairment of function[5.6.7.8].

It is recognised that patients are usually asymptomatic for many years with chronic pulmonary regurgitation. Over time, and this may be as long as 20-25 years after initial repair, the right ventricle dilates and may eventually fail. This is associated not only with the development of symptoms but also an increased risk of ventricular tachyarrhythmia and sudden cardiac death[9]. Pulmonary valve replacement has been shown to reverse this remodelling of the RV if performed below a threshold of RV dilatation[10]. Widespread clinical practice is therefore to offer pulmonary valve replacement for severe pulmonary regurgitation when one or more of the following are present: symptoms, objective evidence of exercise limitation (of no alternative cause), progressive RV dilatation, impairment of RV systolic function, or arrhythmia[11]. Optimal timing of pulmonary valve (PV) replacement however remains to be defined, with the above criteria under constant refinement. The identification of additional biomarkers of early subclinical right ventricular dysfunction, which may help in the timing of cardiac surgery in an individual patient, is therefore highly desirable.

Tetralogy of Fallot

Tetralogy of Fallot (TOF) is one of the most common forms of congenital heart disease, and is one of the most common reasons for intervention in the first year of life.[12] It is characterized by the following four anatomical abnormalities:

1) Ventricular septal defect (VSD)
2) Right ventricular outflow tract (RVOT) obstruction
3) Overriding aorta
4) Right ventricular hypertrophy

The precise embryological mechanism that leads to this pattern is unknown however genetic and environmental factors are involved. There is initially anterior and cephalad deviation of the infundibular septum. This results in the listed abnormalities 1-3, with hypertrophy of the right ventricular occurring as a result of increased workload as a consequence of right ventricular outflow tract obstruction.[13]

Pulmonary Stenosis

Pulmonary stenosis may be detected either as a single entity or as part of more complex congenital heart disease. The treatment of choice for isolated pulmonary stenosis in the 1960s and 1970s was surgical valvotomy. This has largely been replaced with percutaneous balloon valvuloplasty. Both procedures are associated with subsequent pulmonary regurgitation, although less so with the percutaneous approach.

Ebstein's Anomaly

Ebstein's anomaly is a relatively rare congenital malformation of the tricuspid valve. There is great variety in morphology but in general the valve leaflets are malformed and apically displaced. The anterior leaflet of the tricuspid valve is abnormally elongated and 'sail-like' and usually arises correctly from the tricuspid annulus. The posterior and septal leaflets are displaced apically and may be attached to the right ventricular endocardium. The defect causes the right ventricle to be divided into two; the proximal atrialised RV and the apical functional RV. The tricuspid valve is typically incompetent (regurgitant) into a dilated right atrium. Associated conditions include patent foramen ovale or atrial septal defect (typically secundum ASD), ventricular septal defect, patent ductus arteriosus, coarctation of the aorta, atrial arrhythmias, and rarely left heart lesions. The clinical presentation depends on age at presentation and includes cyanosis, heart failure and arrhythmia. Management and follow up requirements are determined by the clinical manifestation. In the adult this may include management of arrhythmias, pacemakers, heart failure, referral for surgery to repair the abnormal/Ebstein tricuspid valve when severe tricuspid regurgitation is associated with symptoms and or progressive dysfunction of right sided chambers.

Follow up and Monitoring of Pulmonary Regurgitation

The natural course of chronic pulmonary regurgitation necessitates regular follow up, generally on an annual basis. Evidence suggests that replacing the pulmonary valve before the right ventricle dilates to a threshold measured by end-diastolic volumes of 160ml/m2 – 170ml/m2, or end-systolic volumes of 80-85ml/m2 is associated with reversal of abnormal remodeling[14,15,16]. Pulmonary valve replacement is in the form of bioprosthetic or homograft valves which have a limited lifespan. Therefore finding the right time to replace the valve so that it is neither too late nor too early is the challenge of the adult congenital heart disease specialist.

The investigations used to monitor cardiac function include transthoracic echocardiography (TE), cardiac magnetic resonance imaging (cMRI), and cardiopulmonary exercise testing (CPET).

Echocardiography is the first line diagnostic modality which provides information on pulmonary regurgitation, residual right ventricular outflow tract obstruction, tricuspid regurgitation, and right and left ventricular size and function. There are well known limitations in assessing the right ventricle due to its peculiar shape. Assessing right ventricular volumes, mass and function necessitates geometrical assumptions which introduces error in its calculation. Echocardiography is also at times limited by poor acoustic windows, with the pulmonary valve a particularly difficult valve to image and assess.

Cardiac MRI provides more accurate information in some conditions within adult congenital heart disease. In particular, the evaluation of right ventricular volumes and ejection fraction, and pulmonary regurgitation fraction. It is also useful in evaluating the right ventricular outflow tract, conduits between the right ventricle and pulmonary artery, and branch pulmonary arteries[17]. It has the advantage of an unlimited view, producing high quality images without ionizing radiation.

Cardiopulmonary exercise testing is used to quantify exercise intolerance in adult congenital heart disease. The evidence suggests that objective measures of reduced exercise tolerance are more prevalent than either patient or physician may suspect[18]. CPET provides prognostic information which is similar to its role in heart failure. A symptom limited exercise test is performed with simultaneous measurement of cardiac and respiratory function including respiratory oxygen uptake (VO2), carbon dioxide production (VCO2), and several ventilatory variables. Pre-operative CPET testing can predict surgical outcome and is considered a routine part of the pre-operative evaluation[19].

Effects of central venous pressure on the liver

Central venous hypertension such as seen in the Fontan circulation causes chronic pressure overload of the hepatic venous circulation. This leads to a series of histological changes characterized by centrilobular parenchymal atrophy, sinusoidal and terminal hepatic venular distention and perisinusoidal collagen disposition, conferring a risk of cirrhosis, regenerative nodules and hepatocellular carcinoma. The presence and severity of fibrosis are important prognostic factors for the risk of progression to cirrhosis[20,21,22,23].

