PhD (Clinical Research) Thesis

Insights from **R**ight and **L**eft Ventricular **M**echanics During Exercise Stress in Relation to Functional Capacity and Recovery Following Surgery in Patients with Tetralogy of Fallot and Pulmonary Regurgitation

(IRLM-TOF)

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Statement of Originality

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Details of publications:

- **Alborikan S,** Pandya B, Von Klemperer K, Walker F, Cullen S, Badiani S, Bhattacharyya S Lloyd G. "Cardiopulmonary Exercise Test (CPET) in patients with repaired Tetralogy of Fallot (rTOF); A systematic review." International Journal of Cardiology Congenital Heart Disease 1 (2020): 100050.

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Abstract

Long term complications management of adult patients with repaired TOF mainly result from chronic severe pulmonary regurgitation (PR). Guidelines are based on observational data and highlight the balance between symptoms, biventricular volume, and severity of PR as predictors of exercise ability or indication for reintervention. The determinates of exercise capacity in this population remain poorly understood, so I undertook two formal meta-analyses which showed clearly that there was a marked reduction in exercise capacity and this reduced exercise capacity was associated with increased serum levels of blood biomarker (plasma brain natriuretic peptide, NT-proBNP). I have demonstrated that elevated serum levels of NT-proBNP were not age or sex related, but they were dependent on surgical selection and advanced surgical techniques in addition to their direct link to higher risks of adverse cardiovascular outcomes in a large percent of TOF population. In this thesis, I sought to critically evaluate the unique capabilities of combined cardiopulmonary exercise testing (CPET) and echocardiography to describe right and left ventricle structure, volume, function and provide mechanistic insights of echocardiography in a prospective cohort, in addition to gather data from normal individuals to better understand RV augmentation during stress echocardiography. Biventricular functional mechanics in these patients are complex so I sought to understand these in more detail by critically evaluating the best haemodynamic determinants of exercise capacity. I extended the methodology further by constructing sub studies to evaluate functional mechanical techniques sometimes for the first time in this context, so I have put special emphasis on describing the reproducibility and coefficient of variations for these techniques. Finally, I undertook a novel study to evaluate blood biomarkers including NT-proBNP and soluble suppression of tumorigenicity-2 (sST2) to understand their relation to structural and functional parameters in this population. In this thesis, I have demonstrated that the relationship between exercise ability and myocardial function is complex, but not predominantly effected by the severity of PR and is more a function of contractile reserve potential of the right and left ventricles in adult survivors of TOF.

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I would like to thank all of the patients who took part in the project and contributed to this thesis. Without their participation, I would not have been able to successfully progress through various levels of my PhD. I am extremely grateful to the Saudi Arabian government and Ministry of Health who funded my doctoral research fellowship.

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Abbreviations

CPET= Cardiopulmonary Exercise Test

VO_2 (ml/min) = Peak absolute maximum oxygen uptake

VO_2 (ml/kg/min) = Peak relative maximum oxygen uptake

VO_2 (%) = Peak percent of predicted maximum oxygen uptake

RER=Respiratory exchange ratio

SPR= Severe pulmonary regurgitation

RV= Right ventricle

LV= Left ventricle

TAPSE= Tricuspid annular plane systolic excursion

FAC= Fractional area change

RVS'= Right ventricular systolic velocity

RVGLS= Right ventricular global longitudinal strain

RVGFWS= Right ventricular global free wall stain

RVEDV= Right ventricular end diastolic volume

RVEDVI= Indexed right ventricular end diastolic volume;

RVESV= Right ventricular end systolic volume

RVESVI= Indexed right ventricular end systolic volume

RVEF= Right ventricular ejection fraction

RVSV= Right ventricular stroke volume

4D RVSVI= Indexed right ventricle stroke volume

PG= Pulmonary regurgitation pressure gradient

PHT= Pulmonary regurgitation pressure half time

LVS'= Left ventricular systolic velocity

LVEF= Left ventricular ejection fraction

MAPSE= Mitral annular plane systolic excursion

LVGLS= Left ventricular global longitudinal strain

LVEDV= Left ventricular end diastolic volume

LVEDVI= Indexed left ventricular end diastolic volume

LVESV= Left ventricular end systolic volume

LVESVI= Indexed left ventricular end systolic volume

LVEF= Left ventricular ejection fraction

LVSV= Left ventricular stroke volume

LVSVI= Indexed left ventricular stroke volume

ΔRVS'= Contractile reserve of right ventricular systolic velocity

ΔFAC= Contractile reserve of fractional area change

ΔTAPSE= Contractile reserve of tricuspid annular plane systolic excursion

ΔLVS'= Contractile reserve of left ventricular systolic velocity

ΔLVGLS= Contractile reserve of left ventricular global longitudinal strain

SES-VO_2 /S'= Systolic Efficiency Slope of average slope of LV systolic longitudinal function to oxygen uptake

MES-VO_2/GLS= Myocardial Efficiency Slope of average slope of LV global longitudinal stain function to oxygen uptake

LES= Longitudinal Efficiency Slope of average RV longitudinal function to oxygen uptake

LVMW= Left ventricle myocardial work

GWI= Global work index

GCW= Global constructive work

GWW= Global wasted work

GWE= Global work efficiency

LV MD= Left ventricular mechanical dispersion

RV MD= Right ventricular mechanical dispersion

NT-proBNP= Plasma brain natriuretic peptide

sST2= Soluble suppression of tumorigenicity-2

Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Chapter 9

Chapter 10

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Chapter 9

Chapter 10

Chapter 1 Introduction and literature review

1.1 Tetralogy of Fallot (TOF)

Tetralogy of Fallot (TOF) is the most common form of cyanotic congenital heart disease (CHD) with incidence approaching 10 percent of all forms of CHD that occurs equally in female and male (Perloff and Marelli, 2012, Sahn, 2001). It was first described by Niels Stenson in 1671 and William Hunter who have illustrated a more precise anatomical description at St Georges Hospital Medical School in London in 1784 (Steno, 1671). It is a combination of anatomical congenital malformations including a mal-alignment of the septum leading to a sub aortic ventricular septal defect (VSD) with the overriding of aorta, right ventricular outflow tract (RVOT) obstruction and consequent right ventricular hypertrophy (Fallot, 1888) (Figure 1.1). These malformations occur as a result of anterior and cephalad deviation of the infundibular septum. A non-restrictive VSD is located in the peri membranous region of the septum and rarely can extend to the muscular region (Figure 1.1). Associated cardiac anomalies occur in 40 percent in TOF patients in which the right aortic arch accounts for approximately 25 percent and abnormalities of the coronary arteries which occur in 9 percent (Perloff and Marelli, 2012, Dabizzi et al., 1980). Occasionally, patients will have aortopulmonary collateral, patent ductus arteriosus, complete atrioventricular septal defects, and multiple ventricular septal defects (Perloff and Marelli, 2012).

The degree of RVOT, the relative pressures in the right and left ventricles, and the proportion of aorta overriding will control the pathophysiological consequences and the clinical presentation of TOF. Acyanotic patients (pink Fallot) are presented with a predominately left-to-right shunt if the resistance to blood flow across the RVOT obstruction is less than the resistance across the aorta, while cyanotic patients with right-to-left shunt which occurs with higher degree of obstruction that can result in a large volume of desaturated blood in the systemic circulation (Fallot, 1888, Perloff and Marelli, 2012).

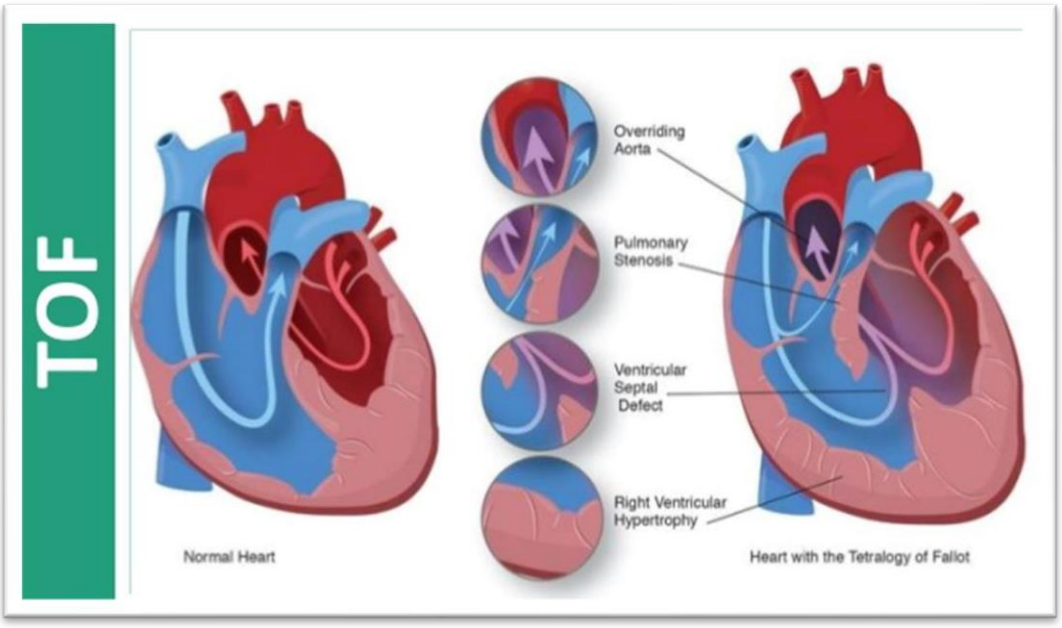


Figure 1. 1. Characteristics of TOF in relation to normal heart (Anderson and Weinberg, 2005).

Complete surgical correction in these patients is usually performed electively in the first year of life, typically before six months of age (Al Habib et al., 2010). In a minority of infants, palliative shunts before surgical correction are required in infants with severe RVOT obstruction and in those who cannot undergo intra-cardiac repair. The main expected outcomes from the primary surgery are complete separation between pulmonary and systemic circulation by VSD patch closure, enlargement of the RVOT by complete relief of pulmonary flow obstruction through resection of infundibular and sub-infundibular muscle bundles and/or by a transannular patch if needed (between the right ventricle (RV) into the pulmonary arteries), maintaining the right ventricular function and minimising the post-operative pulmonary valve incompetency (Al Habib et al., 2010, Gerrah et al., 2015).

1.2 Late functional outcomes after repair in adult patients with TOF

Successful long-term outcomes with survival rates of up to 85 percent with advances in operative techniques, diagnosis and postoperative outcomes have been observed in adult patients with repaired TOF (Gerrah et al., 2015, Ternestedt et al., 2001, Bacha et al., 2001). A randomised large centered study of 734 patients who underwent primary repair in early childhood (median age 17 months) with median follow-up of 12.5 years between 1986 and 2007, has reported an excellent survival rate of 95, 93 and 93 percent at 10, 20 and 25 years respectively (Park et al., 2010). This was also observed in other large cohorts (Lindberg et al., 2011, Engelfriet et al., 2005, Hickey et al., 2012, Frigiola et al., 2013). Despite excellent long-term outcomes, about one-third of adult survivors of TOF repair patients require reoperation and most commonly for pulmonary valve replacement (PVR) (Park et al., 2010, Hickey et al., 2012, Frigiola et al., 2013).

Adult patients with surgically repaired TOF are an increasing population and the knowledge about late clinical outcomes is still evolving. Although the haemodynamic burden following TOF repair is often tolerated well during childhood and adolescence, the incidences of exercise intolerance, arrhythmias, heart failure and death are seen frequently in adulthood (Khairy et al., 2010, Nollert et al., 1997). Pulmonary regurgitation (PR) is a frequent adult complication following childhood repaired TOF patients, leading to progressive RV dilatation and dysfunction, exercise intolerance, arrhythmia and sudden cardiac death (Wijesekera et al., 2016). Chronic PR can result after the primary intra-cardiac repair and in those who have monocusp valves, or who have conduit valves from the right ventricle to pulmonary artery (Nollert et al., 1997). PR with subsequent ventricular dilation have been identified as an important independent determinants of arrhythmia (Bacha et al., 2001). RV dilatation and dysfunction caused by surgical approach, such as RVOT patch insertion, infundibulectomy and PR, carry a significant risk of morbidity and mortality late after repair of TOF ((Valente et al., 2014a, Valente et al., 2014b). In the setting of long-standing severe PR, global RV dysfunction results from failure of compensatory mechanisms to accommodate chronic volume and/or pressure loads. Although the stages of RV dysfunction have been studied in detail, the pathophysiology of RV dysfunction in late survivors of TOF repair remains incompletely understood.

Left ventricular (LV) function is also frequently abnormal and progression to symptomatic heart failure is extremely variable. Furthermore, abnormalities of the LV caused by ventricular interdependence are frequent (Dragulescu and Mertens, 2010). LV dysfunction is linked to adverse outcome in patients with repaired TOF and it is a poor prognostic factor in this population (Wijesekera et al., 2016, Orwat et al., 2016, Stanton et al., 2009). The mechanism of changes in LV volume and function is not known, however, shared myocardial fibers between ventricles and the influence of septal shift caused by RV volume and pressure overload, may account for the LV dysfunction (Tzemos et al., 2009). Ventricular-ventricular interaction has been used to define the association between worsening of RV dysfunction and deterioration of LV dysfunction (Valente et al., 2014a). Therefore, early assessment of RV and LV function should be a part of comprehensive risk scores together with clinical history and exercise capacity for effective management in adult patients with repaired TOF.

1.3 Pulmonary valve replacement (PVR)

PVR has become the commonest re-intervention in repaired TOF although patient selection to undergo intervention remains controversial (Dragulescu et al., 2014, Orwat et al., 2016). Bioprosthetic pulmonary valve replacements whether surgical or transcatheter typically last approximately 10 years and clinicians have to weigh up the risks of committing the patient to several further pulmonary interventions versus chronic RV overload leading to RV dysfunction. Currently literature and guidelines use the combination of symptoms and ongoing adverse remodelling of RV and volumetric measurements to determine optimum timing of surgical intervention (Frigiola et al., 2013, Oosterhof et al., 2007). PVR in these patients is important to restore pulmonary valve competency and RV function (Buechel et al., 2005, Ferraz Cavalcanti et al., 2013). It can be performed either surgically or percutaneously; surgical PVR has an excellent reported survival of 97, 96 and 92 percent at one, three and five years of follow up (Babu-Narayan et al., 2014). PVR with pulmonary homograft is associated with good long-term outcomes without the need for anticoagulation if it is compared with the mechanical prosthesis (Oosterhof et al., 2007). However, mechanical prostheses have the advantages of low re-operation rates. More recently, percutaneous pulmonary valve replacement has been shown its efficiency with low mortality and morbidity rates (Khambadkone et al., 2005).

However, the optimal timing of PVR with severe PR is contentious, as the decision should be balancing between the risks of re-intervention and irreversible RV failure.

Ideally, PVR should be considered before irreversible RV changes, as RV function together with exercise capacity may not improve after surgery in the presence of severe RV dysfunction (Lee et al., 2012, Ammash et al., 2007, Therrien et al., 2000). Randomised evidence covering this concept is lacking and the current models poorly describe the relationship between symptoms, right ventricular volumes, and postoperative RV reverse remodelling (Oosterhof et al., 2007, Nakamura et al., 2014). After PVR, cardiac performance enhancement is expected and this has been confirmed by many studies (Buechel et al., 2005, Vliegen et al., 2002, Frigiola et al., 2006). Although it is known that PVR may result in reduction of RV volume, it is still unknown whether a threshold can be found after which no RV remodelling occurs after PVR (Therrien et al., 2005, Geva et al., 2010, Buechel et al., 2005, Oosterhof et al., 2007). Moreover, it is yet to be clarified if the presence of preservation of normal RV volume is associated with better long-term prognosis in these patients. According to data using magnetic resonance imaging (MRI), the threshold degree for RV end diastolic volume is 160 to 170 ml/m^2 and 80 to 85 ml/m^2 for end systolic volume beyond which the restoration of RV volume cannot be achieved (Therrien et al., 2005, Lee et al., 2012).

It is worth restating that physical performance in these patients is reduced and symptoms are related to RV dilation and dysfunction (Chiu et al., 2012). As a result, in the last 15 years this has been a marked increase in asymptomatic patients undergoing PVR. The literature documenting the improvement in either symptoms or prognosis is, however, scarce and sometimes conflicting. One meta-analysis found that PVR is effective in reducing RV volume and PR fraction but with no direct effect on RV ejection fraction (Cheung et al., 2009). Similarly, in more recent study observed a reduction in RV volume after re-intervention with no improvement in the RV function by using cardiovascular magnetic resonance imaging (Hallbergson et al., 2015). It is important to mention that identifying a clear cut-off point for RV size and volume below which complete reverse remodelling would persist was unreachable (Wijesekera et al., 2016). The current focus has moved towards earlier re-intervention before developing RV dysfunction through early assessment of right and left ventricular size and function by the current feasible advanced cardiac imaging for more objective evaluation before

the onset of the symptoms. However, literature remains unclear about the exact haemodynamic improvement after PVR which makes the identification of the right timing of reintervention a complex approach in this population.

1.4 Role of echocardiography in adult patients with repaired Tetralogy of Fallot

Echocardiography has become the primary non-invasive imaging tool in the diagnosis and assessment of cardiac function in patients with CHD, including patients with repaired TOF. It is an ideal tool for anatomical evaluation, haemodynamic and myocardial strain mechanics assessment in these patients. It is the most effective first line modality for qualitative and quantitative assessment of the right ventricle, due to its non-invasive nature, portability and efficiency in providing reliable information about global and regional myocardial function. Right ventricular dysfunction is the key determinant outcome in TOF patients and the assessment of RV size and function is mandatory. It is an ideal tool for longitudinal follow up, however, RV volumetric and functional measurements assessed by echocardiography remains a challenge due to its complex geometry and limited acoustic windows in many patients (Ho and Nihoyannopoulos, 2006, Haddad et al., 2008, Valente et al., 2014a). Conventional echocardiographic techniques for the evaluation of the anatomical, haemodynamic changes and myocardial strain mechanical changes based on 2D modalities or M-mode are load dependent and technically challenging. Additionally, their inability to provide any regional assessment for both RV and LV function is considered an important limitation (Kalogeropoulos et al., 2009).

A routine echocardiography is recommended every two years in adult patients with repaired TOF (Valente et al., 2014a). The main areas that need to be covered in each comprehensive echocardiographic examination for adult patients with TOF are: severity of pulmonary regurgitation, presence and size of any residual septal defects, detection of any residual RVOT obstruction, right and left ventricular size and function, coronary anatomy and detection of any aortic root dilation and/or regurgitation (Valente et al., 2014a). Despite the presence of current guidelines for RV assessment in these patients, only limited information exists on the reproducibility and prognostic value of echocardiographic parameters in adult patient with repaired TOF.