There is currently a spectrum of adult congenital heart disease lesions which ultimately lead to impairment of right ventricular function. Hepatic dysfunction is regarded as a later manifestation in patients with right-sided heart failure[24,25]. The primary pathophysiology involved in these cases is hepatic dysfunction either due to passive congestion or acute cellular injury due to poor perfusion to the liver; with the latter more important in cases of ischaemic hepatitis[26,27]. The optimal time to replace or repair either or both the pulmonary and tricuspid valves to provide competency - in the hope of preventing right ventricular failure and “cardiac cirrhosis” - is still not clear. PR in the presence of normal RV systolic function and no more than mild tricuspid valve regurgitation should not cause significant chronic elevation of systemic venous pressure and is not associated with clinically apparent hepatic dysfunction[28]. However it is worth noting that the only published study used standard liver function tests and no structural or functional measures of fibrosis or cirrhosis.

Whilst there are clear imaging indices in TE and cMRI that describe right ventricular haemodynamics and function, at present there have been few studies which have attempted to identify novel biomarkers of right ventricular dysfunction. The development of such biomarkers may help in the timing of cardiac surgery. Guha et al investigated structural and functional hepatic factors (by means of serum fibrosis markers – the Enhanced Liver Fibrosis (ELF) score, and Indocyanine Green Clearance (IGC)) in stable patients with Fontan circulation versus those with

proven compensated viral cirrhotic patients. They were able to show that both groups had similar global hepatic function and degree of fibrosis. However unlike the viral cirrhotic group, the Fontan group did not display a significant correlation between global liver function and degree of fibrosis[29]. In a recent prospective study of patients with congenital heart disease, transient elastography was used to show a close correlation between liver stiffness and central venous pressure (CVP). They suggested a cut-off value of 8.8 kPa on Fibroscan would predict a CVP > 10mm Hg[30].

Assessment of hepatic structure, fibrosis and function

The gold standard for staging of liver fibrosis is liver biopsy. Given the well documented drawbacks and risks of this procedure, non-invasive tests including fibrosis scores, advanced biochemical markers and imaging studies have been developed[31,32]. Sensitivity, specificity and positive/negative predictive values vary according to the test panel used and the population group screened[33,34].

Magnetic Resonance Elastography has been investigated and validated in several studies for the assessment of liver fibrosis and cirrhosis. MR Elastography is a non-invasive method, and is considered the imaging counterpart to the skill of palpation by a physician. The basic technique involves generating shear waves on the surface of the skin and then imaging the propagation of this shear wave using a modified phase contrast MR sequence. This measure of tissue stiffness is measured in kPa and correlates closely with the extent of fibrosis. A meta-analysis that included 12 studies of MRE found the following test characteristics[35]:

- Detecting any fibrosis (F≥1): Optimal cutoff 3.45 kPa, sensitivity 73 percent, specificity 79 percent
- Detecting significant fibrosis (≥F2): Optimal cutoff 3.66 kPa, sensitivity 79 percent, specificity 81 percent
- Detecting advanced fibrosis (≥F3): Optimal cutoff 4.11 kPa, sensitivity 85 percent, specificity 85 percent
- Detecting cirrhosis (F4): Optimal cutoff 4.71, sensitivity 91 percent, specificity 81 percent

Ultrasound-based Transient Elastography is another non-invasive imaging technique providing a measure of liver stiffness. Fibroscan® (Echosens, Paris, France) is a 1-dimensional ultrasound Transient Elastography which measures the velocity of a low-frequency (50 Hz) elastic shear wave propagating through the liver. This velocity is directly related to tissue stiffness, called the elastic modulus (expressed as $E = 3 \rho v2$, where v is the shear velocity and ρ is the density of tissue, assumed to be constant). The stiffer the tissue, the faster the shear wave propagates. Advantages of TE include a short procedure time (<5 min), immediate results, and the ability to perform the test at the bedside or in an outpatient clinic.

The Enhanced Liver Fibrosis panel (ELF Panel) is a serological test used as a direct marker of fibrosis. ELF is a proprietary algorithm that takes into account hyaluronic acid level, amino-terminal propeptide of type III collagen level, and TIMP-1. In a study that included 1021 patients with chronic liver disease who were undergoing liver biopsy, a threshold score of 0.102 was associated with a sensitivity of 87 to 90 percent and a specificity of 41 to 51 percent for diagnosing moderate or severe fibrosis. If a threshold of 0.457 was used, the specificity increased to 95 percent.

This study aims to establish:

4. Whether right heart lesions are associated with subclinical abnormality of hepatic structure and/or function.
5. If so, whether the presence of hepatic abnormality correlates with right ventricular function (as assessed by echocardiography and cardiac MRI)
6. For participants with PR, whether any hepatic abnormality correlates with established prognostic markers such as degree of RV dilatation and peak oxygen uptake on exercise[18]. As such may this contribute to definition of the optimal timing of pulmonary valve replacement?

Future studies should investigate whether any hepatic abnormalities seen normalise after pulmonary valve replacement.

Study Design and Methodology

Study Design: This study is designed as a cross-sectional observational study.

Setting: The primary location will be Norfolk and Norwich University Hospital (NNUH). The research team may consider recruiting patients from Guy's and St.Thomas' Hospital and Papworth Hospital in the future should recruitment at NNUH prove suboptimal. Separate R&D approval will be sought should this be required.

Sample size: In this study we plan to recruit 40 patients but we are requesting permission to recruit up to 60 if there are patients whose results cannot be used – either because they are not able to participate or there are technical problems. A power calculation was considered not necessary given the exploratory nature of the study. This is the first piece of research in this area and consequently we do not have any prior knowledge to calculate a sample size. The sample size target was determined by feasibility. The intention is that the data collected from this study will allow power calculations for any future trials.

Participants: The study will select patients with repaired Tetralogy of Fallot, Ebstein's anomaly or prior pulmonary stenosis from the adult congenital heart disease NORPAP database at NNUH. The database has 2547 patients of whom 17% have a diagnosis of Fallot's Tetralogy or pulmonary valve disease. Selected patients will be divided into three groups based on previous investigations.

Group 1: will have moderate to severe PR
Group 2: will have no more than mild PR
Group 3: will have at least mild TR associated with Ebstein's anomaly

The aim is to enrol at least 10 patients in each group to achieve a total sample of 40 patients. This will be carried out by appropriate screening of patient registers, assessing eligibility and inviting patients to participate. Once the required number of patients is recruited in one group, priority will be given to recruiting to other groups.

Inclusion Criteria:
1) All patients will have a prior diagnosis of Tetralogy of Fallot, or Ebstein's anomaly of the tricuspid valve (associated with tricuspid regurgitation), or prior pulmonary stenosis treated with valvotomy/valvuloplasty with subsequent pulmonary regurgitation.

Exclusion criteria:
1) Any other haemodynamically significant lesion* with Vmax > 3m/s, with the exception of tricuspid regurgitation.
2) Contraindications to MRI **
3) Pre-existing chronic liver disease ***
4) Currently Pregnant ****
5) Unable to complete the study protocol for non cardiac reason
6) Age <17 or >70
7) Lack of capacity to consent

* Including any cause of RV outflow tract obstruction for example pulmonary stenosis, sub-pulmonary stenosis, supravalvar pulmonary stenosis, branch pulmonary artery stenosis.
** Non-compatible cardiac pacemakers, intracerebral or eye metallic clips
*** Pre-existing liver disease will be defined as known diagnosis of Cirrhosis of any cause, Non-alcoholic fatty liver disease, alcoholic liver disease, viral hepatitis (hepatitis B or C infection), haemochromatosis, alpha-1-antitrypsin disease, primary biliary cirrhosis, primary sclerosing cholangitis or autoimmune liver disease.
**** Determined by history during telephone conversation

Definitions

Normal Right Ventricular Function:

- TAPSE (triscupid annular plane systolic excursion) > 1.6cm
- Ejection Fraction (assessed on cardiac MRI) as below

Right ventricular function will be assessed on TE and cMRI. We will use the standard British Society of Echocardiography reference range for tricuspid annular plane systolic excursion[36]. The European Society of Cardiology has defined normal ranges for right ventricular ejection fraction in their 2013 publication 'Cardiovascular Magnetic Resonance - Pocket Guide' [37]. This is outlined below:

RV EF%	Age <35	Age>35
Males	57 ± 5 (47-67)	61 ± 6 (49-73)
Females	61 ± 3 (55–67)	64 ± 7 (50-78)

Given the nature of the gender & age defined reference ranges for normal ejection fraction, right ventricular function will initially be considered as a categorical variable (i.e. normal or abnormal). Further subgroup analysis as a continuous variable between gender & age groups and hepatic structure/function will also be performed.

Pulmonary Regurgitation:

Expert consensus forms the basis of the reference range for pulmonary regurgitant fraction on cardiac MRI, which is agreed by both cardiology supervisors in this study. Pressure-half time reference range is taken from the British Society of Echocardiography guidelines[36].

Parameter	Mild	Moderate	Severe
PR Fraction	<20%	20-40%	>40%
Pressure half-time			<100ms
Technician qualitative assessment	Mild	Mod	Free/severe

Tricuspid Regurgitation: [36,38]

Parameter	Mild	Moderate	Severe
Colour Flow Jet	Small, central	Intermediate	Very large central jet or eccentric wall inpinging jet
Vena Contracta Width mm	Undefined	<7	>7

Presence or absence of tricuspid regurgitation will be recorded. A further exploratory analysis will be performed by dividing participants into those with no more than mild TR versus those with moderate to severe TR.

Right atrial size will be recorded, and this is determined by using RA end systolic area (cm^2) as recommended by the American Society of Echocardiography[39]. Normal range will be considered as 10-18 cm^2.

Note: All parameters will be considered when classifying RV function, PR and TR; all borderline cases will be discussed with either or both consultant cardiologists supervising this study.

Consent:

Information on this study will be provided to participants in both written (using the study information sheet in Appendix 1) and verbal format. Adequate time will be allocated to allow the patients to consider the information and their involvement before making a decision – typically at least 24 hours. Formal consent will then be gained by asking the patient to read and sign the consent form (Appendix 2). GPs will also be notified of the participants' inclusion into the trial (Appendix 3) with the participants' consent.

Incidental Findings:

Any incidental findings on scans or investigations will be reported to the responsible clinician for that patient. A discussion will take place and a low threshold to inform the patient will be adopted. GPs will be notified (with the patient's consent) to organise further investigations or to instigate referrals.

Reporting of Serious Adverse Events:
A Serious Adverse Event (SAE) is defined as any untoward occurrence that may: a) result in death b) is life-threatening c) requires hospitalisation, or prolongation of existing inpatients' hospitalisation d) results in persistent or significant disability or incapacity e) consists of a congenital anomaly or birth defect. SAEs will be reported according to Good Clinical Practice and NNUH R&D Standard Operating Procedure 205 v2.2 including the reporting time frame. All SAEs considered to be related to the study procedure will be reported to the sponsor in an expedited manner. This may include haematoma or vascular injury from venepuncture although this is considered highly unlikely.

This study is classified as non-CTIMP (Clinical Trial of Investigational Medicinal Product). SAEs considered to occur as a result of the underlying condition specifically will be reported on the trial case report form and not in an expedited manger. These events include:
1) Death
2) Arrhythmia including atrial and ventricular tachyarrhythmias, heart block
3) Admission for heart failure
4) Investigations such as angiography or other invasive interventions.

Sampling Methods: All potentially eligible participants will be screened from the NORPAP database by the Chief Investigator using the inclusion and exclusion criteria. All eligible participants will be sent written information by the CI. A follow up phone call will be made by the CI where potential participants will be invited to discuss the study in more detail and to sign the consent form to enter the study. Patients will be invited to attend NNUH for a one day visit to complete their investigations and interview. Patients will also be approached directly by the clinical cardiology consultant supervisors should they attend for routine clinic reviews on an ad hoc basis.

Data Collection:

Data will be collected on a paper case report form (CRF) on the categories listed below. Test results within the preceding 12 months prior to inclusion in the trial (with consent being the entry point) will not be repeated. Results will be drawn from the various hospital web systems available at NNUH including ICE (Integrated Clinical Environment) and Cadran Image Platform. We expect most patients to have up to date echocardiography, electrocardiogram and cardiac MRI. A small proportion of patients may require a repeat cardiopulmonary exercise test as part of routine clinical care.

Blood tests that are required will be taken from the patient during their visit – venepuncture will be performed by the Chief Investigator.

Hepatology structural and functional testing in the form of blood tests, Fibroscan, MR Elastography, and ultrasound of the liver will be performed on the same day when the patient attends for their interview. Blood will be stored for Enhanced Liver Fibrosis Panel blood tests when this becomes available. Results will be available on the hospital web systems in the few days following their attendance.