1.4.1 Key echocardiographic parameters

1.4.1.1 Right ventricle

2D modality is the most common used imaging modality to interpret an echocardiogram which allows qualitative and quantitative evaluation of the right size in these patients including right atrium, right ventricle, pulmonary arteries (PAs), RVOT, pulmonary valve (PV), tricuspid valve, and atrial and ventricular septa, and RV longitudinal function. Doppler echocardiography is used for haemodynamic assessment of the RV and PA pressures assessment. The most important functional parameters for RV are; systolic longitudinal velocity (RVS') using tissue Doppler imaging (TDI), tricuspid annular plane systolic excursion (TAPSE) using M-mode, RV fractional area change (FAC) using 2D, and RV longitudinal strain using two-dimensional speckle-tracking deformation imaging (2DSTE) (Mor-Avi et al., 2011). RVS' is used to calculate peak RV systolic velocity (Figure 1.2). TAPSE is used to calculate the global RV longitudinal function by measuring the distance between maximum and minimum excursion in the lateral tricuspid annulus using M-mode obtained from a four-chamber apical view (Figure 1.3). FAC is used to calculate the global index of RV function by measuring the area difference between end-diastole and end-systole from apical 4-chamber view (Figure 1.4). However, they are both limited by angle, loading dependency and their ability to measure the global function only.

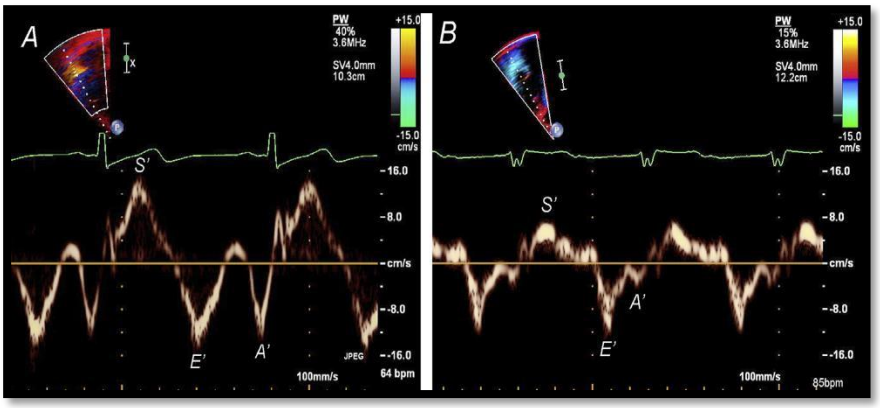


Figure 1. 2. Normal tissue Doppler imaging for RV systolic function (a) vs abnormal RV systolic function (b) (Mor-Avi et al., 2011).

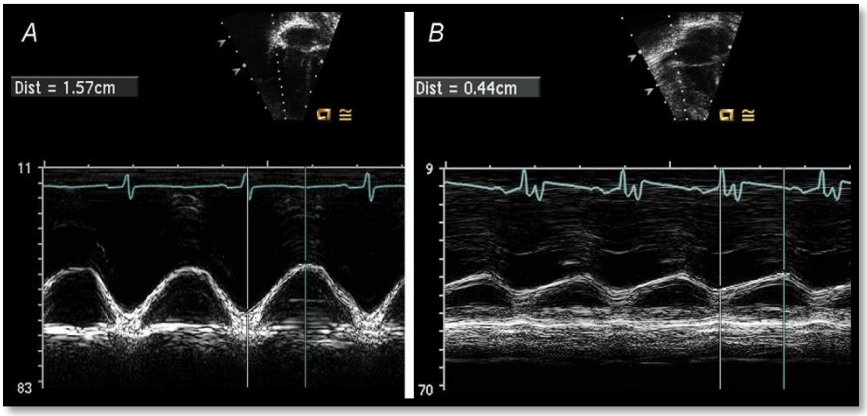


Figure 1. 3. TAPSE of a normal (a) and (b) reduced RV systolic function (Rudski et al., 2010).

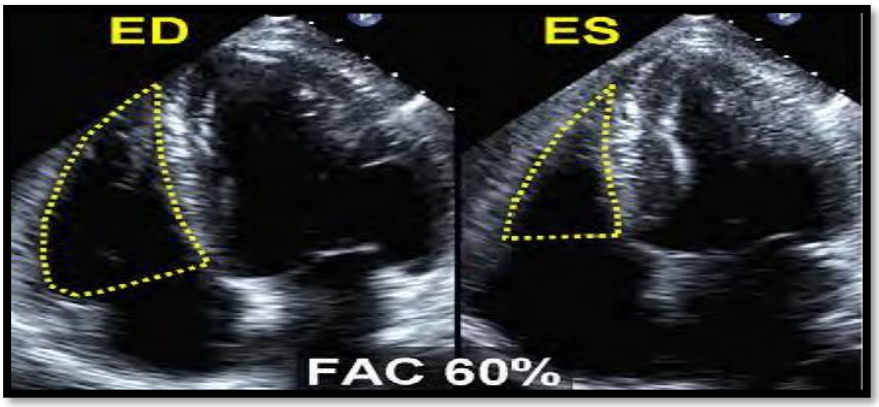


Figure 1. 4. RV FAC calculation in apical 4-chamber view in normal subject (Rudski et al., 2010).

There are two principles echocardiographic techniques to study myocardial deformation; tissue Doppler imaging (TDI) and speckle tracking echocardiography (STE) (Friedberg and Mertens, 2009). TDI has higher temporal resolution compared to STE; however, it is limited by load and angle dependency, but STE has lower inter and intra observer variabilities (Koopman et al., 2010, Dragulescu and Mertens, 2010, Forsey et al., 2013).

For RV volume, recent advances in four-dimensional modality have significantly increased the accuracy and reproducibility of RV volume, and ejection fraction (Kjaergaard et al., 2006). 4D modality is a new comprehensive assessment for RV and it is a computerised automated or semi-automated detection software. The software assesses RV endocardial borders in three views (apical 4 chamber, coronal, and basal-short axis views). Manual adjustment of RV endocardial border can be used when needed. The software automatically calculates a final data panel that displays right ventricle end diastolic volume (RVEDV), indexed right ventricular end diastolic volume (RVEDVI), right ventricle end systolic volume (RVESV), indexed right ventricle end systolic volume (RVESVI), right ventricle stroke volume (RVSV), indexed right ventricle stroke volume (RVSVI), right ventricle ejection fraction (RVEF), tricuspid annular plane systolic excursion (TAPSE), and fractional area change (FAC) (Figure 1.5).

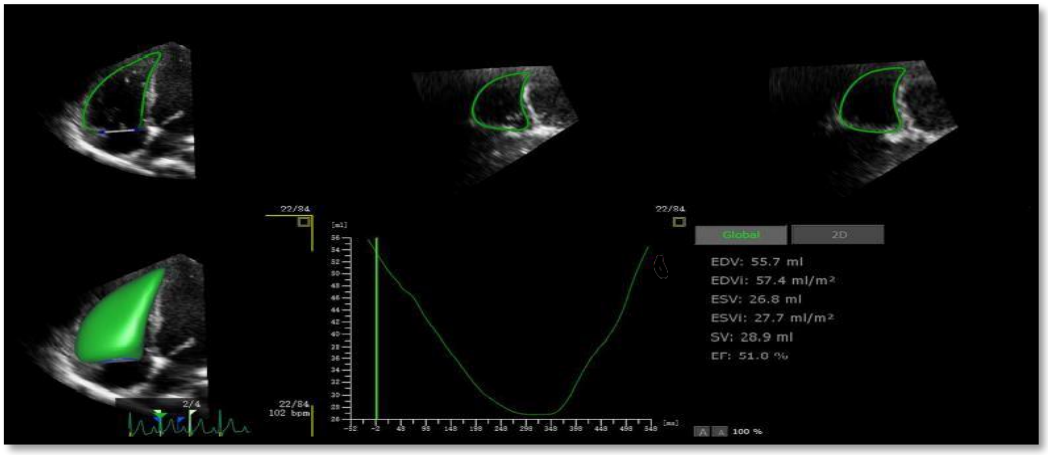


Figure 1. 5. An example of 4D Auto RVQ analysis (adapted from study cases).

1.4.1.2 Left ventricle

Assessment of LV in patients with repaired TOF is essential which include 2D size, function and volume. The most important functional parameters for LV to assess are systolic longitudinal velocity (LVS') using tissue Doppler imaging (TDI), mitral annular plane systolic excursion (MAPSE) using M-mode, and LV global longitudinal strain (LVGLS) using two-dimensional speckle-tracking deformation imaging (2DSTE) (Lang et al., 2015). LVS' is used to calculate maximum LV systolic velocity in TDI imaging (Figure 1.6). MAPSE is used to calculate the global LV function by measuring the distance between maximum and minimum excursion in the lateral mitral annulus using M-mode obtained from a four-chamber apical view (Figure 1.7).

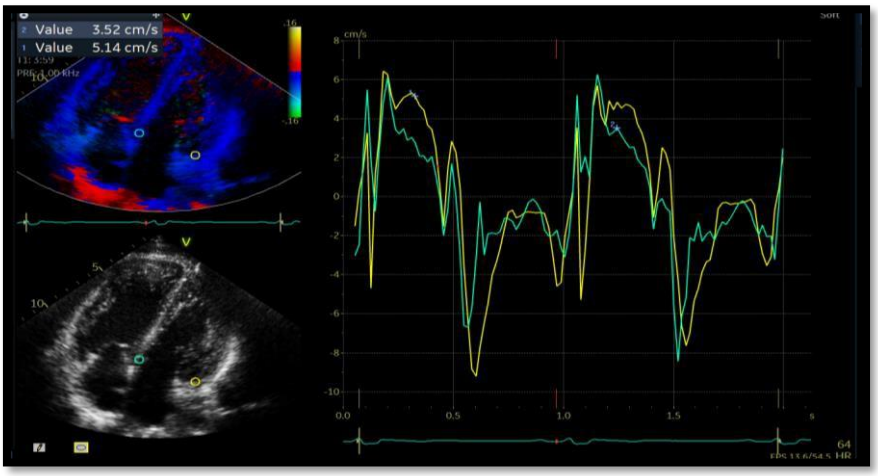


Figure 1. 6. Colour tissue Doppler: an example of abnormal LV systolic longitudinal function (LVS') in the lateral and septal walls (adapted from study cases).

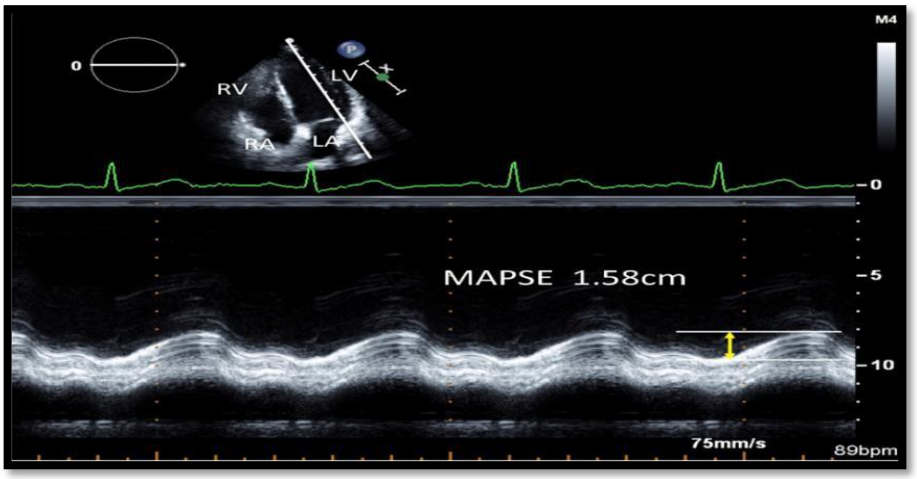


Figure 1. 7. An example of normal LV MAPSE from LV lateral wall by M-mode (MAPSE>10mm, (Lang et al., 2015)).

For LV volume, recent advances in four-dimensional modality have significantly increase the accuracy and reproducibility of LV volume, and ejection fraction (Mor-Avi et al., 2004, Nikitin et al., 2006). 4D modality for LV is a computerised automated or semi-automated detection software. The software requires a manual input of only three points (two points at mitral borders and one at the apex), one in end-diastolic frame, and in end-systolic frame. Manual adjustment of LV endocardial border can be used when needed (Figure 1.8). The software automatically calculates final data panel that displays left ventricle end diastolic volume (LVEDV), left ventricle end systolic volume (LVESV), left ventricle ejection fraction (LVEF), stroke volume, cardiac output, and heart rate values (Figure 1.9).

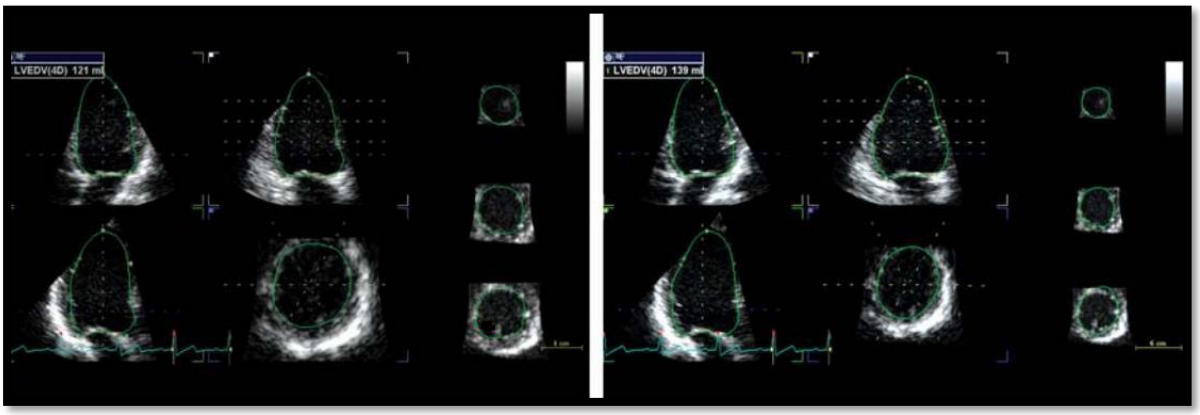


Figure 1. 8. An example of fully automated (left) vs manually corrected (right) endocardial contour of LV using 4D AutoLVQ software (Muraru et al., 2010).

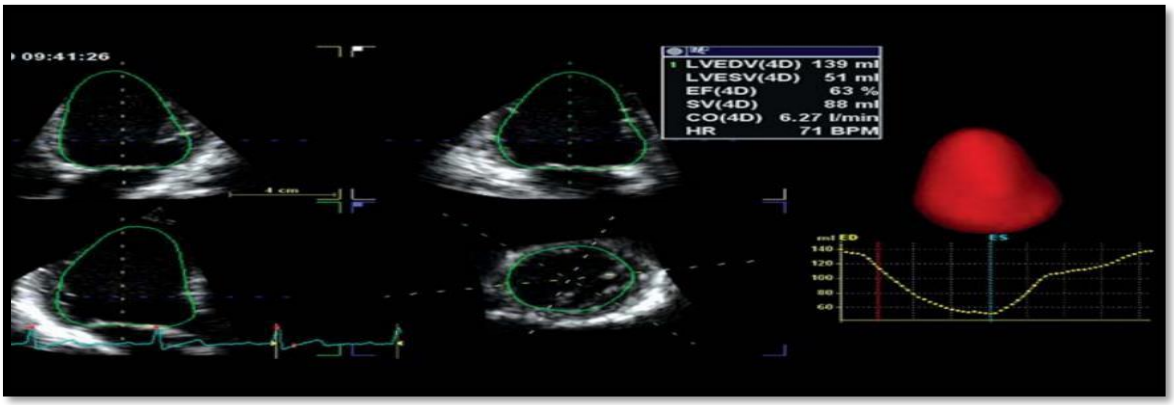


Figure 1. 9. An example of 4D AutoLVQ end results (Muraru et al., 2010).

1.4.2 Longitudinal strain mechanics detected by speckle tracking echocardiography (STE)

New echocardiographic techniques, such as 2D and 4D speckle tracking echocardiography for the assessment of the global and regional right and left ventricular longitudinal function have demonstrated their efficiencies in the field. The ability of these techniques to detect subclinical dysfunction is well recognised in the field of CHD. Furthermore, they have been proved to be better predictor of all-cause mortality than conventional measures of ventricular function and volume with low inter and intra-observer variabilities (Brown et al., 2009, Cho et al., 2006). However, correlation between these echocardiographic novel techniques and cardiac magnetic resonance (CMR) derived velocity vector imaging is still an area of debate. 2D-STE has one limitation, as the speckle can only be tracked in the plane of acquisition not a movable plane motion like 3D STE. Further advances in 4D strain imaging have emerged to provide better insights into the assessment of the global and regional function with better resolution (temporal and spatial). It is a robust tool, as it has proven its concordance with gold standard cardiac magnetic resonance (Seo et al., 2009).

STE is considered the primary modality for the assessment of myocardial deformation in CHD which has a great role in research initiation. The term 'strain' is a dimensionless parameter that defines the direction and local magnitude of the myocardium shortening, lengthening, or thickening. It is the fractional change in the length of the myocardial segments relative to its original length expressed as a percentage (Dandel and Hetzer, 2009). This technique is superior to displacement (TDI-derived strain), as the STE reflects the global and regional function independently and it is angle independent, which can measure myocardial motion in any direction with more reproducible results (Koestenberger, 2012). The term LV global strain refers to the average myocardial deformation of longitudinal, circumferential and radial strain in the LV. While in the RV, strain is defined as the percentage of changes in myocardial deformation (longitudinal shortening).

The muscle fibers in the right ventricle are responsible for chamber movement, which consist of superficial circumferential and endocardial longitudinal fibers that together are responsible for systolic contraction. However, the predominant motion of the RV is the longitudinal direction, as it has a greater role in RV emptying during systole compared to LV. This is believed to be due to the movement of the RV base toward the apex during systole is linked to the contribution of interventricular septum (IVS). This occurs at the same time with RV ejection to pulmonary circulation under the collaboration of IVS (Torrent-Guasp et al., 2001).

Practically, the term of RV global longitudinal strain (RVGLS) is calculated as the percentage of systolic shortening of the RV free wall and the average of septal segmental strain, measured from the apical 4-chamber (Figure 1.10).