Baseline clinical assessment

1) Clinical history including:

- New York Heart Association Score (NYHA)
- Awareness of rhythm disturbances
- Sensation of abdominal fullness or right upper quadrant pain
- Drug and Alcohol history
- Previous Intravenous Drug use
- Screening for risk factors associated with metabolic syndrome and non-alcoholic fatty liver disease

2) Physical examination and Body Mass Index. To include:

- Assessment of jugular venous pressure
- Presence of palpable liver edge
- Nature of second heart sound (split or not)
- Cardiac murmurs on auscultation
- Presence of peripheral oedema or ascites
- Height, weight, BMI

Blood tests

3) Full Blood Count, Urea & Electrolytes, NT Pro BNP, Cholesterol, HbA1c, glucose.

Cardiovascular

4) Electrocardiogram (ECG)

- Rhythm
- QRS duration
- Presence/absence of right bundle branch block
- Presence/absence and location of T wave inversion

5) Cardiopulmonary exercise testing (CPET)

- Baseline HR and BP
- Heart rate response
- Peak oxygen uptake
- VE/VCO2 ratio
- Maximum workload
- Exercise duration
- Respiratory exchange ratio > 1

6) Transthoracic Echocardiogram (as per NNUH protocol)

- Tricuspid Annular Plane Systolic Excursion (TAPSE)
- Tissue Doppler derived tricuspid lateral annular systolic velocity (S') (<10cm/s will be considered abnormal [39])
- Vena Contracta Width
- Right Atrial size
- Pressure half time across pulmonary valve

7) Cardiac MRI (as per Guy's and St Thomas' Hospital protocol)

- Right Ventricular Ejection Fraction
- LVEDV:RVEDV
- Right Ventricular end diastolic volume (indexed to BSA ml/m^2)
- Right Ventricular end systolic volume (indexed to BSA ml/m2)

Hepatology

1) Bloods including Alkaline Phosphatase (ALP), Bilirubin, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Gamma glutamyl transpeptidase (GGT), Albumin, and Clotting Screen including INR.

2) Patients will have a panel taken for fibrosis markers; this will include the Enhanced Liver Fibrosis (ELF) Panel - hyaluronic acid level, amino-terminal propeptide of type III collagen level, and TIMP-1. Blood will be stored until the ELF Panel tests are available. The ELF Panel blood tests will be sent to the Royal Free Hospital in London for analysis when this arrangement has been finalised. In addition we will use non invasive parameter scores that include FORNS and APRI.

3) Storage of serum sample to aid additional sub-analysis if required. This may include transcriptione analysis and proteinomics.

4) Fibroscan is performed on a patient lying supine, with the right arm elevated to facilitate access to the right liver lobe. The tip of the probe is contacted to the intercostal skin with coupling gel in the 9th to 11th intercostal space at the level where a liver biopsy would be performed. The operator, assisted by a time-motion image, locates a liver portion at least 6 cm deep and free of large vascular structures. The operator then presses the probe button to start the measurements ("shots"). TE measures LS in a volume that approximates a cylinder 1 cm wide and 4 cm long, between 25 mm and 65 mm below the skin surface. The software determines whether each measurement is successful or not. When a shot is unsuccessful, the machine does not return a value. The entire procedure is considered to have failed when no value is obtained after ten shots. The final result of a TE session can be regarded as valid if the following criteria are fulfilled: 1) a number of valid shots of at least 10; 2) a success rate (the ratio of valid shots to the total number of shots) above 60%; and 3) an interquartile range (IQR, reflecting the variability of measurements) less than 30% of the median LS measurements (M) value (IQR/M $\leqslant$0.30%). The results are expressed in kilopascals (kPa), and range from 1.5 to 75 kPa with normal values around 5 kPa, higher in men and in patients with low or high body mass index (BMI) (U-shaped distribution). It is required that patients are fasted for 3 hours prior to the Fibroscan.

5) MR Elastography - The patient is in the supine position within the MRI room, and then a pneumatic driver device is strapped to the patient over the right side of the lower rib cage centered approximately over the mid-point of the liver, beneath the MRI coil. When the device is activated, the patient will feel gentle vibrations under this area due to the pressure waves. Each MR elastography examination of the liver only takes about 30 seconds, although the overall set up and calculation should take 30-40 minutes.

6) Ultrasound of the liver – The patient is in the supine position with their abdomen exposed. Standard ultrasound probe will be used aided by ultrasound gel. Routine assessment of liver size and structure will be performed followed by Doppler assessment of the major liver vessels.

Summary of Data Categories / Investigations

Category/Investigation	Pre-Study Visit	Study Visit 1
History and Examination		✓
Routine Blood Tests		✓
Electrocardiogram	✓	
Cardiopulmonary Exercise Test	✓	
Echocardiogram	✓	
Cardiac MRI	✓	
ELF Panel Blood Tests (storage only)		✓
Fibroscan		✓
MR Elastography of Liver		✓
Ultrasound Liver		✓

Note: The majority of patients will have up to date cardiac investigations. In the event that a participant has not had one of these tests in the preceding 12 months, this will be organised as a part of routine clinical care (which may involve an additional visit to NNUH).

Use of Human Tissue Samples

The only type of human tissue/biological sample will be in the form of blood sampling. This will be collected from study participants by the CI. Informed consent will be gained prior to taking blood samples. Samples will be stored in linked anonymised form. Blood samples will be stored in the Norfolk and Norwich University Hospital laboratories and analysis will be completed here. One set of bloods for each patient will be stored for the ELF panel. This particular test is not done at NNUH and Dr. Simon Rushbrook (hepatology supvervisor) is collaborating with the Royal Free Hospital, London. At the end of the study samples will be transferred to the research tissue bank at Norfolk & Norwich University Hospital in accordance with the Human Tissue Act.

Data Collection, management and analysis

Data will be collected by the CI onto a paper CRF. All data will be entered into an excel spreadsheet and stored on the secure NNUH network. Data will be anonymised to only include the study identification number. The trial master file will contain the transfer sheet with study identification number and patient's full name, hospital number, age and date of birth.

Basic descriptive methods will be used to present the data on study participants for all baseline clinical assessments as described in the data collection section (in total and for each condition). The 4 primary outcomes will be the ELF panel blood tests, the high Fibroscan result, ultrasound liver results and the MR elastography score.

Correlation analysis will be performed between the 4 primary outcome tests and right ventricular systolic function (Ejection Fraction and TAPSE) within all conditions.

Correlation analysis will also be performed between the hepatic primary outcomes and right ventricular end diastolic volume (indexed to BSA ml/m2), right ventricular end systolic volume (indexed to BSA ml/m2), ratio of LVEDV:RVEDV, Pulmonary Regurgitant Fraction and peak oxygen uptake on CPET.

We will also test the hypothesis that there is no difference in these 4 outcomes between participants that have moderate to severe PR versus those that have only mild PR using either the Wilcoxon Rank Sum test or the 2 sample t-test (two-tailed, 5% significance level) depending on whether the data can be assumed to be from a normal distribution. As a subsidiary analysis we will investigate the effect of duration of regurgitation, participant age on all three primary outcomes, and potentially BMI for the Fibroscan score using a linear mixed model. To adjust for potential age and duration of regurgitation effects, age and duration of regurgitation will be included in the mixed model as random effects. The adjusted Least Square means of the differences and their respective 95% confidence intervals will be reported. For any of the primary outcome measures that are not normally distributed the outcome measure will be transformed (e.g. log transformation) so that the residuals are normally distributed.

Further exploratory analysis will be carried out splitting the Moderate to severe group into 2 further groups, one with no more than mild tricuspid regurgitation and the other with moderate to severe tricuspid regurgitation. The same analysis as above will be carried out comparing these 2 groups against the no more than mild PR group.

If there is evidence of a difference between the two groups in their hepatic function an exploratory correlation analysis will be conducted between right ventricular function and established prognostic markers such as right ventricular dilation and reduction in peak oxygen uptake on CPET.

Sensitivity analysis will be performed between hepatic imaging modalities to test the hypothesis that MR Elastography has a higher sensitivity than Fibroscan in diagnosing liver fibrosis as indicated in one study. [40]

An interim analysis will be carried out when the study has achieved 20 patients. If no significant difference in hepatic structure and function is seen between conditions, the study investigators may extend the inclusion criteria to include other participants from the adult congenital heart disease population.

Data will be analysed using Microsoft Excel and SAS. Analysis will be performed by Ian Nunney (Statistician - UEA) and Dr. Dilip Abraham.

Study administration and ethical issues:

The day to day running of the trial will be managed by the Chief Investigator Dr. Dilip Abraham. His role will include screening the NORPAP database, checking test results and archived clinical letters to ensure inclusion and exclusion criteria are met. Information will then be sent to patients. This will be followed up by telephone calls to ask if patients would be willing to enter the trial. Regular meetings have been organised with the steering group (Dr. Leisa Freeman, Dr. Catherine Head, Dr. Simon Rushbrook, Prof. Marcus Flather) to review trial progress and to identify any emerging logistical or safety issues. Monthly meetings will be documented in the Trial Master File.

Following the collection of blood samples, Fibroscan, ultrasound of liver, and MR Elastography, data will be entered onto the Microsoft Excel Spreadsheet. Data will be anonymised by allocation of

a Patient Identification Number. This data will subsequently be analysed using Microsoft Excel and SAS with input from the named statistican Ian Nunney.

Ethical Approval

The trial will comply with the Declaration of Helsinki (http://www.wma.net/) on research involving human subjects. The IRAS forms, study protocol, patient information sheet, consent form, and GP letter will be submitted to the Research Ethics Committee for approval.

Funding:

Funding is in place from the F019 Charitable fund Cardiac. The holder of this fund is Dr. Leisa Freeman. Her interest in this study is to add to the current knowledge and improve clinical practice and outcomes for patients.

Sponsorship:

This study is sponsored by The University of East Anglia.

Resource requirements:

This study requires input from the following departments – cardiology, hepatology/gastroenterology and radiology. In addition the biochemistry, haematology, immunology and microbiology laboratories will be utilised for processing of blood samples.

Costing:

Most of the tests included in the data collection are considered routine clinical tests that would be performed in the absence of this study. This includes the following blood tests: Full Blood Count, Urea & Electrolytes, Liver Function Tests (Bilirubin, ALT, ALP, AST, GGT), Albumin, Clotting Screen/INR, BNP, lipids & cholesterol, glucose and HbA1c.

ECG, Echocardiography, Cardiac MRI and CPET are also considered to be routine clinical tests and if requested will not incur additional costs to the source of funding of this study. Fibroscan will be performed by the research registrar and will therefore also incur no extra cost.

Patients will be reimbursed for their travel/parking/food expenses on the day up to a maximum agreed fee of £20.

The ELF Panel will be analysed by the Royal Free Hospital when this becomes available. Discussions are underway for this test to be provided for free. In the unlikely event that this carries a cost, the F019 Charitable Fund has sufficient funds to meet this cost. Blood will be stored at NNUH until this arrangement is finalised.

The study specific tests have been costed below.

Item	Cost per item	Cost for n = 40	Cost for n = 60
Hyaluronic acid (HA) (Elf Panel)	-	-	-
Procollagen III amino terminal peptide (PIIINP) (Elf Panel)	-	-	-
Tissue inhibitor of metalloproteinase 1 (TIMP-1) (Elf Panel)	-	-	-
Ultrasound Liver	£84.00*	£3360.00	£5040
Magnetic Resonance Elastography of Liver	£90.26*	£3610.40	£5415.60
Travel/Food Expenses	£20.00	£800.00	£1200
Total £		£7770.40	£11655.60

* Cost quoted by Radiology Department for UEA Sponsored research/Academic rate.

Study Plan

R&D/Ethics Approval: November 2015 - December 2015

Data Collection: December 2015 – May 2017

Data Analysis: May 2017 – June 2017

Writing up of Report/Results: June 2017 – July 2017

Draft MD Thesis: April 2017 – July 2017

Supervision for student projects

The academic supervisor for this project is Professor Marcus Flather who will supervise the MD thesis of Dr. Dilip Abraham. Weekly meetings have been arranged with both the clinical supervisors and academic supervisor.

Dissemination and outcome

Results from this observational study will be submitted for publication to a major cardiology journal, and for presentation to relevant cardiology conferences. Papers on other aspects of the study, or sub-studies may be published during the course of the study.