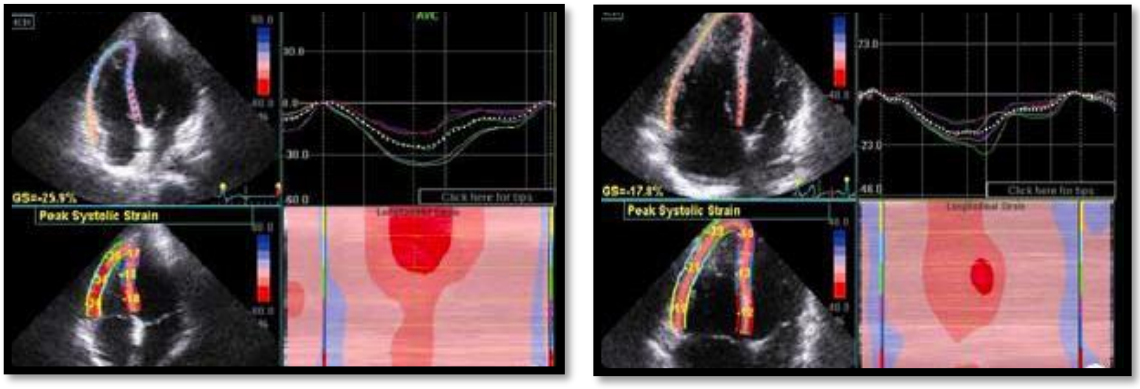


Figure 1. 10. Normal RVGLS in normal (left) versus abnormal in adult patients with repaired TOF (right) (-25%vs -17%) (adapted from study cases).

STE for the LV allows presentation of myocardial deformation in 3 directions: longitudinal, circumferential and radial strain (Mor-Avi et al., 2011, Dandel and Hetzer, 2009) (Figure 1.11). Apical views are used for longitudinal strain while parasternal short axis view is used to obtain circumferential and radial strain (Figure 1.11). The validity testing in 3D and 4D echocardiography in repaired TOF demonstrates a high level of variability (van der Zwaan et al., 2010, Grewal et al., 2010, Khoo et al., 2009). Normal STE reference values are lacking to allow a routine assessment of the 3D and 4D echocardiography for quantitative assessment of the RV and LV function and volume in adult patients with repaired TOF. The current major interests for research should focus on standardisation of the new STE-derived data in clinical practice in this population.

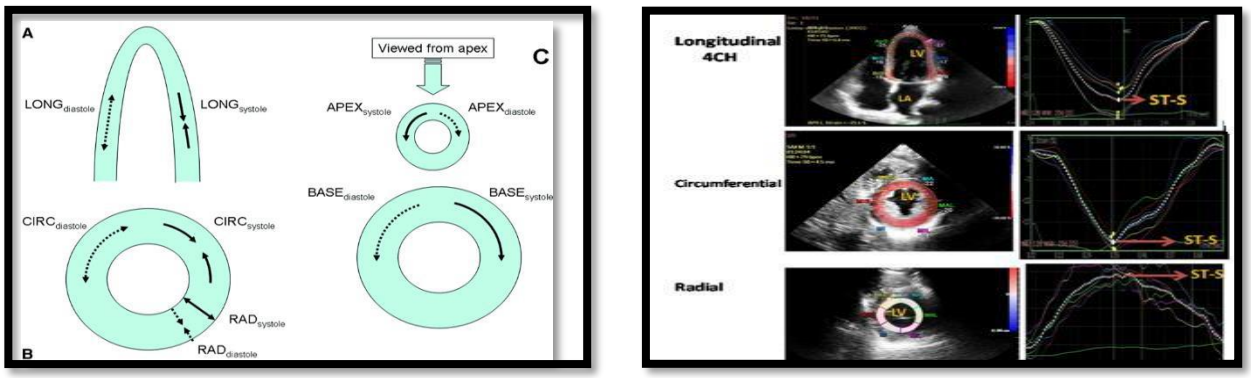


Figure 1. 11. (left) principle of LV myocardial deformation: longitudinal (A) from apical views, circumferential (B) and radial (C) from short axis views. (right) longitudinal, circumferential and radial strain curves of LV deformation (Lang et al., 2015).

Mechanical dispersion (MD) by 2D STE reflecting right and left ventricular contraction heterogeneity that has been suggested as a novel index to predict ventricular arrhythmias and sudden cardiac death (Haugaa et al., 2010a, Haland et al., 2016). However, little is known about biventricular mechanical dispersion in patients with repaired TOF (Thambo et al., 2004). MD is calculated automatically by the same software used for STE build for GLS measurements and defines as the standard deviation (SD) of contraction duration from R wave on electrocardiogram (ECG) to peak negative longitudinal strain (Figure 1.12).

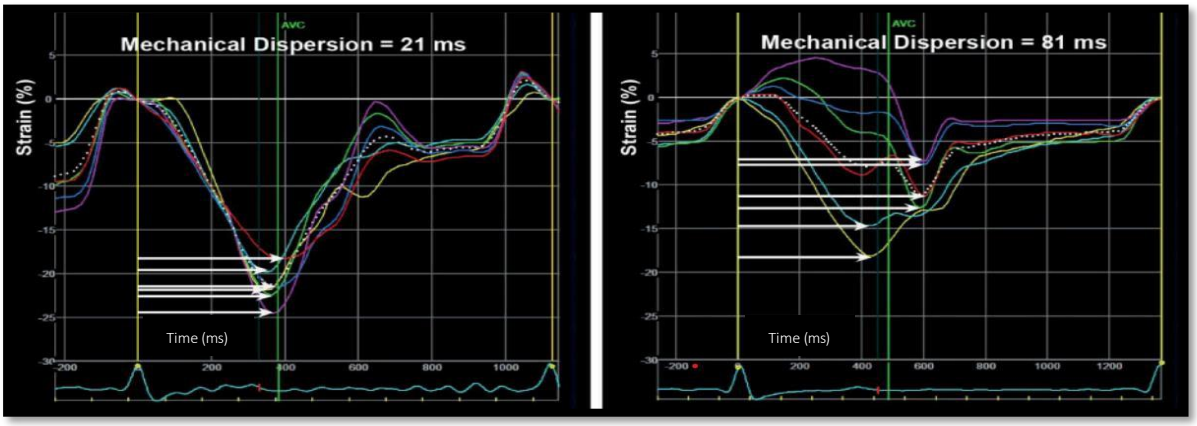


Figure 1. 12. Strain curve in a subject with normal (left), and pathological (right) mechanical dispersion measurements. MD defined as the standard deviation of contraction duration of all segments (adapted from study cases).

The most recent LV assessment is LV myocardial work (MW), which is derived from LV pressure-strain loop analysis incorporating non-invasive blood pressure and strain measures (Russell et al., 2012). It has been suggested that LV myocardial work is superior to LVGLS in identifying patients with acute coronary syndrome (Boe et al., 2015). Despite that, there are very few data on normal values of LV myocardial efficiency in healthy individuals (El Mahdiui et al., 2019), and there is no data to date in patients with repaired TOF. LV MW is an alternative tool to assess cardiac mechanics that is less load dependent and can be measured from the same STE based method (Boe et al., 2015). The components of MW are global constructive work (GCW), global wasted work (GWW), global work index (GWI), and global work efficiency (GWE), that calculated from the apical views (4, 2, and 3 chamber views) and with manual entry of blood pressure (Figure 1.13).

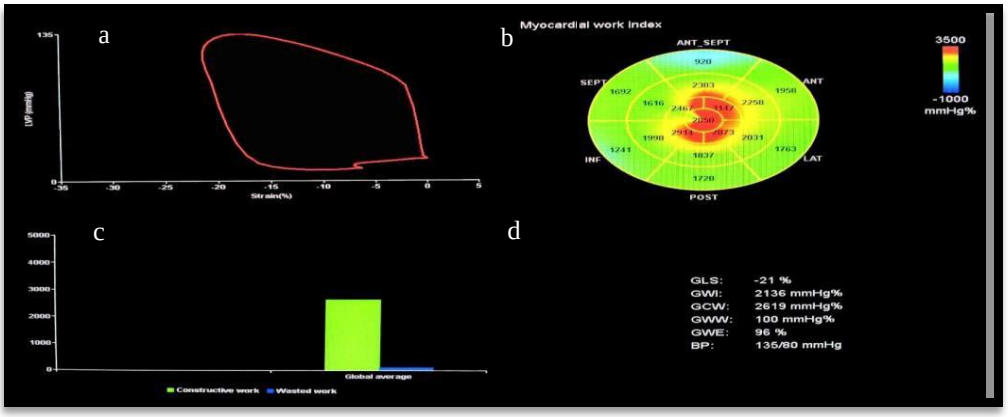


Figure 1. 13. Measurements of normal LV MW. (a) LV pressure-strain loop; (b) Bull's eye of GWI; (c) bar graph representing GCW and GWW; and (d) LV MW results analysis; global constructive work (GCW), global wasted work (GWW), global work index (GWI), and global work efficiency (GWE) (adapted from study cases).

1.5 Role of cardiopulmonary exercise test (CPET) in adult patients with repaired Tetralogy of Fallot (TOF)

Cardiopulmonary exercise testing (CPET) remains a valuable diagnostic and prognostic tool that allows a comprehensive cardiopulmonary assessment, which can predict outcomes in adult patients with TOF. It allows very detailed myocardial assessments when it is combined with echocardiography in response to high physiological demand that can provide objective measures of exercise capacity (Dallaire et al., 2017). Assessment of cardiac myocardial strain and haemodynamical changes by cardiopulmonary exercise test and echocardiography in repaired TOF patients is yet to be investigated, although it has been involved in the prognosis in these patients (Orwat et al., 2016). Reduction in peak oxygen consumption (VO_2) has been associated with poor prognosis in different cardiovascular abnormalities including CHD (Diller et al., 2005). In patients with repaired TOF, VO_2 is a predictor of early mortality and it is related to the need for PVR (Babu-Narayan et al., 2014, Giardini et al., 2007). It is important to mention that there is a lack of understanding in the current practice in how to incorporate exercise prognostic values into management protocols. The literature remains uncertain about the extent of exercise capacity limitations that could indicate the need of PVR in this population.

Poor exercise capacity in adult patients with CHD identifies who is at risk of hospitalisation or death (Diller et al., 2005). To clarify, patients with worse exercise capacity are more likely to be admitted than patients with higher peak VO_2 value. It has been reported that many of these patients even in asymptomatic presentation have exercise intolerance and the underlying mechanisms behind it are not well understood (Mahle et al., 2002, Rowe et al., 1991, Wessel et al., 1980). Additionally, its role in risk stratification for re-intervention and patient selection in adult patients with repaired TOF remains unclear. They tolerate long-standing limited exercise capacity and are often unable to recognise their exercise limitation, and consequently, underreported symptoms will occur. The potential reasons behind the exercise intolerance in adult patients with repaired TOF are multifactorial including pulmonary regurgitation, impaired lung function, chronotropic impairment and ventricular dysfunction (Gatzoulis et al., 1995, Sutton et al., 2008, Roest et al., 2002, Meadows et al., 2007, Fredriksen et al., 2002, Diller et al., 2005).

In this population, there are few studies evaluating the relationship between exercise capacity and its relationship with echocardiographic changes. However, several cross-sectional studies have evaluated exercise capacity in these patients and have shown an average oxygen consumption (VO_2) of 51% to 95% (Mahle et al., 2002, Rowe et al., 1991, Wessel et al., 1980, Gatzoulis et al., 1995, Sutton et al., 2008, Samman et al., 2008, Fredriksen et al., 2002, Buys et al., 2011, Diller et al., 2005). It has to be taken into account that evaluation of peak VO_2 in CHD might be underestimated, as the peak VO_2 is an effort dependent measure. Therefore, evaluation of sub-maximum exercise parameters including ventilatory threshold (VT), oxygen uptake efficiency slope (OUES) and ventilatory efficiency (V_E/VCO_2) is essential in these patients. VT occurs as a consequence of increasing serum lactic acid concentration secondary to anaerobic metabolism once lactate decomposition pathways in the liver have been overcome; the excess acid is buffered by HCO3-/CO_2 causing an increased requirement to expire excess carbon dioxide (Diller et al., 2005). This can be detected by both an increase in VCO_2 slope and ventilation slope. OUES is plotting VO_2 against the logarithm of ventilatory efficiency (VE) and it is clinically useful for two reasons, as it can be obtained from submaximal exercise data and even with minimal data after the ventilator threshold has been achieved. Moreover, VE is the sum of volume of breaths per minute; has been suggested that it can predict event-free survival in patients with repaired TOF, not the mortality or ventricular arrhythmias in a big study (n= 875) who underwent CPET (Diller et al., 2005).

V_E/VCO_2 relationship is a measure of ventilatory efficiency, which reflects the increases in ventilation in response to carbon dioxide (CO_2) production during exercise. A high value of V_E/VCO_2 indicates poor ventilation-perfusion matching and poor cardiopulmonary reserve that can occur in either with structural lung disease or heart failure (Clark et al., 1995). In adult patients with repaired TOF, higher V_E/ VCO_2 slope has been associated with right ventricular dysfunction, pulmonary hypertension, and reduced exercise capacity (Yang et al., 2012). An elevated V_E/VCO_2 is associated with high risk of mortality and hospitalisation and it is known as a strong predictor of poor outcomes in these patients (Giardini et al., 2007). The possible factors that contribute to this slope elevation in these patients are pulmonary blood flow mal-distribution and the presence of ventilatory/perfusion mismatch (Yang et al., 2012). Its association with peak VO_2 has been reported by several cross-sectional studies, which have shown a strong negative correlation between inadequate exercise capacity and the degree of VE to VCO_2 slope elevation (Kipps et al., 2011, Clark et al., 1995). Although the evaluation of maximum and sub-maximum parameters and their association to peak oxygen consumption in adult patients with TOF have been tested in several studies, many of these studies are relatively small cross-sectional studies that cannot be generalised (Mahle et al., 2002, Rowe et al., 1991, Wessel et al., 1980, Gatzoulis et al., 1995, Sutton et al., 2008, Samman et al., 2008).

The evaluation of echocardiographic haemodynamic changes during exercise in adult patients with repaired TOF and their associations with exercise parameters are important in prognosis that could become a powerful tool in decision-making for re-intervention and also for sudden cardiac death prevention (Giardini et al., 2007). The effect of chronic PR on RV function and size in these patients has been recognised as an understudied area due to the lack of reliable tools to measure specific variables. Furthermore, this contributes to the uncertainty in the timing of PVR in these patients. With the development of CPET, its efficiency in predicting prognosis, and its promising ability to disclose abnormal echocardiographic haemodynamic changes will consequently feed the ongoing debates in the field (Eyskens et al., 2000, Yang et al., 2015). Inconsistent findings were reported when evaluating the effect of PR on RV size and haemodynamic functional changes during exercise (Rebergen et al., 1993, Niezen et al., 1996, Helbing et al., 1996). Furthermore, it was shown that there was limited impact of RV dilation on exercise performance when biventricular function is preserved (Davlouros et al., 2002, Silvilairat et al., 2011). So, the presence of biventricular dysfunction has been recognised as a marker of exercise capacity impairment.

In the literature, there is limited evidence to support the use of stress echocardiography to better understand RV function in both adults and children with repaired TOF (Ait-Ali et al., 2014, Apostolopoulou et al., 2007), while cardiopulmonary exercise testing with magnetic resonance has been used to unmask latent RV dysfunction (Parish et al., 2013). Several studies have been conducted in assessing the functional changes by stress echocardiography using conventional techniques to unmask biventricular function even in asymptomatic patients (Ait-Ali et al., 2014, Baspinar and Alehan, 2006, Roche et al., 2010). Nevertheless, in terms of myocardial strain mechanics evaluated by advanced echocardiographic techniques and their association to exercise capacity, very limited data has been proposed (Cifra et al., 2015). One study that has evaluated LV strain found that circumferential strain has a critical role in determining peak VO_2 in patients with repaired TOF (Cheung et al., 2009). Moreover, many authors have demonstrated the impact of different LV parameters on exercise capacity while RV derived parameters linkage to exercise capacity still needs more research (Frigiola et al., 2012, Schwartz et al., 2012, Silvilairat et al., 2011). The effect of ventricular-ventricular interaction in adult patients with repaired TOF and the mechanism that links RV and LV interaction are not known, however, shared myocardial fibers between ventricles and the influence of septal shift caused by RV volume and pressure overload, have been suggested to account for the LV dysfunction (Tzemos et al., 2009). Despite this literature, which comes from different investigators but in small studies without common methodologies, ambiguity behind the structural response to exercise and exercise capacity is still present (Rebergen et al., 1993, Niezen et al., 1996, Helbing et al., 1996, Davlouros et al., 2002).

One study with cardiopulmonary exercise in patients with repaired TOF found a reduction in pulmonary regurgitation fraction (PRF) during exercise with abnormal exercise response. In this study, abnormal RV response to exercise characterised by increased end-diastolic volume and lack of reduction in end-systolic volume with no changes in ejection fraction (RVEDV has increased from 132 ml/m^2 to 137 ml/m^2; p=.041). Significant correlation was observed between the amount of PR and RV volume (r=.74, p=.002), however, they did not find a correlation between PR and exercise intolerance (Roest et al., 2002). These results confirm that the presence of RV dilatation resulted from RV volume overload at rest is an important factor of causing RV dysfunction during exercise in these patients. These findings are consistent with previous reports that found no relationship between poor exercise capacity and pulmonary

regurgitation fraction with changes in right ventricular dimensions (Rowe et al., 1991, Meadows et al., 2007, Wald et al., 2009, Marx et al., 1988). However, these are low powered cross-sectional studies that cannot be generalised.

Nevertheless, in terms of the association between the PRF changes and the exercise capacity, these findings contrast a recent study which found such association. This study concluded that the main determinants of reduced peak VO_2 in adult patients with repaired TOF were PR fraction, age at repair and LV function (Yang et al., 2015). Determining the precise relationship between functional limitations in this population and the haemodynamic echocardiographic changes is a complex approach due to the incomplete understanding of the major determinants behind exercise intolerance. It has been found that the main determinants of diminished peak VO_2 are RV/LV function, degree of PR severity (Samman et al., 2008, Giardini et al., 2006, Cetin et al., 2008), older age at total repair (Fredriksen et al., 2002, Diller et al., 2005), age at cardiopulmonary exercise test (Fredriksen et al., 2002), residual shunt (Diller et al., 2005), pulmonary arterial hypertension (Diller et al., 2005), peak heart rate (Diller et al., 2005), and indexed LV and RV end-diastolic volumes (Yap et al., 2013).