Study Protocol References:

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Appendix B - Ethics Approval

NHS
Health Research Authority
East of England - Cambridgeshire and Hertfordshire Research Ethics Committee
Royal Standard Place
Nottingham
NG1 6FS
Telephone: 01158839390

20 January 2016

Dr Dilip Abraham
Clinical Research Registrar in Cardiology
Norfolk and Norwich University Hospitals NHS Foundation Trust
Norfolk and Norwich University Hospital
Colney Lane
Norwich
NR4 7UY

Dear Dr Abraham

Study title:	**The Hepatic Effects of Right Heart Lesions in Adults with Congenital Heart Disease**
REC reference:	**15/EE/0440**
IRAS project ID:	**189485**

Thank you for your letter of 11th January 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Georgia Copeland, nrescommittee.eastofengland-cambsandherts@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the

study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper [Cover Letter]	1	15 October 2015
Covering letter on headed paper [Cover Letter]	1	08 January 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [UEA-Insurance & Indemnity]	1	20 October 2015
GP/consultant information sheets or letters [GP Letter with trackmarks]	2	04 January 2016
IRAS Checklist XML [Checklist_11012016]		11 January 2016
Letter from funder [Funding Letter]	1	15 October 2015
Letter from sponsor [sponsor email]	1	14 October 2015
Letter from statistician [statistician letter/email]	1	15 October 2015
Letters of invitation to participant [Patient Invitation/Cover Letter]	1	04 January 2016
Participant consent form [Consent form with trackmarks]	4	04 January 2016
Participant information sheet (PIS) [PIS]	6	04 January 2016
Participant information sheet (PIS) [PIS with trackmarks]	6	04 January 2016
REC Application Form [REC_Form_09112015]		09 November 2015
Research protocol or project proposal [Research Protocol]	9	04 January 2016
Summary CV for Chief Investigator (CI) [CV]	1	15 October 2015
Summary CV for supervisor (student research) [Academic Supervisor CV - Marcus Flather]	1	31 October 2015
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Flow Chart]	2	29 October 2015
Validated questionnaire [SF-36 Questionnaire (non-commercial/original version)]	1	15 October 2015

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

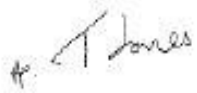
HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/EE/0440	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely



Professor Barry Hunt
Chair

Email:nrescommittee.eastofengland-cambsandherts@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

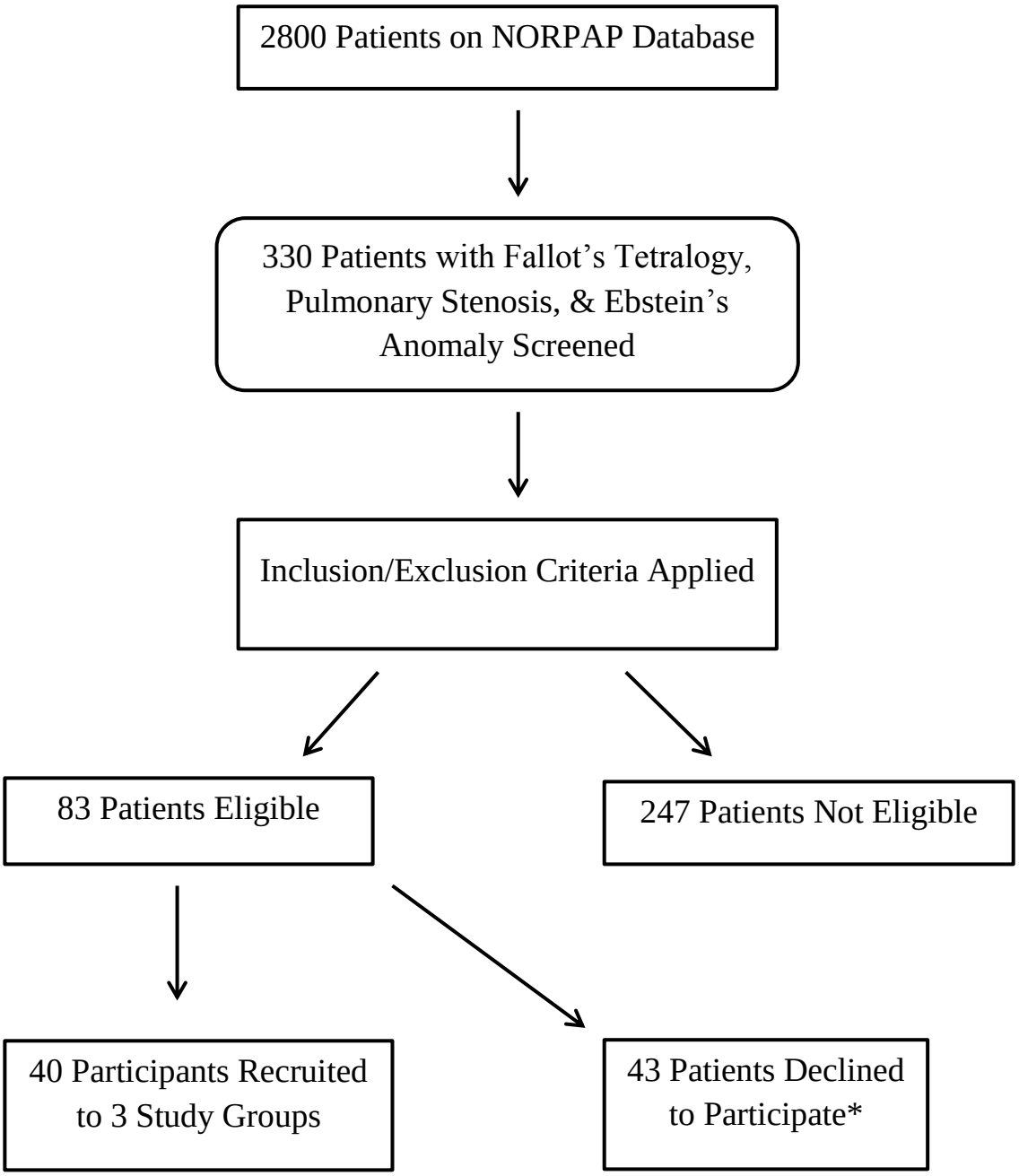
Copy to: *Mrs Deborah Graver*
Mrs Lisa Chalkley, R&D Norfolk and Norwich University Hospital

Appendix C - Glossary of Abbreviations

AT	Anaerobic threshold
CHD	Congenital Heart Disease
CMR	Cardiovascular Magnetic Resonance Imaging
CPET	Cardiopulmonary exercise test
ECG	Electrocardiogram
Echo	Transthoracic Echocardiography
ELF	Enhanced liver fibrosis score/panel
LV	Left ventricle
LVEDV	Left ventricular end diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end systolic volume
LV SV	Left ventricular stroke volume
MRE	Magnetic resonance elastography of liver
MRI	Magnetic resonance imaging
MVO_2	Maximum volume of oxygen consumed
NNUH	The Norfolk & Norwich University NHS Foundation Trust
NT-Pro-BNP	N-terminal prohormone of brain natriuretic peptide
O_2 Pulse	Oxygen pulse
PR	Pulmonary regurgitation

PVR	Pulmonary Valve Replacement
PS	Pulmonary stenosis
QRS	QRS duration on 12 lead electrocardiogram
r	Pearson's correlation coefficient
R^2	Coefficient of Determination
RV	Right ventricle
RVEDV	Right ventricular end diastolic volume
RVEF	Right ventricular ejection fraction
RVESV	Right ventricular end systolic volume
RVOTO	Right ventricular outflow tract obstruction
RV SV	Right ventricular stroke volume
RVSP	Right ventricular systolic pressure
TOF	Tetralogy of Fallot
TR	Tricuspid Regurgitation
UEA	University of East Anglia
US	Ultrasound
VE/VCO_2	Minute ventilation to carbon dioxide ratio
VSD	Ventricular septal defect

Appendix D - Participant Selection



Flow Chart depicting screening and recruitment process. * Background information on eligible patients not recruited shown in tables that follow.

Characteristics of eligible patients not recruited (N=43)

Diagnoses

- Fallot's Tetralogy: 21/43
- Pulmonary Stenosis: 14/43
- Ebstein's Anomaly: 8/43

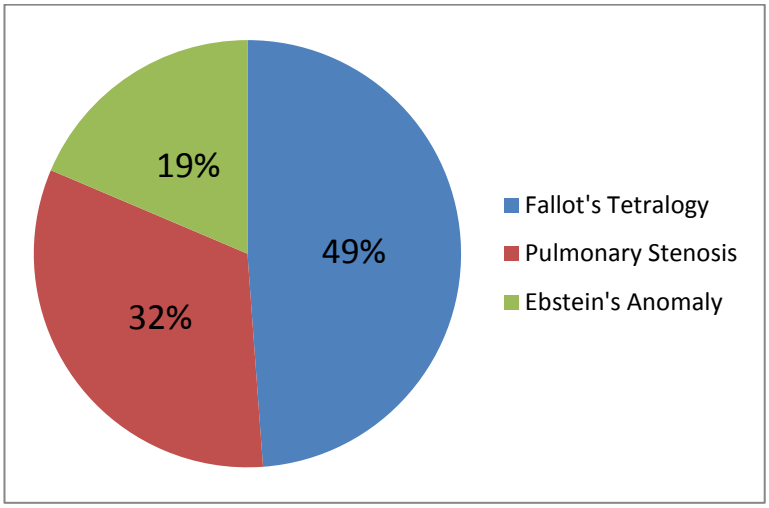


Fig 1 – App.D Distribution of diagnoses in eligible patients.

Planned Study Group Allocation

Screening entailed allocating patients to potential study groups based on clinical details sourced from the NORPAP database, clinic letters and test results.

- Group 1 Moderate to Severe PR: 19/43
- Group 2 Mild PR: 16/43
- Group 3 Ebstein: 8/43

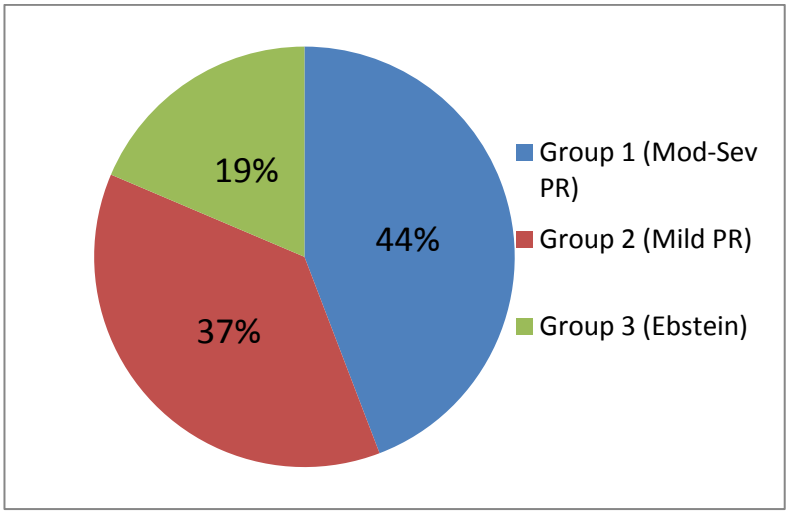


Fig 2 – App.D Planned study group allocation in those eligible but not recruited.

Gender

- Male: 23/43
- Female: 20/43

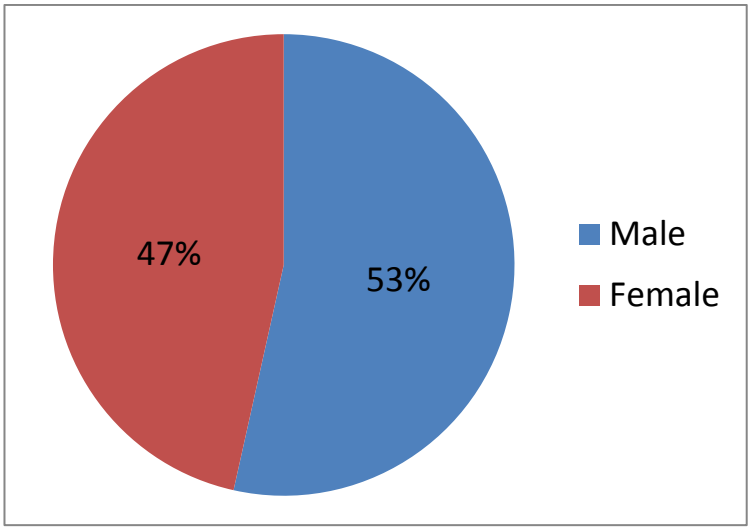


Fig 3 – App.D Gender distribution in those eligible but not recruited

Age

Measure	Years
Minimum	19
Maximum	79
Median	32
Mean	38
Standard Deviation	16

Table 1 – App.D: Age of eligible patients (n=43) not recruited – descriptive statistics.