To date, LV/RV haemodynamic changes during stress and their association to RV volume and exercise intolerance remains an understudied area (Nakamura et al., 2014, Davlouros et al., 2002, Frigiola et al., 2012). Correlation between peak VO_2 and right ventricular function and whether RV systolic and diastolic parameters could predict exercise capacity are still an area of debate. Likewise, predictors of the change in exercise capacity in adult patients with TOF following surgical re-intervention is an area of debate. It has been shown that PVR increases the subjective exercise tolerance with reduction of the right ventricular end-diastolic volume (Apostolopoulou et al., 2007, Parish et al., 2013, Baspinar and Alehan, 2006, Tsang et al., 2010). However, the changes of exercise capacity in these studies and others were inconsistent, as the number of patients who underwent PVR using CPET were limited (Parish et al., 2013, Roche et al., 2010, Cifra et al., 2015).

This discussion highlights the opportunity of CPET in combination with echocardiography and CMR to further understand how cardiac haemodynamic changes during exercise could reveal the extent and recoverability of underlying haemodynamic dysfunction in these patients. The unresolved questions regarding the management of TOF are still related to finding the optimal timing of PVR, management of residual lesions of the disease, RV/LV functional changes and exercise capacity. Capturing haemodynamic deterioration earlier in clinical follow ups is important in these patients that could change the timing of reintervention consequently. Therefore, early identification of subclinical RV and LV dysfunction by stress echocardiography in relation to further justification of exercise capacity as more objective measures will be life transforming in these patients. This will advance our understanding in pathophysiology and treatment strategies. Ongoing research aims to identify more sensitive indicators of systolic dysfunction for both RV and LV in asymptomatic repaired TOF patients. The controversies in findings and ambiguities behind the association between exercise capacity, pulmonary regurgitation and RV/LV functional and strain parameters evaluated by advanced cardiac imaging techniques are the basis for this research area in adult patients with repaired Tetralogy of Fallot.

We have developed a detailed systematic review of the available evidence of CPET in the next chapter for further understanding the exercise performance in a large population of TOF patients, and to review the most important determinants of exercise capacity in adult patients with repaired TOF; providing strong evidence before introducing the main result chapters.

1.6 Proposed aims of the thesis

This thesis used highly quantifiable and validated measures of CPET and advanced echocardiographic techniques for detailed description of haemodynamic and physiological augmentation measures during exercise echocardiography and investigated to what extent they are related to functional limitations in adult patients with repaired TOF. This complex description could help in establishing the best haemodynamic predictors of exercise ability in patients with repaired TOF and severe PR using advanced myocardial analyses. The IRLM-TOF study was the main project of my PhD which will be presented in chapter 5, and all subsequent sub-studies were drawn from the same population, except study 4 (presented in chapter 8).

1.6.1 Hypotheses

We hypothesised using CPET with exercise echocardiography:

I. Exercise echocardiography could predict CPET derived exercise capacity in this population.

II. Known biventricular function impairment is associated with reduced exercise capacity in patients with repaired TOF.

1.6.2 Aims

This study was designed to fill some of the gaps in the current literature by:

I. Evaluation of maximum exercise capacity (VO_2) in this population.

II. Description of resting and stress induced echocardiographic differences between severe pulmonary regurgitation group and a control group (TOF patients with no or mild pulmonary regurgitation) during exercise.

III. Establishing the relationship between RV free wall and LV global strain, biventricular function, and volume with peak exercise capacity judged by concurrent cardiopulmonary exercise testing.

IV. Description of pathophysiology of RV and LV dysfunction in these patients.

V. Critical evaluation of exercise predictors to identify which are the best echocardiographic haemodynamic parameters that could be surrogate for peak VO_2 in this patient group.

1.6.3 Primary end points

I. VO_2 peak during cardiopulmonary exercise.

II. To establish the true burden of symptoms (< 80% VO_2 peak) in this population.

1.6.4 Secondary end points

I. To establish an expected range of contractile reserve of the RV and LV which could relate to exercise ability in patients with repaired TOF.

II. To establish the reproducibility and feasibility of all measures.

1.7 Sub-studies

1.7.1 Reproducibility and Repeatability of biventricular function/volume, and strain parameters by 2D and 4D echocardiography (chapter 4)

Background: Validity assessment of stress echocardiography has never been assessed in patients with repaired TOF**,** and there is no recommended strategy for proper biventricular assessment during stress echocardiography in the current guidelines.

Aim: To perform a detailed assessment of inter and intra observer variability as well as test-retest variability.

1.7.2 Complex myocardial mechanics in adult patients with repaired TOF- A novel study assessing myocardial work and mechanical dispersion at rest and during exercise (chapter 6)

Background: The novel LV myocardial work and biventricular mechanical dispersion in adult patients with repaired TOF are unknown.

Aims: To describe LV myocardial work, biventricular dispersion of myocardial contraction, and to relate to exercise ability.

1.7.3 Contribution of LV longitudinal systolic and myocardial strain functional augmentation to exercise intolerance in adult survivors of TOF (chapter 7)

Background: The shape of LV systolic augmentation curve during exercise echocardiography and the relative contribution to exercise capacity in patients with repaired TOF are not known.

Aim: To develop a novel mechanistic insight into the relationship between LV systolic augmentation to the incremental rise in oxygen uptake (VO_2).

1.7.4 Normal right ventricular augmentation during stress echocardiography- A comparative study (chapter 8)

Background: RV contractile reserve in normal individuals is poorly described.

Aims: To describe normal RV contractile reserve in verifiably healthy adults and to compare those against patients with defined RV compromise (patients with repaired TOF), in addition to develop a novel mechanistic insight into the relationship between RV systolic augmentation to the incremental rise in oxygen uptake (VO_2).

1.7.5 Blood biomarkers in adult patients with repaired TOF- A novel study evaluating blood biomarkers in adult survivors of repaired TOF (chapter 10)

Background: Plasma brain natriuretic peptide (NT-proBNP), and Soluble suppression of tumorigenicity-2 (sST2) are poorly described in patients with repaired TOF.

Aims: To provide the first description of combined blood biomarkers in asymptomatic adult survivors of TOF repair. To describe the association between blood biomarkers and adverse haemodynamic echocardiographic findings at rest and during exercise, and to what degree they are related to impaired functional capacity.

Chapter 2 Cardiopulmonary Exercise Test (CPET) in patients with repaired Tetralogy of Fallot (rTOF); A Systematic Review

This chapter is based on the peer-reviewed publication below:

Alborikan S, Pandya B, Von Klemperer K, Walker F, Cullen S, Badiani S, Bhattacharyya S Lloyd G. "Cardiopulmonary Exercise Test (CPET) in patients with repaired Tetralogy of Fallot (rTOF); A systematic review." International Journal of Cardiology Congenital Heart Disease 1 (2020): 100050.

My contribution was performing all comprehensive research of literature, statistical analyses, and writing the whole manuscript.

2.1 Abstract

Background: Cardiopulmonary exercise testing (CPET) provides a comprehensive objective assessment in patients with repaired Tetralogy of Fallot (rTOF). However, the evidence underpinning this practice is scanty as are the mechanisms which drive exercise ability.

Objectives: To describe the current evidence linking CPET data and prognosis in addition to review the most important determinants of exercise.

Methods: The preferred reporting items (PRISMA) guidelines were followed. A systematic search of CPET studies, with/without echocardiography was carried out on PubMed MEDLINE, EBM review-Cochrane Database, Wiley Online library and EBM reviews.

Results: Of 400 studies identified, 21 met the inclusion criteria. 17 articles (81%) were reported in young adults, and 4 articles (19%) in a younger group. The sample size ranged from 15 to 875, and the publication year ranged from 2002 to 2019. Mean age was 25±7 years. Overall mean predicted VO_2 peak was 68±2.8% (95% CI.62.3-74%). There was no difference in mean predicted VO_2 between older and younger groups (68±2.7vs 69±2.9, %, p>.05). Peak predicted VO_2 was found to be higher in contemporary studies than historic investigations (76±6 vs 59±13, %, p=.001). Submaximal measures were rarely reported. Determinants of exercise capacity were reported in 9 studies (43%). Prognostic findings qualitatively suggested that mild exercise intolerance with preserved ventilatory-equivalent for carbon dioxide is associated with better outcomes and lower mortality rate.

Conclusions: The literature showed a high degree of heterogeneity which limited comparability. Marked reduction in functional capacity in patients with repaired TOF seems to be more dependent on surgical selection and developing technique than advancing age.

2.2 Introduction

Tetralogy of Fallot (TOF) is the most common form of cyanotic congenital heart disease (CHD) and accounts for 10 percent of all forms of CHD (Perloff and Marelli, 2012). The population of adult patients who have undergone repair of TOF is increasing and knowledge about how objective exercise assessment to predict adverse late clinical outcomes is still evolving. Although the residual problems after primary repair are often well tolerated during childhood and adolescence, exercise intolerance, heart failure, arrhythmias and premature death are common in adulthood (Wessel et al., 1980, Frigiola et al., 2013). The majority of patients with repaired TOF have been shown to have limitation in functional capacity which worsens overtime (Rowe et al., 1991). Subjective evaluation of symptoms may be a poor guide of exercise intolerance, so cardiopulmonary exercise testing (CPET) is routinely performed for an objective assessment (Wessel et al., 1980, Frigiola et al., 2013, Rowe et al., 1991, Mahle et al., 2002). There are however no interventional or randomised trials to specifically support this practice. Several haemodynamic and physiobiological factors contribute to exercise intolerance in this population and has been shown to relate to pulmonary regurgitation (PR), chronotropic impairment, impaired lung function and ventricular dysfunction (Gatzoulis et al., 1995, Sutton et al., 2008, Roest et al., 2002, Meadows et al., 2007, Fredriksen et al., 2002, Diller et al., 2005). To what extent each of these factors is responsible for impaired exercise capacity in this population remains poorly understood (Mueller et al., 2013).

We therefore undertook a systematic review evaluating determinants of cardiopulmonary exercise test performance in this patient group and based on current literature how it can best be used to predict clinical outcomes and plan intervention.

2.3 Material and Methods

2.3.1 Systematic review analysis

We used the Preferred Reporting Items for Systematic reviews (PRISMA) guidelines for this systematic review. The flow diagram in figure 2.1 demonstrates the literature search based on the PRISMA guidelines.

2.3.2 Inclusion and exclusion criteria

Inclusion criteria were (a) prospectively or retrospectively conducted cohort, cross sectional, single and multicenter studies and (b) English language; (c) CPET included in methodology; (d) adult and adolescent patients with repaired TOF. Studies excluded from the review were those: (a) without CPET data; (b) other types of exercise test (e.g., Dobutamine exercise); (c) not published in English; (d) non rTOF.

2.3.3 Information sources and search strategy

A comprehensive retrospective search of the literature was conducted using MEDLINE, Pubmed, EBM review-Cochrane Database of systematic review, Wiley Online library and EBM reviews, utilising a combination of the following search keywords: “Tetralogy of Fallot’”, “Stress echocardiography”, “cardiopulmonary exercise test in TOF with severe pulmonary regurgitation “,” Exercise capacity in TOF “, “Severe pulmonary regurgitation and functional capacity”. There was no limitation on age. The search included all studies between 1990 and 2019.

2.3.4 Study selection and eligibility criteria

The following steps were performed (Figure 2.1). (1) Identification of titles through database searching. (2) Removal of duplicates. (3) Titles and abstracts screening. (4) Full text sources for further screening. Eligible studies were either retrospective or prospective studies.

2.3.5 Data extraction

A search strategy was created by one reviewer (Sahar Alborikan, S.A) who determined the eligibility of the studies by screening the titles and abstracts of all identified literature and verified by a second reviewer (Guy Lloyd, G.L). To prevent bias, the screening was performed independently. The following data were extracted: first author's name, country, year of publication, number of patients included, study design, age at CPET, predicted VO_2 peak (%), respiratory exchange ratio (RER), CPET protocol, ventilatory-equivalent for carbon dioxide (VE/VCO2), ventilatory threshold (VT) and oxygen uptake efficiency slope (OUES) when available and the conclusion together with main findings of the study. We have chosen to report oxygen uptake as a percentage of predicted VO_2 because of the wide ranges of age included in the study and many of selected studies have reported oxygen uptake as a percentage of predicted (%). Older group was defined as studies with participants who were older than 18 years old and younger group who were under that age. Contemporary studies were defined as those published between 2011 to present and historic studies prior 2008.

2.3.6 Statistical Analysis

Studies were divided into (i) those that reported in an adult population and (ii) those reported in a population younger than 18 years old. Data were presented as mean differences (MD) ± standard deviation for the continuous outcome variables. The overall means of age and different reported exercise parameters in 21 studies were calculated. The overall means of multiple exercise parameters in older and younger groups were calculated independently**.** Comparison of mean exercise parameters was evaluated by paired student t-test with $p<.05$, level of significance. Statistical analyses were conducted using SPSS statistics version 23 (IBM corp, London, United Kingdom).

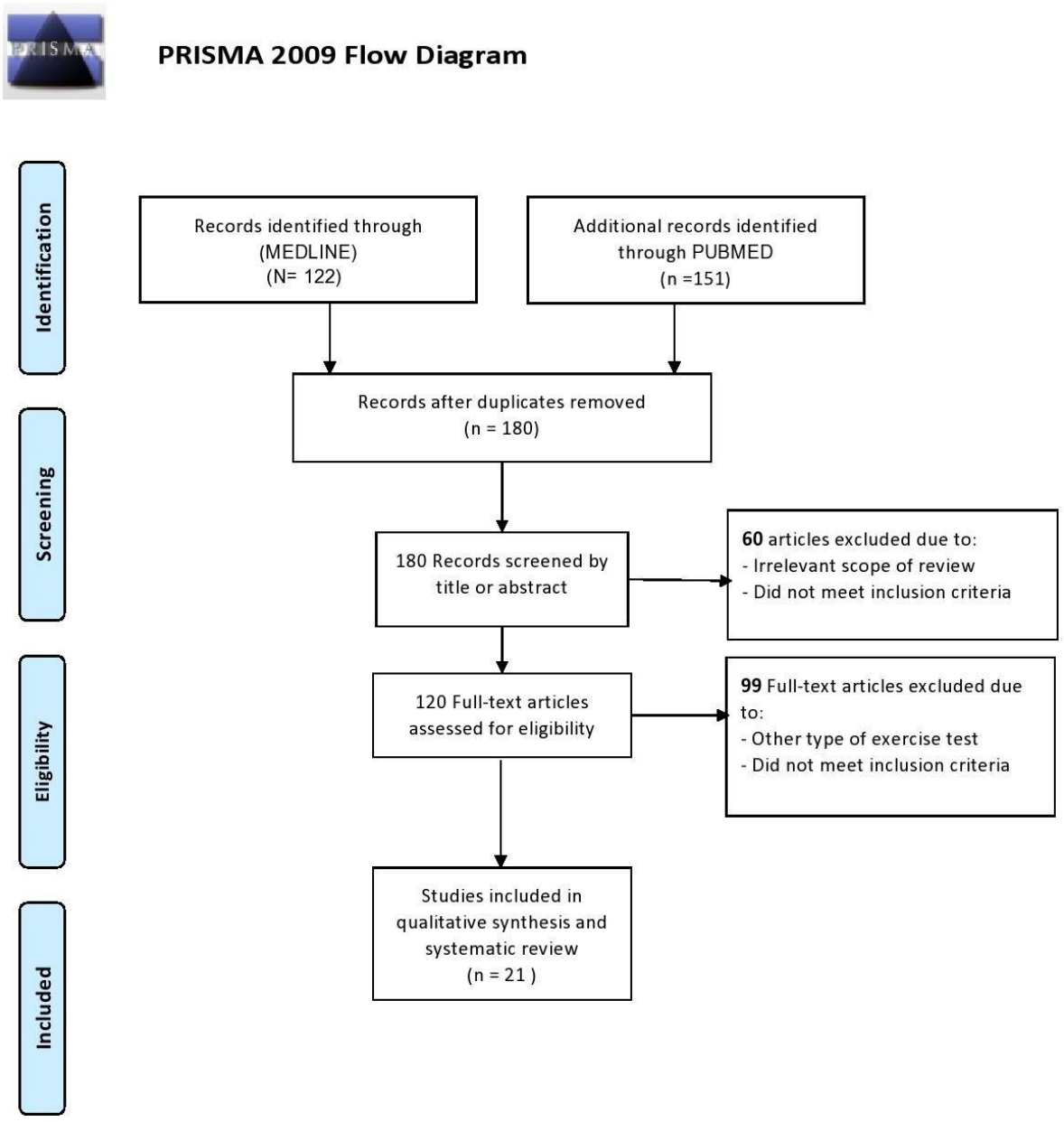


Figure 2. 1. The PRISMA flow chart for the systematic selection of studies (Alborikan et al., 2020).

2.4 Results

2.4.1 Literature search outcomes

The literature search identified a total of 400 potentially eligible studies. After the exclusion of irrelevant studies, 273 were screened thoroughly though abstract and/or full text. 93 articles were excluded due to duplication. Of 180 articles, 60 articles were excluded, as they did not meet the inclusion criteria. Full text article assessment was performed for 120 articles and 99 articles were further excluded, leading to a total of 21 articles included in this review. Figure 2.1 shows the PRISMA flow chart displaying exact details of the selection process.

2.4.2 Characteristics of selected studies

Of the 21 articles, 17 (81%) were reported in adult survivors of TOF (Fredriksen et al., 2002, Diller et al., 2005, Bhatt et al., 2019a, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yang et al., 2015, Müller et al., 2015, Eindhoven et al., 2014, Yap et al., 2013, Buys et al., 2011, O'Meagher et al., 2012, Kipps et al., 2011, Samman et al., 2008, Izbicki et al., 2008, Giardini et al., 2007, Giardini et al., 2006, Norozi et al., 2005), while 4 studies (19%) were reported in younger group (Mahle et al., 2002, Roest et al., 2002, Hock et al., 2019, Fernandes et al., 2012). 8 studies (38%) were cross- sectional studies (Bhatt et al., 2019a, Yap et al., 2013, O'Meagher et al., 2012, Samman et al., 2008, Izbicki et al., 2008, Giardini et al., 2007, Giardini et al., 2006, Norozi et al., 2005). 12 studies (57%) were cohort studies (Mahle et al., 2002, Roest et al., 2002, Fredriksen et al., 2002, Diller et al., 2005, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yang et al., 2015, Eindhoven et al., 2014, Buys et al., 2011, Kipps et al., 2011, Hock et al., 2019, Fernandes et al., 2012) . 6 studies (29%) were conducted prospectively (Roest et al., 2002, Bhatt et al., 2019b, Yang et al., 2015, Hock et al., 2019, Fernandes et al., 2012) and 10 studies (48%) were conducted retrospectively (Mahle et al., 2002, Fredriksen et al., 2002, Diller et al., 2005, Dłużniewska et al., 2018, Meierhofer et al., 2017, Müller et al., 2015, Yap et al., 2013, Buys et al., 2011, Kipps et al., 2011, Samman et al., 2008). Sample size ranged from 15 to 875, and the publication year ranged from 2002 to 2019. Mean age of included participants ranged from 12 to 34 years. All included studies were single center designed except one big multicenter study with retrospective evaluation (Müller et al., 2015). Bicycle ergometry protocol was the most frequently used protocol. Table 2.1 shows overall baseline characteristics of the included studies and CPET parameters.

Table 2. 1. Baseline characteristics of the included studies and CPET parameters.

Baseline characteristics	**N= 2239***	**Older n=17****	**Younger n=4*****	**P value**
Mean age±SD , years	25±7	27±4	13±2	**p >.05**
Study type	20, Single Centre One, multicentre	-	-	-
CPET parameters				
Peak predicted VO_2 (% pred)	68±2.8 % CI (62.3-74%)	68 ± 2.7 %	69±2.9%	**p >.05**
RER max	1.18±.09	1.2±.1	1.1±.1	**p >.05**
VE/VCO_2#	29±2.2	29±2.4	29.6[#]	**p >.05**
OUES (% pred) ^	90.3 %[?]	90.3	-	-
Mode of CPET				
Bicycle ergometry protocol (n, %)	19 (90%)	-	-	-
Treadmill (n, %)	2 (10%)	-	-	-

VO_2 (% pred) =maximum predicted oxygen uptake; RER=respiratory exchange ratio; VE/VCO_2 =ventilator-equivalent; OUES =oxygen uptake efficiency slope**;** *total number of patients in all studies; **Number of studies in adult survivors of TOF repair; ******* Number of studies in younger group; ^ OUES derived from one adult study; #VE/VCO_2 derived from one study in younger group.

2.4.3 Cardiopulmonary exercise testing parameters

In total, data was collected on 2239 young patients with repaired TOF with a mean age of 25±7 years. Mean age of the older group was 27±4 years in 17 studies in young adults and the mean age of the younger group was 13±2 years in 4 studies in adolescents. All 21 articles have reported maximal exercise effort data as predicted VO_2 peak (%). A higher proportion of population had undergone transannular patch repair (TAP) in 10 studies (48%) (relatively less frequent surgical approach in recent years) (Mahle et al., 2002, Roest et al., 2002, Fredriksen et al., 2002, Bhatt et al., 2019a, Yang et al., 2015, Müller et al., 2015, Kipps et al., 2011, Giardini et al., 2006, Norozi et al., 2005, Fernandes et al., 2012). Respiratory exchange ratio (RER) was reported in 13 studies (61%) with a mean of 1.18±.09 (Mahle et al., 2002, Roest et al., 2002, Fredriksen et al., 2002, Bhatt et al., 2019a, Dłużniewska et al., 2018, Yang et al., 2015, Müller et al., 2015, Eindhoven et al., 2014, Yap et al., 2013, Buys et al., 2011, O'Meagher et al., 2012, Kipps et al., 2011, Samman et al., 2008, Fernandes et al., 2012). 19 studies (90%) have used a bicycle ergometer protocol in their exercise methodology while only 2 (10%) studies have used a treadmill protocol. An overview of the included 21 studies is provided in table 2.2.

Table 2. 2. Summary of CPET studies and their findings in patients with repaired TOF included in this review.

Author	Country	Study type	N*	Age at CPET (Years)	Peak VO_2 (%)	RER	VE/VCO_2	OUES	Conclusion
Hock et al., 2019	Germany	Single-centre, prospective cohort	55	13.5±3.8	80.4±16.8	n/a	29.6±3.6	n/a	-Limited exercise capacity -No predictive analysis
Bhatt et al., 2019	USA	Single-centre, cross sectional Prospective	29	18.3± 4.83	75±10	1.24	31±4.2	n/a	-There were low and high exercise performers -Chronotropic response was a predictor of exercise capacity, neither RV or PR
Dluzniewska et al., 2018	Poland	Single-centre, retrospective cohort	52	29±8.9	67.2±16.7	1.1±.2	25.8± 6.2	n/a	-Markedly reduced exercise tolerance -Impaired RV function was associated with exercise intolerance
Meierhofer et al., 2017	Germany	Single-centre, retrospective cohort	132	29±11	77.5±19.3	n/a	n/a	n/a	-Limited exercise capacity -RVSP predicts functional capacity (not RV or PR)
Yang et al., 2015	Taiwan	Single-centre, Prospective cohort	158	29.5± 12.2	68±14	1.09	27.1± 5.3	90.3± 14.1	-Impaired exercise capacity -PR fraction, LV function and age at repair were the most important exercise determinants
Muller et al., 2015	Germany	Multicentre, retrospective	875	25.5± 11.7	67.6±19.7	1.11±. 16	30.5± 7.3	n/a	-Impaired exercise capacity -Poor exercise capacity was associated with poor outcomes (death and arrhythmias)
Eindhoven et al., 2014	Australia	Single-centre, prospective cohort	177	34±11.8	77±12	1.37	n/a	n/a	-Impaired exercise capacity -No predictive analysis
Yap et al., 2013	Singapore	Single-centre, cross sectional retrospective	36	30±10	83±18	1.1	n/a	n/a	-Near normal exercise capacity -Indexed RV and LV volume were associated with exercise capacity -No predictive analysis
Fernandes et al., 2012	Canada	Single-centre, prospective cohort	124	12±3	66±15	1.01	n/a	n/a	-Impaired exercise capacity -No predictive analysis
Buys et al., 2011	Netherland	Single-centre, retrospective	98	25.6±7.7	74±15	1.13	26.2± 5.5	n/a	-Impaired exercise capacity -Exercise duration and chronotropic response were predictors of all-cause mortality and hospitalisation
O'Meagher et., 2012	England	Single centre, cross sectional	55	26±8.8	85±15	1.27±. 12	28±4	n/a	-Preserved exercise capacity -No predictive analysis

Kipps et al., 2011	USA	Single-centre, retrospective	70	27.8±15	78±19	n/a	28.2 ±4.6	n/a	-Low exercise capacity that decline overtime -No predictive analysis
Samman et al.,2008	Canada	Single-centre, cross sectional retrospective	99	34±11	66±13	1.1	n/a	n/a	-Impaired exercise capacity -RV/ LV function was associated with exercise intolerance
Izbicki et al., 2008	Israel	Single-centre, cross sectional	50	29 ±11	75±7	n/a	n/a	n/a	-Mild limitation of exercise capacity -No predictive analysis
Giardini et al.,2007	Italy	Single-centre, cross sectional	118	24±8	58±17	n/a	31.1 ±4.6	n/a	-Impaired exercise capacity -Peak VO_2 and VE/VCO_2 were the most important predictors for poor outcomes and plan intervention
Giardini et al.,2006	Italy	Single-centre, cross sectional	61	23.1±12.1	54±18	n/a	32± 7	n/a	-Impaired exercise capacity -CPET could be used to guide intervention -Severe PR and RV dysfunction was associated with exercise intolerance
Norozi et al.,2005	Germany	Single-centre, cross sectional	50	30.5±1.6	64±3	n/a	n/a	n/a	-Impaired exercise capacity -RV dysfunction was associated with exercise intolerance
Diller et al., 2005	England	Single-centre, retrospective	107	31±11	56±20	n/a	n/a	n/a	-Impaired exercise capacity
Fredriksen et al.,2002	Canada	Single-centre, retrospective	168	32±15	51±12	1.1± .10	n/a	n/a	-Impaired exercise capacity -No predictive analysis
Roest et al., 2002	Netherland	Single-centre, prospective cohort	15	17.5±2.5	40±12	1.16 ±.05	n/a	n/a	-Impaired exercise capacity -First exercise with MRI -PR and RV Dysfunction were not associated with exercise intolerance
Mahle et al., 2002	USA	Single-centre, retrospective	57	12.5±3.2	80±10	1.12 ±.07	n/a	n/a	-Nearly normal exercise capacity

VO_2 (% pred) =maximum predicted oxygen uptake; RER =respiratory exchange ratio; VE/VCO_2 =ventilatory-equivalent; OUES =oxygen uptake efficiency slope; RV =right ventricle; LV =left ventricle; PR=pulmonary regurgitation; RVSP=right ventricular systolic pressure. *N=number of patients in each stud; n/a =not available

2.4.4 Maximum oxygen uptake (VO_2)

Overall mean peak predicted VO_2 in patients with repaired TOF was 68±2.8%, ranged from 40 to 85%. Despite small individual studies showing a relationship with age (Mahle et al., 2002, Diller et al., 2005, Bhatt et al., 2019a, Kipps et al., 2011, Hock et al., 2019), there was no overall difference in mean predicted VO_2 between older and younger participants (68±2.7 vs 69±2.9, %, p>.05) (Table 2.1). Contemporary studies (Bhatt et al., 2019a, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yang et al., 2015, Müller et al., 2015, Eindhoven et al., 2014, Yap et al., 2013, Buys et al., 2011, O'Meagher et al., 2012, Kipps et al., 2011, Hock et al., 2019, Fernandes et al., 2012) have demonstrated higher peak predicted VO_2 comparing to historic investigations (Mahle et al., 2002, Roest et al., 2002, Fredriksen et al., 2002, Diller et al., 2005, Samman et al., 2008, Izbicki et al., 2008, Giardini et al., 2007, Giardini et al., 2006, Norozi et al., 2005) (76 ±6 vs 59±13 %, p=.001). Among all studies, lower exercise performers were reported in populations where TAP was used as a surgical approach (reported in 10 studies (48%)) (Mahle et al., 2002, Roest et al., 2002, Fredriksen et al., 2002, Bhatt et al., 2019a, Yang et al., 2015, Müller et al., 2015, Kipps et al., 2011, Giardini et al., 2006, Norozi et al., 2005, Fernandes et al., 2012). Distribution of exercise capacity in TAP and non-TAP studies (other type of surgery or unknown) is shown in figure 2.2.

Moderate exercise intolerance (when max VO_2 40 to < 65% of predicted), was observed in 6 studies (29%) (Roest et al., 2002, Fredriksen et al., 2002, Diller et al., 2005, Giardini et al., 2007, Giardini et al., 2006, Norozi et al., 2005). Mild exercise intolerance (when max VO_2 65 to 80 % of predicted) was observed in 13 studies (62%) (Mahle et al., 2002, Bhatt et al., 2019b, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yang et al., 2015, Müller et al., 2015, Eindhoven et al., 2014, Buys et al., 2011, Kipps et al., 2011, Samman et al., 2008, Izbicki et al., 2008, Hock et al., 2019, Fernandes et al., 2012). Borderline exercise intolerance (when max VO_2 81 to 85 % of predicted) was observed in 2 studies (9%) (Yap et al., 2013, O'Meagher et al., 2012). The highest mean predicted VO_2 peak was reported in only one study performed by O'Meager et al., 2012 who showed preserved oxygen consumption in young adults with repaired TOF with mean predicted VO_2 of 85% (O'Meagher et al., 2012) (Table 2.2).

2.4.5 Exercise parameters as a predictor of death, arrhythmias and the need of reintervention

The use of CPET to predict cardiac related mortality and the need for reintervention was reported only in two studies included in this review (10%) (Müller et al., 2015, Giardini et al., 2007). Peak oxygen uptake and V_E/VCO_2 were reported as the most powerful predictors of cardiac related mortality and hospitalisation (Giardini et al., 2007). Poor exercise capacity was associated with increased risk for death and sustained ventricular arrhythmias (Müller et al., 2015). Peak oxygen uptake < 36% of predicted value was found to have the highest sensitivity and specificity to predict cardiac-related outcomes and death (sensitivity 89% and specificity 99%) (Giardini et al., 2007). While a cut off value of peak predicted VO_2 of 62 % was identified as an optimal cut-off value for 5-year freedom from death or ventricular arrhythmia (sensitivity 82% and specificity 63%) (Müller et al., 2015). No studies evaluated whether intervention could influence objective exercise performance.

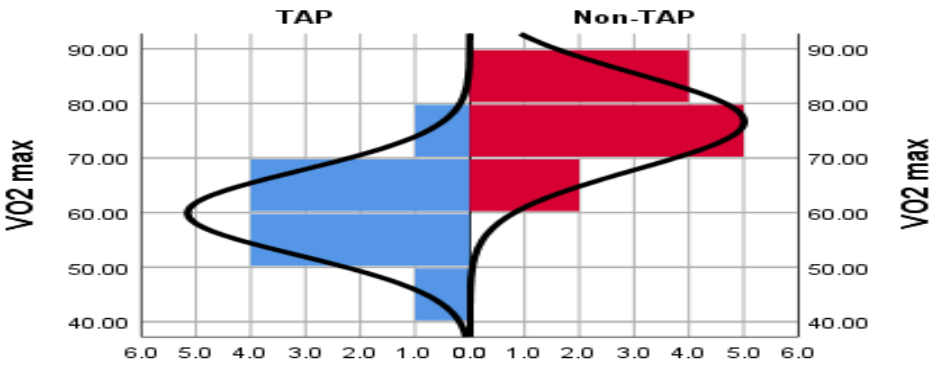
2.4.6 Sub maximal exercise effort data

Only one study in this review reported OUES in adult patients with repaired TOF with a normal mean value of 90.3% (normal >80%) (Yang et al., 2015, Ramos et al., 2014). Minute ventilation (V_E) to carbon dioxide production (VCO_2) slope was reported in ten studies (48%); one in younger group and 9 studies in adults (43%) (Bhatt et al., 2019b, Dłużniewska et al., 2018, Yang et al., 2015, Müller et al., 2015, Buys et al., 2011, O'Meagher et al., 2012, Kipps et al., 2011, Pescatello et al., 2014, Giardini et al., 2006, Hock et al., 2019). The observed mean V_E/VCO_2 slope was normal with a mean value of 29±2.2 (normal< 30) (Dallaire et al., 2017). In two studies, V_E/VCO_2 was used as a predictive tool to determine outcomes following pulmonary re-intervention (Müller et al., 2015, Giardini et al., 2007). A value of V_E/VCO_2 >39 has higher sensitivity to predict cardiac-related mortality and the need for reintervention in this group of patients (sensitivity 89% and specificity 99%) (Giardini et al., 2007). While a value of V_E/VCO_2 >31 is at highest risk of cardiac related events or death (sensitivity 82% and specificity 63%) (Müller et al., 2015). These values were proposed as a risk model guidance for surgical intervention. Ventilatory threshold (VT) was reported only in two studies (Yap et al., 2013, Buys et al., 2011). Minute of ventilation (VE) was reported in five studies (23%)

(Fredriksen et al., 2002, Dłużniewska et al., 2018, Izbicki et al., 2008, Giardini et al., 2007, Giardini et al., 2006).

2.4.7 Determinants of exercise capacity

Haemodynamic determinants of exercise performance were reported in 9 studies (43%). These studies used echocardiography in addition to CPET. Impaired exercise tolerance was mainly explained by a variety of different measured echocardiographic parameters at rest and augmentation during exercise (Bhatt et al., 2019a, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yang et al., 2015, Yap et al., 2013, Kipps et al., 2011, Samman et al., 2008, Giardini et al., 2006, Norozi et al., 2005). At rest, severe PR and left ventricle (LV)/ right ventricle (RV) dysfunction were reported the most important determinants of exercise capacity in some studies (Yang et al., 2015, Samman et al., 2008, Giardini et al., 2006), although this was not observed in all (Bhatt et al., 2019b, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yap et al., 2013). Reduced LV stroke volume at peak exercise was related to more exercise deterioration in one study (Kipps et al., 2011), while peak RV systolic pressure was associated with exercise impairment in two reports (Meierhofer et al., 2017, Norozi et al., 2005).



TAP = studies with transannular patch; non-TAP = studies with other type of surgery (e.g., pulmonary valvotomy) or not known.

Figure 2. 2. Distribution of exercise capacity in TAP and non-TAP studies.

2.5 Discussion

CPET has entered routine clinical cardiovascular practice as a reduced VO_2 peak has been associated with poor prognosis in both ischemic heart disease and heart failure assessment (Diller et al., 2005). This has also been applied to patients with congenital heart disease and in particular those with repaired TOF patients, where CPET is used to predict early mortality and to provide clinically relevant data to determine optimal timing of pulmonary valve replacement (PVR) (Diller et al., 2005, Müller et al., 2015, Giardini et al., 2007, Babu-Narayan et al., 2014). However, the literature remains unclear about the extent of limitation, the causes of any limitation and there is scant data demonstrating an improvement in exercise performance after PVR.

It has been extensively reported that asymptomatic rTOF patients have exercise intolerance (Müller et al., 2015, Giardini et al., 2006, Norozi et al., 2005, Fernandes et al., 2012, Clark et al., 1995). Identification of early functional impairment by CPET is intuitively important even though the underlying mechanisms may not be well understood (Wessel et al., 1980, Rowe et al., 1991, Mahle et al., 2002). Most studies included in this review were single-centre cohort studies and few were originally designed for assessing the functional status in these patients. There were significant methodological weaknesses or limitations; for instance, predicted VO_2 values were reported but infrequently with the associated RER. It cannot therefore be verified that these were maximal effort scans; other submaximal measures such as OUES, VT and V_E/VCO_2 were rarely reported. Finally, the majority of the tests were performed using a bicycle ergometer protocol, and although this allows for more accurate blood pressure, electrocardiogram and other physiological measures, treadmill protocol produces higher maximum oxygen uptake (Astrand, 1967, Pescatello et al., 2014). This finding of rare documentation of submaximal effort in the current evidence was highlighted also in a big recent systematic review in cardiopulmonary exercise test in healthy subjects (Ramos et al., 2014). A summary of CPET studies and their findings in patients with repaired TOF is shown in table 2.2.

Overall, a marked reduction in exercise tolerance and functional capacity was noted in patients with repaired TOF with an overall mean peak predicted VO_2 of 68±2.8% (95% CI. 62.3- 74 %). 17 adult (81%) studies have shown impaired functional capacity with a mean peak of predicted VO_2 of 68±2.7% (ranging from 51 to 85 %). 16 of these were single-center studies with either prospective or retrospective design (Fredriksen et al., 2002, Diller et al., 2005, Bhatt et al., 2019a, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yang et al., 2015, Eindhoven et al., 2014, Yap et al., 2013, Buys et al., 2011, O'Meagher et al., 2012, Kipps et al., 2011, Samman et al., 2008, Izbicki et al., 2008, Giardini et al., 2007, Giardini et al., 2006, Norozi et al., 2005). Mild exercise intolerance was the most reported level of functional capacity in this review (13 studies, 62%). This was in keeping with the only multicenter study that performed by Muller et al., 2015 who have reported mild exercise intolerance with an overall peak of predicted VO_2 of 76 % (Müller et al., 2015).