Eligible Patients – Reason Not Recruited (N = 43)

Reason	Frequency	Percentage
Language Barrier	1	2.3%
Death *	1	2.3%
Frailty	1	2.3%
Did Not Attend to Planned Study Visit	2	6.6%
Childcare Commitments	3	6.9%
Declined **	8	18.6%
Transport/Distance to Study Site ***	9	20.9%
Failed to respond to invitation letter/did not return calls	18	41.8%
Total	43	100%

Table 2 – App.D: Reason eligible patients not recruited

* Died from non-cardiac cause. Did not participate in study.

** Predominantly no interest in participating in research, none cited health reason.

*** Some patients on the database work, study, or had moved away from the vicinity of the study site.

Appendix E - Time Intervals

Time interval between study visit date and cardiac magnetic resonance imaging (CMR)

Most participants underwent all liver investigations on the study visit date. In some this was not feasible and a particular test was rearranged, usually within one month. For simplicity, study visit date is used here to represent the date of all liver assessments.

Intervals in months are shown as positive numbers (i.e. there is no 'start date', particularly as CMR used were clinical investigations organised at the discretion of the responsible consultant and may have pre or post-dated the study visit date.)

Table 1 – App.E: Time interval between study visit date and cardiac magnetic resonance imaging (CMR)

Study ID	Group	Study Visit Date	CMR	Interval CMR to Study Visit (months)
1	1	30.03.16	17.09.15	6
2	1	06.04.16	01.09.15	7
3	1	04.04.16	16.02.17	10
4	1	27.04.16	12.09.16	5
5	1	20.04.16	16.05.17	13
6	1	13.04.16	09.06.16	2
7	1	20.07.16	16.07.16	0
15	1	29.06.16	01.06.16	0
18	1	01.06.16	24.12.15	6
25	1	12.10.16	23.04.15	18
30	1	27.07.16	23.10.16	3
31	1	29.06.16	01.04.16	2
33	1	10.08.16	23.01.16	7
35	1	14.09.16	11.11.15	10
49	1	28.12.16	18.05.17	5
17	1	20.04.16	15.08.16	4
8	2	10.08.16	05.12.16	4
9	2	11.05.16	22.08.16	3
11	2	27.04.16	21.07.16	3

12	2	11.05.16	10.12.16	7
13	2	18.05.16	17.07.15	10
14	2	01.06.16	05.09.16	3
16	2	26.10.16	29.09.14	25
19	2	25.05.16	08.09.16	4
20	2	06.04.16	27.06.16	2
22	2	22.06.16	31.08.16	2
23	2	15.06.16	12.03.17	9
27	2	03.08.16	02.02.15	16
28	2	27.07.16	19.09.16	2
29	2	29.06.16	13.04.15	14
34	2	27.07.16	12.09.16	2
36	2	21.09.16	07.11.16	4
38	3	19.10.16	18.12.14	22
37	3	28.09.16	01.09.16	0
39	3	05.10.16	09.12.16	2
40	3	14.09.16	15.10.16	1
43	3	28.09.16	10.11.16	2
44	3	05.10.16	10.11.16	1
46	3	09.11.16	09.02.16	9
48	3	07.12.16	25.11.16	1
Range				**0-25**
Median				**4**
Mean				**6**
Standard Deviation				**6**

Time Intervals – Corrective Surgery to Study Inclusion

Comparative tables showing date of corrective surgery and study visit date for those with pulmonary regurgitation. Time intervals are shown in years. Where participants had multiple procedure, the date of corrective surgery is used (rather than prior palliative shunts for instance).

Table 2 - App.E: Time interval between date of surgery and study visit date for participants in Group 1 (Mod-Sev PR).

Group	Study ID	Date of Surgery	Study Visit	Interval - Years
1	1	1995	30.03.16	21
1	2	1973	06.04.16	43
1	3	1993	04.04.16	23
1	4	1982	27.04.16	34
1	5	1983	20.04.16	33
1	6	1967	13.04.16	49
1	7	1988	20.07.16	28
1	15	1972	29.06.16	44
1	18	1988	01.06.16	28
1	25	1969	12.10.16	47
1	30	1983	27.07.16	33
1	31	1984	29.06.16	32
1	33	1986	10.08.16	30
1	35	1994	14.09.16	22
1	49	1998	28.12.16	18
1	17	1964	20.04.16	52
Range				**18-52**
Median				**32**
Mean				**34**
Standard Deviation				**10**

In Group 2 (Mild PR), 3 participants had pulmonary valve replacement as adults (highlighted with asterix *). In these subjects, date of pulmonary valve replacement (rather than initial corrective repair date) is shown.

Table 3 - App.E: Time interval between date of surgery and study visit date for participants in Group 2 (Mild PR).

Group	Study ID	Date of Surgery	Study Visit	Interval - Years
2	***8**	**2014**	**10.8.16**	**2**
2	9	1984	11.05.16	32
2	11	1971	27.04.16	45
2	12	1967	11.05.16	39
2	13	1976	18.05.16	40
2	14	1973	01.06.16	43
2	16	1974	26.10.16	42
2	19	1973	25.05.16	43
2	***20**	**2009**	**06.04.16**	**7**
2	22	2000	22.06.16	16
2	23	1981	22.06.16	35
2	27	2002	03.08.16	14
2	***28**	**2010**	**27.07.16**	**6**
2	29	1975	29.06.16	41
2	34	1991	27.07.16	25
2	36	1989	21.09.16	27
Range				**2-45**
Median				**33**
Mean				**28**
Standard Deviation				**15**

Appendix F - Right Ventricular Restrictive Physiology

Table 1 - App.F: Right ventricular restrictive physiology from transthoracic echocardiography for those with pulmonary regurgitation

Group 1 (Mod-Sev PR)		**Group 2 (Mild PR)**	
Study ID	**RV Restrictive Physiology**	**Study ID**	**RV Restrictive Physiology**
1	No	8	No
2	No	9	No
3	No	11	No
4	No	12	No
5	No	13	No
6	No	14	No
7	No	16	No
15	No	19	No
18	No	20	No
25	No	22	No
30	Yes	23	No
31	Yes	27	Yes
33	No	28	No
35	Yes	29	No
49	No	34	No
17	No	36	No
Frequency	**3/16 = 18.75%**		**1/16 = 6.25%**