Exercise performance in patients with repaired TOF is thought to decline with age, however, we found no difference in mean predicted VO_2 between populations drawn from older and younger age groups suggesting that young patients are equally affected by functional limitation (68± 2.7 vs 69±2.9, %, p>.05) (Diller et al., 2005, Kipps et al., 2011). Similar findings were reported in two adolescents' studies included in this review as they have demonstrated an exercise intolerance of 66 and 40% (Roest et al., 2002, Fernandes et al., 2012). However, Bhatt et al found that in their small but prospectively conducted recent study; there was a better functional capacity with higher performance in younger patients (Bhatt et al., 2019b). This also has been reported in other two contemporary studies in adolescents (Mahle et al., 2002, Hock et al., 2019). In this review, more contemporary studies have shown higher predicted VO_2 peak comparing to historic investigations irrespective of age and this is most likely to be related to an era of more careful surgical selection and contemporary technique used to repair TOF (Bhatt et al., 2019b, Dłużniewska et al., 2018, Meierhofer et al., 2017, Yang et al., 2015, Müller et al., 2015, Eindhoven et al., 2014, Yap et al., 2013, Buys et al., 2011, O'Meagher et al., 2012, Kipps et al., 2011, Hock et al., 2019, Fernandes et al., 2012) (Figure 2.2). Because of the high level of heterogeneity, it is difficult to draw firm conclusions, but our analysis suggests that subsequent exercise tolerance is more a function of surgical technique than a function to age.

2.5.1 Determinants of exercise capacity in patients with repaired TOF

Due to multifactorial interaction behind the progressive exercise intolerance in adult survivors of TOF, determining the precise relationship between each facet of heart function exercise intolerance is challenging; consequently, the major determinants of exercise capacity in patients with repaired TOF are not yet completely understood (Bhatt et al., 2019a, Samman et al., 2008, Giardini et al., 2006, Cetin et al., 2008, Cifra et al., 2015). It has been found that the main determinants of diminished peak VO_2 are: RV/LV function, degree of PR severity (Samman et al., 2008, Giardini et al., 2006, Cetin et al., 2008), older age at total repair (Fredriksen et al., 2002, Diller et al., 2005), age at cardiopulmonary exercise test (Fredriksen et al., 2002), residual shunt (Diller et al., 2005), pulmonary arterial hypertension (Diller et al., 2005), peak heart rate (Diller et al., 2005), and indexed LV and RV end-diastolic volumes (Yap et al., 2013). Approximately half of studies, 9 articles (43%) have used echocardiography in addition to CPET. In the literature, there is limited evidence to support the use of stress echocardiography and CPET to better understand RV and LV function in relation to exercise intolerance (Cifra et al., 2015, Apostolopoulou et al., 2007, Parish et al., 2013, Baspinar and Alehan, 2006, Roche et al., 2010, Ait-Ali et al., 2014, Frigiola et al., 2012). Some studies have demonstrated the limited impact of RV dilation on exercise performance when the biventricular function is preserved (Davlouros et al., 2002, Silvilairat et al., 2011), in contrast to current clinical understanding. Others found that severe PR and RV dysfunction were not predictors of exercise capacity in this population (Bhatt et al., 2019b, Meierhofer et al., 2017). These findings are consistent with previous reports that found no relationship between exercise intolerance and pulmonary regurgitation fraction (Rowe et al., 1991, Meadows et al., 2007, Wald et al., 2009, Marx et al., 1988). However, these were low powered cross-sectional studies.

2.6 Clinical implication

The use of CPET to identify patients who are at greater risk for adverse outcomes is clinically relevant. However, only two studies have demonstrated the importance of CPET testing in which the primary end point was VO_2 and V_E/VCO_2 in the other (Müller et al., 2015, Giardini et al., 2007). For clinical application, these findings from this systematic review suggest that a value of less than 62% is associated with higher risk of adverse outcomes.

An elevated V_E/VCO_2 in adult survivors of TOF could be associated with higher risk of mortality and hospitalisation (Müller et al., 2015, Kipps et al., 2011, Giardini et al., 2007). The potential reasons for enhanced ventilatory response in this population to exercise are poorly understood. However, the possible factors that contribute to this slope elevation in these patients are pulmonary blood flow mal-distribution, numerous surgical sternotomies and thoracotomies, and the presence of ventilatory/perfusion mismatch (Yang et al., 2012). Although the objective value of submaximal measures and their relation to exercise intolerance have been evaluated, these studies are relatively small cross-sectional studies that cannot be generalised (Yang et al., 2015, Müller et al., 2015, Buys et al., 2011, Kipps et al., 2011, Giardini et al., 2007).

2.7 Study limitations

Review limitations include cross-sectional cohorts, heterogeneity in reporting functional capacity and the absence of reporting standards. Quality data can be further improved by performing reliability and reproducibility analyses. Large, longitudinal, prospective and randomised studies are needed to determine whether CPET is a valuable investigation for these patients.

2.8 Conclusions

We have demonstrated that despite the widespread use of CPET in clinical practice the literature shows a high degree of heterogeneity. It is clear that there is a marked reduction in exercise capacity in young adults with rTOF with a mean peak predicted VO_2 of 68 %. This seems to be more dependent on surgical selection and technique than advancing age. The comparability of the literature was limited by variable techniques and reported parameters. We strongly recommend that to allow comparison of data sets including RER, OUES, V_E/VCO_2 and VT in addition to peak VO_2. Moderate decline in peak VO_2 and high ventilatory-equivalent for carbon dioxide were the best predictors of adverse outcomes. However, despite its widespread use, further investigation into how CPET can aid the clinician's decision making for intervention is needed.

Chapter 3 Material and general methods

3.1 Overview and study design

IRLM-TOF study was the main research focus, it was prospective observational cohort study of 100 patients with diagnosis of repaired TOF and pulmonary regurgitation (Figure 3.1 for study flow chart proposed at study begin). Barts Health NHS Trust was the sponsor for this study which was based only in the United Kingdom. The main study will be discussed in chapter 5.

Cohort A: Cross-sectional study of 60 patients with previously repaired TOF and severe PR. Resting parameters of left and right ventricular function, volume, and mechanical deformation parameters from echocardiography were compared to maximal exercise capacity (VO_2 peak).

Cohort B: 40 patients with repaired TOF but no or mild pulmonary regurgitation as a control group.

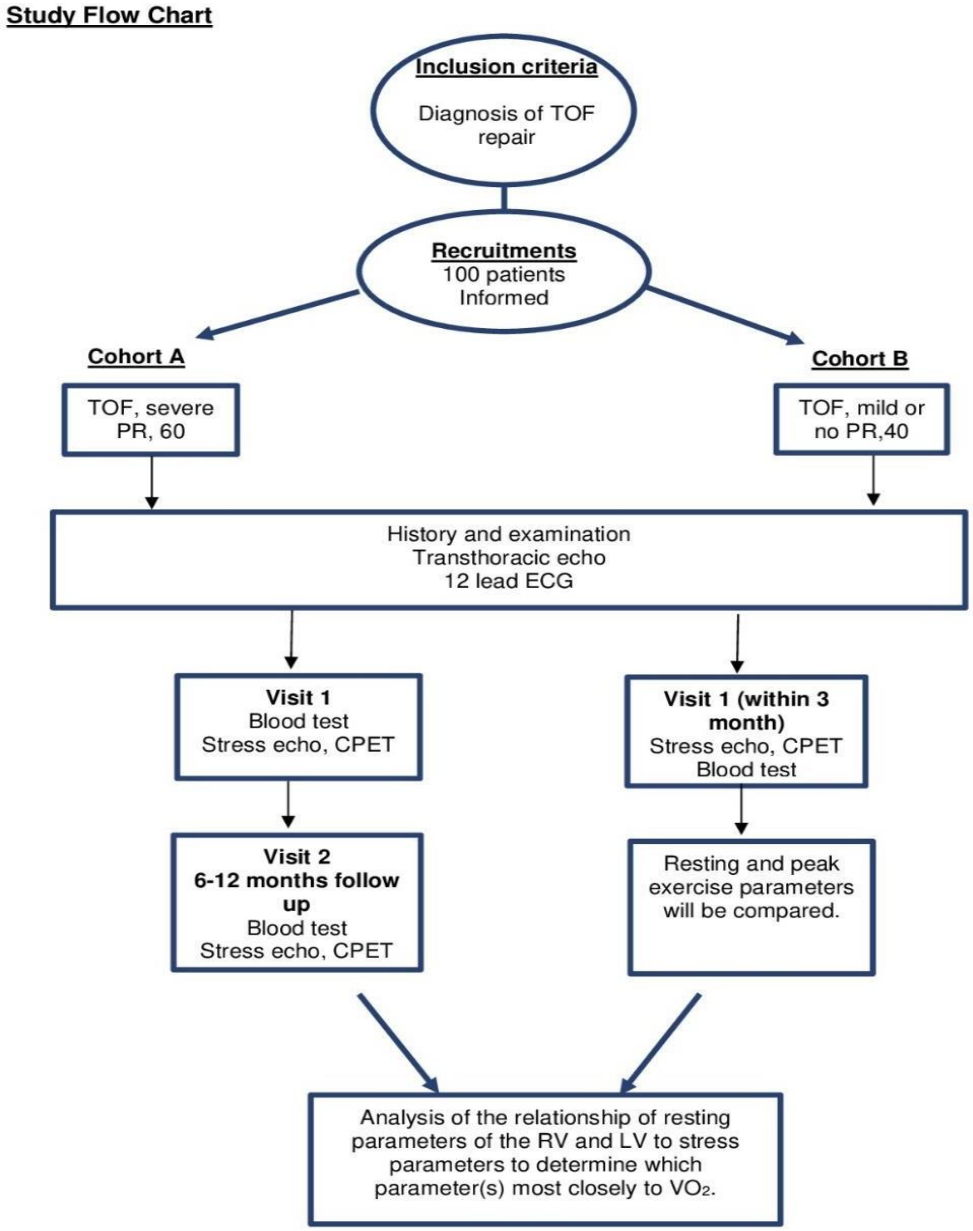


Figure 3. 1. Study flow chart for IRLM-TOF study.

3.1.2 Sub studies

All sub studies were constructed from the same population recruited in the main study (IRLM-TOF), except study 4, which includes heathy subjects that are further described in the specific section. Table 3.1 shows the individual sub studies and their required number of patients.

Table 3. 1. Sub studies and the number of patients per study.

Sub studies	Number of patients
• **Study 1:** Reproducibility and repeatability of biventricular function/volume, and strain parameters by 2D and 4D echocardiography, (chapter 4).	**25, TOF patients**
• **Study 2:** Complex myocardial mechanics in adult patients with repaired TOF-A novel study assessing myocardial work and mechanical dispersion at rest and during exercise, (chapter 6).	**100, TOF patients**
• **Study 3:** Contribution of LV longitudinal systolic and myocardial strain functional argumentation to exercise intolerance in adult survivors of TOF, (chapter 7).	**100, TOF patients**
• **Study 4:** Normal right ventricular augmentation during stress echocardiography- A comparative study, (chapter 8).	**140, 40 healthy subjects, and 100 TOF patients**
• **Study 5:** Blood biomarkers in adult patients with repaired TOF-A novel study evaluating blood biomarkers in adult survivors of repaired TOF, (chapter 10).	**100, TOF patients**

3.2 Ethical Approval

All ethics were approved by the Health Research Authority-Queen Square Research Ethics Committee (18/LO/0092). All subjects provided written informed consent to participate in the study. IRAS number for IRLM-TOF project is 232328 (Appendix A).

3.3 Patients selection

The following inclusion and exclusion criteria were applied to all studies except for study 4 that are described in the related chapter separately.

3.3.2 Inclusion criteria

I. Diagnosis of TOF repair
II. Severe PR
III. Absence of residual pulmonary stenosis
IV. NYHA status less than III
V. Informed consent
VI. Aged 18 years of age or over

3.3.3 Exclusion criteria

I. RVOT obstruction
II. Severe LVOT obstruction
III. Severe pulmonary stenosis
IV. Pulmonary atresia
V. Ventricular arrhythmias
VI. Pacemaker
VII. Associated major cardiac anomalies or systemic disease
VIII. PA systolic pressure >40mmHg
IX. Inability to cycle
X. Poor acoustic windows (one of the limitations of transthoracic echocardiography, which can result in un-interpretable images).
XI. Alternative pathology likely to be life limiting within 2 years
XII. Chronic renal failure (EGFR<30)

3.4 Sample size calculation

Study A: Is powered to assuming a clinically relevant correlation coefficient between regurgitant volume and global longitudinal strain and exercise ability (strength of association = 0.5, (80%) power, allowing for 5 independent covariates).

Study B: Is powered to establish an ICC correlation between CPET and Echocardiographic parameters of greater than 0.8.

Adequate power was calculated to be achieved with a group size of 60, and 40 patients in each cohort, equaling a total sample size of 100 patients to be a realistic recruitment target for our institution.

3.5 Recruitment

Patients were recruited from Grown Up Congenital Heart Disease (GUCH) clinics at Barts Heart Centre. Potential participants were identified by the main investigator or by consultant cardiologists. Participants were approached and given a participant information sheet (PIS) and offered an appointment to see a member of the research team. Figure 3.2 shows the recruitment process from March 2018 to 2020.

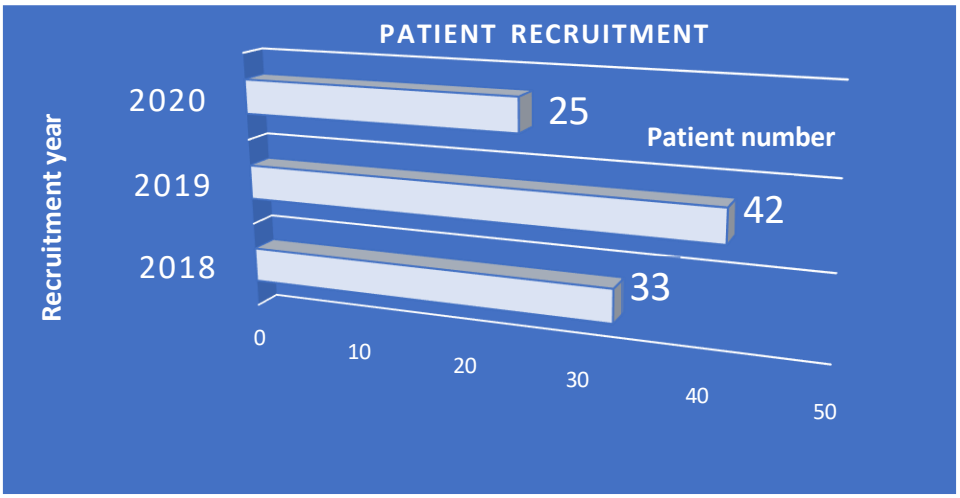


Figure 3. 2. Line chart showing recruitment number from March 2018 to October 2020.

3.6 Standard echocardiographic operating procedures

3.6.2 Transthoracic Echocardiography (TTE) protocol

Echocardiography scans were performed using a GE Vivid 9 platform (Vingmed-General Electric, Horten, Norway) equipped with a phased-array 3.5 MHz transducer. All measurements were made according to the guidelines set by the British Society of Echocardiography. Standard echocardiographic views were obtained using two-dimensional, sectoral Doppler and TVI that were acquired at rest and during exercise for all studies. Offline Q-analysis was used to calculate RV/LV size, function and volume. For STE, QLAB 2D STE quantification software was used. For 4D analyses, offline 4D EchoPac system (QLAB Analyses platform for RV/LV volume and function) was used. All patients had rest and stress echocardiography using BSE/ASE guidelines recommendations following a standardised protocol.

3.6.3 LV/ RV conventional 2D Echocardiography, size and function

For LV size and function, Teichholz formula from the standard 2D short and parasternal long-axis views, and Simpson's biplane method from apical 4, and 3 chamber views were used to calculate LV size and global LV systolic ejection function. For LV longitudinal function, TVI imaging was performed with sample volume placed at mitral annulus in the apical 4-chamber view and stored in a real time. Average LVS′ at baseline and exercise (defined as the highest velocity during systole at the end of isovolumetric contraction) were obtained from the lateral and septal annulus by TDI. Average myocardial velocity during early diastole (E') from both septal and lateral wall at mitral annulus was measured. For LV diastolic function, E, A, E/A were measured using pulsed Doppler through mitral onflow (PW). Global longitudinal function was assessed also by mitral annular plane systolic excursion (MAPSE). All measurements were performed for all 100 patients at rest and at peak exercise (RER >1.0) (Table 3.2).

For RV size and function, RV dimension was measured either in apical 4-chamber view or RV apical focused views at base and at mid-level in end-diastole. Global longitudinal function was assessed by tricuspid annular plane systolic excursion (TAPSE) using M-mode, factional area change (FAC) using 2D apical-four chamber view, and right ventricular systolic velocity (RVS') using TDI from apical 4 chamber view. All measurements were performed for all 100 patients at rest and at peak exercise (RER>1.0) (Table 3.2).

3.6.4 Myocardial strain mechanics measured by 2D STE

2D gray scale specific echocardiographic views were used for offline analysis including parasternal short axis, apical 4, 3 and 2 chamber views in three consecutive cardiac cycles at a frame rate between 35 and 70 frames per second. Offline strain analysis was performed using EchoPac system (QLAB) for apical 4, 2 and 3 chamber views by automatically or manually adjusted tracking (if the latter is needed) of the endocardial border. The endocardial surface was manually identified in three points in apical 4, 2 and 3 chamber views for longitudinal strain (septal, lateral points at the level of basal mitral valve and at the apex). For RV, global longitudinal RV strain (RVGLS) and global free wall strain (RVGFWS) were analysed by apical 4-chamber or RV focused view with semi-automated border detection and manual correction if needed. All measurements were performed for all 100 patients at rest and at low exercise level (RER between .85 to .95) (Table 3.2).

Table 3. 2. 2D parameters of RV and LV used in the main analysis.

2D RV	**2D LV**
RV basal diameter (mm)	LVEDD (mm)
RV mid diameter (mm)	LVESD (mm)
TAPSE (mm)	LVEF (%)
FAC (%)	MAPSE (mm)
RVS' (cm/s)	E/A, E/e'
-	LVS' (cm/s)
2DSTE	
RVGLS (%)	LVGLS (%)
RVGFWS (%)	-

TAPSE=tricuspid annular plane systolic excursion; FAC=fractional area change; RVS'=right ventricular systolic velocity; RVGLS=right ventricular global longitudinal strain; RVGFWS=right ventricular global free wall stain; LVEDD=left ventricular end diastolic diameter; LVESD=left ventricular end systolic diameter; LVEF=left ventricular ejection fraction; MAPSE=mitral annular plane systolic excursion; E/A=ratio between E-wave and A-wave; E/e'=ratio between early mitral inflow velocity and mitral annular early diastolic velocity LVS'=left ventricular systolic velocity; LVGLS=left ventricular global longitudinal strain.

3.6.5 4D Analyses

All 4D echocardiographic images were acquired with a standard 4.0-MHz multiplane transducer and ultrasound scanner adapted for 4D image acquisition. 4D LV and RV full-volume images were acquired from apical views at rest and at peak stress. For LV, AP4C full volume multi beat acquisition was stored with a frame rate not less than 30 (Hz) during end-expiratory breath-hold, when available. For RV, RV focused full volume multi beat acquisition was stored with a frame rate not less than 30 (Hz) during end-expiratory breath-hold, when available and the prob was tilted anteriorly when it was needed to include the whole RV free wall and outflow in the same image.

4D EchoPac software-derived functional parameters including, LV end-diastolic volume (LVEDV), LV end-diastolic volume index (LVEDVI), LV end-systolic volume (LVESV), LV end-systolic volume index (LVESVI), LV stroke volume (LVSV), LV stroke volume index (LVSVI) and LVEF were measured using 4D auto LVEF, manual adjustment was performed when needed. All measurements were performed for all 100 patients at rest and at peak exercise (RER>1.0) (Table 3.3).

In the same way, RV end-diastolic volume (RVEDV), RV end-diastolic volume index (RVEDVI), RV end-systolic volume (RVESV), RV end-systolic volume index (RVESVI), RV stroke volume (RVSV), RV stroke volume index (RVSVI), 4DTAPSE, 4DFAC, and RVEF were measured using 4D auto RVEF, manual adjustment was performed when needed. All measurements were performed for all 100 patients at rest and at peak exercise (RER>1.0) (Table 3.3).

Table 3. 3. 4D RV/LV volume and functional parameters.

4DRV parameters	**4DLV parameters**
RVEDV (ml)	LVEDV (ml)
RVEDVI (ml/m^2)	LVEDVI (ml/m^2)
RVESV (ml)	LVESV (ml)
RVESVI (ml/m^2)	LVESVI (ml/m^2)
RVSV (ml)	LVSV (ml)
RVSVI (ml/m^2)	LVSVI (ml/m^2)
RVEF (%)	LVEF (%)

RVEDV=right ventricular end diastolic volume; RVEDVI=indexed right ventricular end diastolic volume; RVESV=right ventricular end systolic volume; RVESVI=indexed right ventricular end systolic volume; RVEF=right ventricular ejection fraction; RVSV=right ventricular stroke volume; RVSVI=indexed right ventricle stroke volume; LVEDV=left ventricular end diastolic volume; LVEDVI=indexed left ventricular end diastolic volume; LVESV=left ventricular end systolic volume; LVESVI=indexed left ventricular end systolic volume; LVEF=left ventricular ejection fraction; LVSV=left ventricular stroke volume; LVSVI=indexed left ventricular stroke volume.

3.6.6 LV myocardial work

EchoPac speckle tracking-based method was used to evaluate LV MW and estimated by employing brachial artery blood pressure and LVGLS (Automated Functional Imaging, EchoPac, Version 202, GE). The software calculates global constructive work (GCW), global wasted work (GWW), global work index (GWI), and global work efficiency (GWE) as mean of the respective segmental values. All parameters were calculated for all 100 patients at baseline and at low exercise intensity (RER between .85 to .95). These measures are further described in the specific chapter.

3.6.7 Myocardial 2D strain dispersion

The global longitudinal strain for LV from apical views (apical four, three and two chamber views) were used for LV mechanical dispersion (MD) and contraction duration (CD). For RV, the global RV strain from apical 4-chamber view was used for RV MD and CD.

CD was measured for all 100 patients from onset R wave on ECG to maximum LV and RV myocardial shortening. MD was defined as the standard deviation (SD) of CD in 16 LV segments and in 6 RV segments models (three RV free wall segments plus three septal segments) (Haugaa et al., 2010b). All measurements were performed for all 100 patients at rest and at low exercise level (RER between .85 to .95). These measures are further described in the specific chapter.

3.6.8 Assessment of pulmonary regurgitation (PR)

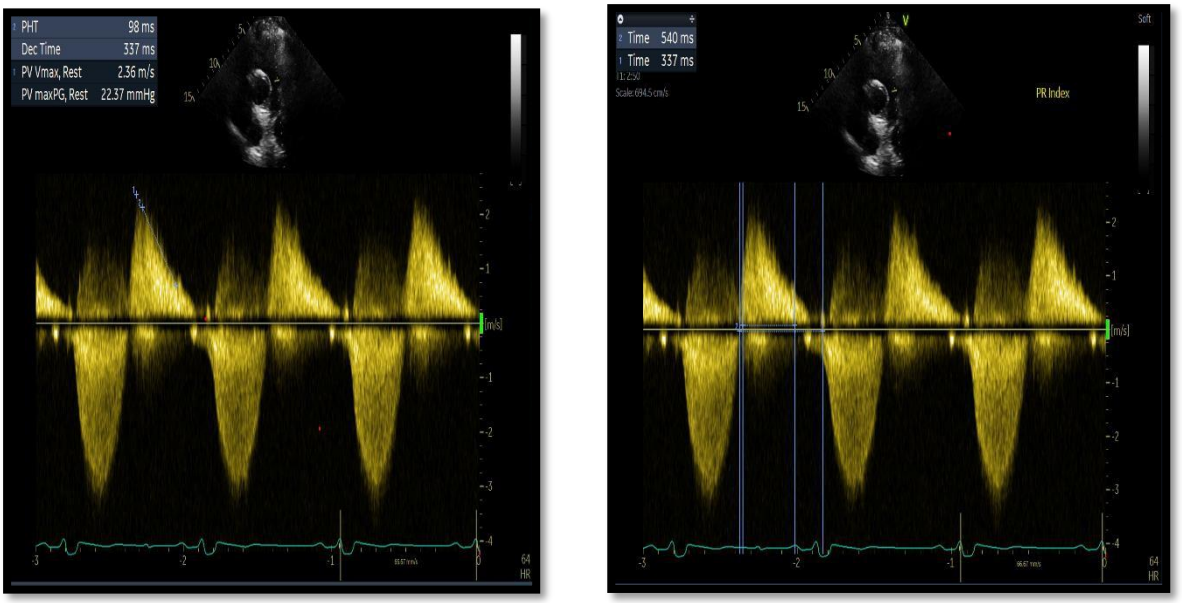
Degree of PR was assessed by color, pulsed and continuous-wave Doppler and severe PR was qualitatively diagnosed when at least one of the following was met: a wide colour Doppler jet occupying more than 2/3 of the right ventricular outflow tract (RVOT), steep and dense continuous wave Doppler jet reaching prior to the end of diastole, or holo-diastolic flow reversal within the main pulmonary artery (Van Berendoncks et al., 2019). All PR assessment was performed at baseline and at peak exercise (when RER>1.0) for all patients.

Severe PR was quantitively assessed by pressure half time (PHT), the ratio of diastolic and systolic time-velocity integrals (DSTVI), PR index, and PR pressure gradient, PG:

- PHT was measured to quantify the deceleration of PR velocity using continuous-wave Doppler from parasternal short-axis view. A PHT of <100 msec for deceleration time was considered severe PR (Figure 3.3, a) (Zoghbi et al., 2017). PR index was calculated as the ratio of PR duration to total diastolic time using continuous-wave Doppler from parasternal short-axis view (Figure 3.3, b). A PR index of <.77 was considered severe PR.

- PR pressure gradient was calculated by Bernoulli equation using continuous-wave Doppler (Figure 3.3, a). DSTVI was measured using continuous-wave Doppler by dividing the area surface of diastolic regurgitation flow by the area of the systolic flow (Figure 3.3, c) (Zoghbi et al., 2017, Van Berendoncks et al., 2019). A DSTVI of >.40 was considered severe PR.

a) **b)**



c)

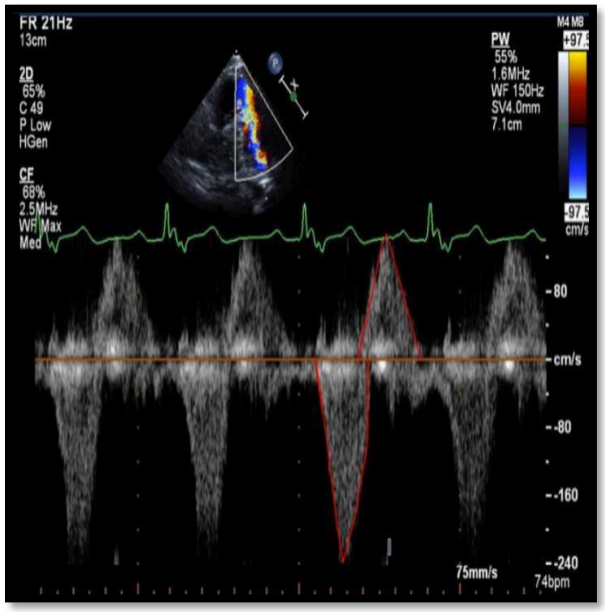


Figure 3. 3. Measurements of severe PR; (a) PG, PHT<100 msec, (b) PR index <.62 (1/2), (c) and DSTVI > .50 (diastolic above the baseline/systolic below the baseline) in repaired TOF patient with severe PR (adapted from study cases).

3.7 Cardiopulmonary stress echocardiography

3.7.2 Cardiopulmonary exercise test (CPET) protocol

Guidelines for contraindications to exercise testing from American Heart Association were used (Fletcher et al., 2001), and the British Cardiovascular Society (The Society for Cardiological Science and Technology, 2008). All patients underwent a semi-recumbent tilting cycle ergometer (ERG 911 S/L, Schiller, Switzerland) with simultaneous echocardiography that were carried out at St. Bartholomew Hospital, London. All tests were conducted with two members of staff present. Patients were asked not to consume alcohol or caffeine 2 hours prior the test, and not to perform any strenuous exercise. All 100 participants were informed of all procedure, risks and benefits relating to the study before providing written consent.

At the start of the test, a 2-min resting period was included followed by a 3-min unloaded warm up period. Exercise protocols were individually determined based on the patient functional status. Work rate (5, 10, 15 or 20 Watt) increased every minute until voluntary exhaustion aiming for 8–10 min of exercise. Patients were asked to stop beta blocker. All participants were paddled at a self-selected rhythm between 60 and 90 revolutions per minute. Heart rate (HR), blood pressure and oxygen saturation were monitored throughout. Oxygen uptake (VO_2), carbon dioxide production (VCO_2) and ventilation (V_E) were continuously measured and derived using a calibrated breath-by-breath analyser (Quark, Cosmed, Italy). A respiratory exchange ratio (RER) >1 was used to indicate a good exercise effort. All tests were terminated if any of the testing guidelines criteria were met (Fletcher et al., 2001).

Echocardiography measurements commenced at low exercise intensity and at peak exercise following specific protocol. Low exercise intensity was identified when the RER between .85-.95 accompanied with 70-80% of peak predicted HR. Peak exercise was identified when patients were close to the end of the test when the RER was more than 1. Patients were verbally encouraged to exercise until maximal exertion. VO_2 peak was expressed as the highest value from an average of 30s during the final stage of the exercise test. A predicted VO_2 of less than 84% was considered to be reduced. The oxygen uptake efficiency slope (OUES) and the V_E/VCO_2 slope were measured using the whole slope as a marker of the effectiveness of ventilation/perfusion matching.

3.7.3 Procedure for exercise echocardiography

A quad-screen format was used for comparative analysis. Echocardiographic assessments following specific protocol taken at baseline, at low intensity, at peak exertion (RER> 1.0), and at recovery with continuous live imaging. A TOF stress echocardiography protocol was created and the following views were obtained at rest, at low intensity and at peak stress (Figure 3.4). The 2D echocardiographic views were taken from parasternal long- and short–axis views, pulmonary artery focused view, apical four chamber, focused RV view, and apical three and two chamber views. 4D LV A4C full volume and 4D focused RV full volume in addition to PW mitral inflow were taken off protocol. Coloured tissue Doppler zoomed optimised pulmonary regurgitation, continuous wave and pulse wave Doppler through the pulmonary valve for pulmonary regurgitation assessment were performed.

Baseline echocardiographic measures including LV size and systolic function, LV diastolic function, left ventricular end-diastolic volume (LVEDV), right ventricular fractional area change (FAC), right ventricular end-diastolic volume (RVEDV), right ventricular size and function, and global longitudinal strain (GLS) for RV and LV, and free wall longitudinal RV deformation were performed at baseline. Once this echo dataset is complete exercise increments was continued to low and peak exercise capacity. Detailed measures of biventricular size, function, volume and strain parameters were described in the standard operating echocardiographic procedures section.

Following acquisition of the baseline imaging, exercise performed using a ramp protocol on a recumbent cycle ergometer (ERG 911 S/L, Schiller, Switzerland) with increasing stress between 5 and 20 W. The test was terminated if limiting symptoms including chest pain, dyspnea or significant adverse haemodynamic changes occurred.

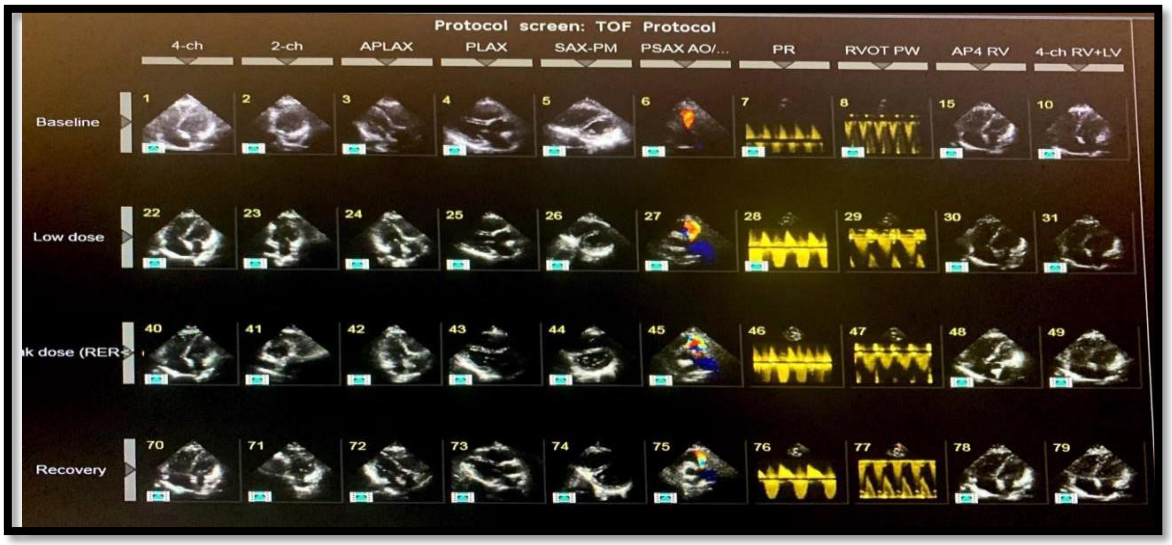


Figure 3. 4. Agreed TOF stress echocardiography protocol.

Echocardiographic offline analysis was performed using the GE Vivid 9 platform EchoPac system (QLAB). Images were optimised for 2D STE analysis in order to ensure coverage of the entire ventricles with a frame rate of 50-100Hz at rest and 70-100Hz during exercise. For left and right ventricular assessment, the depth and the sector size were reduced to include the whole left or right ventricle for further optimisation (higher frame rate).

3.8 Statistical analyses

This study gathered comprehensive data from echocardiography and CPET with the focus on their relationships to exercise testing variables.

3.8.2 Primary endpoint analysis

In cohort A and cohort B

- Resting echocardiographic parameters (function and volume) were compared to stress parameters during CPET. Linear regression was the statistical approach for the predictive analysis of the dependent variable (VO_2) from known value of one or more echocardiographic independent variables.

- Correlation between the exercise capacity and LV/RV function and strain parameters was explored. Pearson correlation test for normally distributed data or Spearman correlation test for non-normally distributed data was chosen for correlation analyses.

- Multivariable linear regression was used to evaluate: (1) independent relationships between measures of RV/LV functional, strain, and contractile reserve parameters, and exercise capacity measures, and; (2) independent relationships of RV/LV volume changes to exercise capacity measures. The presence of severe PR was treated as a binary variable (severe PR / no or minimal PR) in all analyses.

- Intra class correlation coefficient (ICC), and coefficient of variation (COV) were used for interobserver, intraobserver variability, test-retest reliability and agreement for reproducibility, and reliability of all cohort measures.

All statistical analyses were carried out using SPSS 23, 26, 27 and 28 (IBM, Armonk, NY). A p value of <0.05 was considered statistically significant. Detailed statistical analyses are further described in each chapter.

Chapter 4 Reproducibility and repeatability of biventricular function/volume and strain parameters by 2D and 4D echocardiography in adult patients with repaired TOF-A study evaluating the reproducibility of the main study results

4.1 Abstract

Background: The use of 2D and 4D during stress echocardiography to undertake complex measures in complex patients like patients with repaired TOF is challenging and the validity of these measures is not known yet.

Methods: For test-retest variability, 20 patients with repaired TOF with no or mild pulmonary regurgitation were selected randomly and underwent a cardiopulmonary exercise test (CPET) with echocardiography. Intra-observer variability study was performed for all 20 patients by the same observer. Interobserver variability study was performed for 5 patients by different experienced observer. Intraclass correlation coefficient (ICC), and coefficients of variation (COV) were used to quantify reproducibility and variability.

Results: For 2D measures, better reproducibility was observed for semiautomated 2D strain measures than 2D functional measures for biventricular systolic function at baseline and during the stress (ICC>.90 vs >.70, p<0.001), with least COV was observed (COV<10%). 4D semiautomated volumetric measures demonstrated less reproducibility during stress with highest COV was observed for 4D RV volume parameters (COV, 35%), followed by 4D LV volume parameters (COV, 27%). CPET had an excellent agreement of all measures (ICC >.90). with very low COV (<10%).

Conclusions: Semiautomated echo measures outperformed manual measures during stress echocardiography and can be performed with acceptable reproducibility. Variability is at highest for 4D semiautomated measures despite good reproducibility, while lowest variability was observed for 2D semiautomated measures of myocardial deformation.

4.2 Introduction

Exercise stress echocardiography is well established diagnostic tool in clinical decision making in patients with coronary disease and valvular heart disease, however in patients with congenital heart disease and specifically in patients with repaired TOF has not been well established (Cifra et al., 2016, Marwick et al., 1997, Picano et al., 2009, Robbers-Visser et al., 2009). Reproducibility assessment of right and left ventricular systolic function, volume and strain parameters during stress echocardiography in adult patients with repaired TOF is not known. When new techniques are introduced into clinical practice, evaluation of reproducibility and reliability of measurements is of major importance. Our novel results in this thesis need to be validated by further reproducibility analysis in order to utilise the exercise protocol described into the routine clinical assessment in this patient group. We therefore aimed to evaluate the reproducibility of biventricular measurements and reliability of the techniques (stress echocardiography with CPET in adult patients with repaired TOF).

4.3 Methods

4.3.1 Main objectives

To undertake detailed assessment of inter and intra observer variability as well as test-retest variability in this population to validate the study protocol (CPET with stress echocardiography).

4.3.2 Reproducibility analysis

For inter study variability, 20 patients with repaired TOF with no or mild pulmonary regurgitation were selected randomly and underwent a second CPET test with echocardiography. Same protocol was used for CPET and echocardiography which was described in the general method chapter (section 3.7). Intra-observer variability study was performed for all 20 patients. Interobserver variability study was performed for 5 patients that were selected randomly from all population. For intraobserver variability study, all data was analysed by the main observer (Sahar Alborikan, S.A) who was blinded to the former results. For inter-observer variability, a second experienced observer (Ricardo Monteiro, R.M) analysed all data independently who was also blinded to all previous results. Test to retest variability assessment for the advanced echocardiography techniques (4D and 2D strain analysis) was assessed by the same observer for different time acquisitions to assess the test variability across all measurements at baseline and during exercise. Separate data acquisitions with additional evaluation of baseline and exercise echocardiographic images were obtained for all reproducibility assessments within 16 months after the first visit.

4.3.3 Echocardiography analysis

All echocardiographic assessment was performed for detailed reproducibility analyses following the same protocol and echocardiography analyses for functional, volume and strain parameters. Detailed methods of 2D and 4D echocardiographic measurements of right ventricle (RV) and left ventricle (LV) is explained in chapter 3 (section 3.6).

4.3.4 Statistical analysis

Intra and interobserver reproducibility were assessed using the method of intraclass correlation coefficient. The following levels of agreement were used, excellent for ICC values >.75, good for ICC between .60 and .74, fair for ICC .49-.59, and poor for ICC <.49 (Cicchetti, 1994). Data for test-retest variability is presented as mean± SD for all continuous variables, and the systematic difference between two tests were evaluated with Student's paired t test. The intra-class correlation coefficient (ICC) was presented with 95% confident intervals, and coefficients of variation (COV) were used to quantify variability. COV was used in inter-study reproducibility, and were calculated as the SD of the difference between the means of two acquisitions divided by their main value and expressed as a percentage. Any value less than 10% was considered excellent, COV of 10-20% was considered good variation, and COV> 30 % was considered high (Tamborini et al., 2010) . The statistical analysis was performed using IBM SPSS statistics version 28. A p value of <0.05 was considered statistically significant.

4.4 Results

4.4.1 Baseline characteristics

20 adult patients with repaired TOF with no or mild pulmonary regurgitation were selected randomly for the reproducibility analyses. Mean age was 39 ±11 y, and 13 patients (65%) were male and 7 patients (35%) were female. Mean duration between two tests was 16±7 months (Table 4.1).

Table 4. 1. Baseline characteristics of the reproducibility population.

Baseline characteristics	**20 TOF patients**
Age (y), Mean ±SD	39 ±11
Gender (n, %): **Male (%)**	13 (65%)
Female (%)	7 (35%)
Mean duration between tests (months)	16±7

4.4.2 Interobserver reproducibility

5 studies underwent repeated analyses from a different observer analysis. For conventional RV functional measures, RVS', TAPSE (excellent reproducibly at both baseline and at peak exercise) were more reproducible than FAC where the reproducibility was good. For LV functional parameters, LVS' showed an excellent reproducibility at rest and on exertion with ICC >.74 at peak stress (Table 4.2).

For semi-automated RV and LV deformation images, performance was even better with excellent reproducibility for RV and LV strain parameters including RVGLS, RVGFWS and LVGLS at both baseline and at low exercise intensity with all parameters showing and ICC > 0.90 (Table 4.3).

For semi-automated 4D volumetric parameters, for the RV most 4D (volume / indexed volumes and ejection fraction) and derived parameters (TAPSE /FAC) showed good or excellent ICC, with a trend towards weaker correlations on the exercise measured parameters (Table 4.4). 4DLV volume parameters including LVEDV, LVESV, LVSV, and their indexed values which also showed good interobserver variability at baseline. LV 4D volumes at peak stress were more reproducible than 4DRV volumetric parameters $p<0.001$ (Table 4.4).

4.4.3 Intraobserver reproducibility

20 repeated analyses from the same observer were undertaken. For conventional measured, RV functional parameters including RVS' and TAPSE were more reproducible than FAC at baseline and at peak stress (excellent ICC>.83), while good if lower reproducibility for FAC (ICC .70 and .73), at baseline and at peak stress respectively was observed. For LV functional parameters, LVS' showed an excellent reproducibility with ICC >.74 at baseline and at peak stress (Table 4.2). For semi-automated deformation analysis there was an excellent reproducibility for RV and LV strain parameters including RVGLS, RVGFWS and LVGLS at baseline and at low exercise intensity (ICC of >.90) (Table 4.3).

For 4DRV volume parameters reproducibility, 4DRV volume parameters including RVEDV, RVESV, RVSV, and their indexed values showed an excellent reproducibility at baseline (ICC >.80), but again lower reproducibility was observed at peak stress with ICC ranged from fair to good reproducibility (ICC between .50 to .74) (Table 4.4).

For 4DLV volume reproducibility, 4DLV volume parameters including LVEDV, LVESV, LVSV, and their indexed values showed also an excellent reproducibility at baseline (ICC >.80), but with lower reproducibility at peak stress. When compared to 4D RV volume parameters, 4DLV volume parameters at peak stress were more reproducible (4D LV measures ICC ranged from good .74 to excellent .82, while ICC for 4DRV measures ranged from fair .50 to good .70) (Table 4.4).

There were significant differences in all ICC measurements between inter and intra observer reproducibility for 2D and 4D parameters while there was no difference observed for STE measurements (Table 4.2, 4.3 and 4.4). For ICC difference between LV and RV inter and intra observer variability for 4D parameters, there were significant differences in all ICC measurements between inter and intra observer reproducibility in all RV/LV parameters, except inter observer variability for RVEDV and LVEDV (ICC .98 vs .96, p>0.05), RVESV and LVESV (ICC .97 vs .97, p>0.05), and intraobserver variability for RVESVI and LVESVI (ICC .80 vs .82, p.>0.05), RVSVI and LVSVI (ICC .87 vs .85, p>0.05), with relatively higher reproducibility was observed in inter observer variability for the strain and volume analyses (Table 4.4).

Table 4. 2. Inter and intra observer reproducibility of 2D functional parameters.

2D RV longitudinal functional parameters		**ICC_{intra} 95% CI N=20**	**ICC_{inter} 95% CI N=5**	**P value***
FAC (%)	**Baseline**	.70 (.44-.91)	.60 (.59-.99)	**<0.001**
	Peak exercise	.73 (.35-.85)	.70 (.69-.99)	**<0.001**
TAPSE (mm)	**Baseline**	.99 (.97-.99)	.77 (.31-.97)	**<0.001**
	Peak exercise	.93 (.84-.97)	.99 (.90-.99)	**<0.001**
RVS' (cm/s)	**Baseline**	.86 (.65-.94)	.93 (.40-.99)	**<0.001**
	Peak exercise	.84 (.60-.93)	.70 (.31-.96)	**<0.001**
2D LV longitudinal functional parameters LVS' (cm/s)	**Baseline**	.75 (.30-.83)	.70 (.20-.91)	**<0.001**
	Peak exercise	.75 (.21-.87)	.98 (.85-.99)	**<0.001**

FAC=fractional area change; TAPSE=tricuspid annular plane systolic excursion; RVS'=right ventricular systolic velocity; LVS'=left ventricular systolic velocity. Bold values indicate significant level (p<0.001). * p value of the difference between inter and intra observer reproducibility.

Table 4. 3. Inter and intra observer reproducibility of 2D strain parameters.

RV/LV Strain reproducibility		**ICC_{intra} 95% CI N=20**	**ICC_{inter} 95% CI N=5**	**P value***
RVGLS, %	**Baseline**	.94 (.86-.97)	.93 (.39-.99)	NS
	Low intensity exercise	.95 (.85-.97)	.97 (.74-.99)	NS
RVGFWS, %	**Baseline**	.96 (.89-.98)	.98 (.87-.99)	NS
	Low intensity exercise	.94 (.83-.97)	.99 (.85-.99)	NS
LVGLS, %	**Baseline**	.91(.78-.96)	94 (.45-.99)	NS
	Low intensity exercise	.94 (.45-.96)	.96 (.68-.99)	NS

RVGLS=right ventricular global longitudinal strain; RVGFWS=right ventricular global free wall stain; LVGLS=left ventricular global longitudinal strain. * p value of the difference between inter and intra observer reproducibility; NS=non-significant.

Table 4. 4. Inter and intra observer reproducibility of 4DRV and 4DLV volume parameters.

4D RV parameters reproducibility		ICC_{intra} 95% CI N=20	ICC_{inter} 95% CI N=5	P value*	4D LV parameters reproducibility	ICC_{intra} 95% CI N=20	ICC_{inter} 95% CI N=5	P value*	P value RV vs LV	
									Intra	Inter
RVEDV (ml)	**Baseline**	.97 (.84-.99)	.98 (.85-.99)	**<0.001**	**LVEDV (ml)**	.88 (.71-.95)	.96 (.61-.99)	**<0.001**	**<0.05**	NS
	Peak exercise	.70 (.69-.98)	.80 (.79-.99)	**<0.001**		.74 (.38-.90)	.99 (.91-.99)	**<0.001**	**<0.05**	**<0.05**
RVEDVI (ml/m2)	**Baseline**	.96 (.92-.98)	.99 (.91-.99)	**<0.001**	**LVEDVI (ml/m2)**	.93 (.84-.97)	.95 (.61-.99)	**<0.001**	**<0.05**	**<0.05**
	Peak exercise	.65 (.91-.98)	.86 (.85-.99)	**<0.001**		.78 (.45-.91)	.99 (.94-.99)	**<0.001**	**<0.05**	**<0.05**
RVESV (ml)	**Baseline**	.84 (.61-.93)	.97 (.78-.99)	**<0.001**	**LVESV (ml)**	.90 (.73-.95)	.97 (.72-.99)	**<0.001**	**<0.05**	NS
	Peak exercise	.70 (.54-.92)	.75 (.60-.99)	**<0.001**		.77 (.42-.90)	.95 (.60-.99)	**<0.001**	**<0.05**	**<0.05**
RVESVI (ml/m^2)	**Baseline**	.80 (.40-.90)	.83 (.81-.99)	**<0.001**	**LVESVI (ml/m^2)**	.82 (.44-.87)	.96 (.67-.99)	**<0.001**	**NS**	**<0.05**
	Peak exercise	.60 (.59-.95)	.70 (.69-.99)	**<0.001**		.80 (.50-.92)	.98 (.70-.99)	**<0.001**	**<0.05**	**<0.05**
RVEF (%)	**Baseline**	.50 (.21-.69)	.70 (.20-.96)	**<0.001**	**LVEF (%)**	.84 (.59-.93)	.88 (.40-.96)	**<0.001**	**<0.05**	**<0.05**
	Peak exercise	.74 (.40-.91)	.94 (.43- 99)	**<0.001**		.82 (.55-.92)	.90 (.60-.99)	**<0.001**	**<0.05**	**<0.05**
RVSV (ml/m^2)	**Baseline**	.86 (.66-.94)	.98 (.81-.99)	**<0.001**	**LVSV (ml/m^2)**	.81 (.51-.92)	.80 (.34-.96)	**<0.001**	**<0.05**	**<0.05**
	Peak exercise	.50 (.40-.96)	.83 (.80-.99)	**<0.001**		.65 (.20-.86)	.96 (.65-.99)	**<0.001**	**<0.05**	**<0.05**
RVSVI (ml/m^2)	**Baseline**	.87 (.70-.95)	.98 (.86-.99)	**<0.001**	**LVSVI (ml/m^2)**	.85 (.32-.89)	.81 (.40-.91)	**<0.001**	NS	**<0.05**
	Peak exercise	.59 (.50-.96)	.79 (.75-.99)	**<0.001**		.82 (.55-.93)	.95 (.52-.99)	**<0.001**	**<0.05**	**<0.05**
TAPSE (mm)	**Baseline**	.93 (.84-.97)	.99 (.90-.99)	**<0.001**	**CO (l/m)**	.62 (.40-.85)	.72 (.41-.97)	**<0.001**	-	
	Peak exercise	.97 (.93-.99)	.92 (.22-.99)	**<0.001**		.48 (.29-.79)	.93 (.38-.99)	**<0.001**	-	
FAC (%)	**Baseline**	.48 (.30-.79)	.88 (.23-.98)	**<0.001**	-	-	-	-	-	
	Peak exercise	.73 (.32-.89)	.95 (.56-.99)	**<0.001**		-	-	-	-	

RVEDV=right ventricular end diastolic volume; RVEDVI=indexed right ventricular end diastolic volume; RVESV=right ventricular end systolic volume; RVESVI=indexed right ventricular end systolic volume; RVEF=right ventricular ejection fraction; RVSV=right ventricular stroke volume; RVSVI=indexed right ventricle stroke volume; TAPSE=tricuspid annular plane systolic excursion; FAC=fractional area change; LVEDV=left ventricular end diastolic volume; LVEDVI=indexed left ventricular end diastolic volume; LVESV=left ventricular end systolic volume; LVESVI=indexed left ventricular end systolic volume; LVEF=left ventricular ejection fraction; LVSV=left ventricular stroke volume; LVSVI=indexed left ventricular stroke volume; CO=cardiac output. Bold values indicate significant level (p.<0.05). * P value of the difference between inter and intra observer reproducibility; NS=non-significant.

4.4.4 Inter study variability- Agreement

20 patients underwent a second CPET test with echocardiography within 16 months after the first test. Same protocol for CPET and echocardiography was performed. Test to-retest reproducibility of all CPET parameters was excellent with an ICC >.90 of all measures with COV of <10% (Table 4.5).

There were no significant differences in all 4D RV, LV volume parameters means and in 2DSTE strain parameters between two scans (Table 4.6, 4.7, and 4.8). Test to-retest reproducibility of all RV and LV strain parameters was excellent with ICC more than .90, and with COV of <10%, with more reproducibility was observed during low exercise intensity for RVGLS and LVGLS (Table 4.6). Inter test observer variance for RV and LV strain measurements showed a good agreement for all measurements at baseline and during stress with minimal variability (COV<10%) (Table 4.6) (Figure 4.1, a).

4DRV volumetric parameters results were highly reproducible at baseline but with high variability (ICC >.80, COV up to 25%), however, lower reproducibility was observed at peak stress with ICC ranged between fair to good reproducibility with higher variability (ICC ranged between .50 to .74, COV up to 35%) (Table 4.7). 4DLV volumetric parameters showed also high reproducibility at baseline with ICC>.80, and again was less reproducible at peak exercise, however it was more reproducible than 4DRV volume with ICC ranged between good to excellent reproducibility (ICC between .74 to .82, COV up to 27%) (Table 4.8). Inter observer test variance was the highest for 4DRV volume parameters at peak stress (COV, 35%) (Table 4.7), followed by 4DLV volume parameters at peak stress (COV, 25%) (Table 4.8) (Figure 4.1, b), and 4DLV/RV EF (COV, 16 and 9%) (Figure 4.1, c).

Table 4. 5. Test-retest reproducibility of CPET parameters.

CPET parameters	Scan 1 N=20	Scan 2 N=20	P value*	ICC N=20	COV, 95% CI
Exercise duration	9.9±2	9.3±2	NS	.95 (.88-.98)	3%
Exercise protocol	15±1	15±2	NS	.99 (.89-.99)	8%
VO_2 (ml/min)	1910±500	1900±530	NS	.98 (.95-.99)	9%
VO_2 (ml/kg/min)	25±8	25±7	NS	.98 (.96-.99)	5%
Predicted VO_2 (%)	81±15	80±15	NS	.99 (.97-.99)	2%
RER	1.2±.1	1.2±.2	NS	.96 (.66-.97)	1%

VO_2=peak maximum oxygen uptake in absolute (ml/min), relative (ml/kg/min), percent of predicted (%); RER=respiratory exchange ratio. * P value of the difference between scan 1 and scan 2 measurements; NS=non-significant.

Table 4. 6. Test-retest reproducibility of 2D strain parameters**.**

Strain parameters		Scan 1 N=20	Scan 2 N=20	P value*	COV	ICC, 95% CI
RVGLS, %	**Baseline**	-14.6 ±3.5	-14.7±3.5	NS	9.2%	.94 (.86-.97)
	Low intensity exercise	-17±3.7	-17±3.2	NS	10%	.95 (.85-.97)
RVGFWS, %	**Baseline**	-16.8±3.6	-16.9±3.1	NS	10%	.95 (.89-.98)
	Low intensity exercise	-19±4	-19±4.2	NS	12%	.94 (.83-.97)
LVGLS, %	**Baseline**	-15.1±2.1	-15±3.7	NS	4%	.91(.78-.96)
	Low intensity exercise	-17±2.1	-17±2.1	NS	3%	.94 (.45-.96)

RVGLS=right ventricular global longitudinal strain; RVGFWS=right ventricular global free wall stain; LVGLS=left ventricular global longitudinal strain. * P value of the difference between scan 1 and scan 2 measurements; NS=non-significant.

Table 4. 7. Test-retest reproducibility of 4DRV volume parameters.

4D RV parameters reproducibility		Scan 1 N=20	Scan 2 N=20	P value*	COV	ICC, 95% CI
RVEDV (ml)	**Baseline**	135 ±28	134 ±28	NS	25 %	.97 (.84-.99)
	Peak exercise	118 ±30	117 ±27	NS	35%	.70 (.69-.98)
RVEDVI (ml/m2)	**Baseline**	72 ±13	71 ±13	NS	14%	.96 (.92-.98)
	Peak exercise	63 ±13	62 ±13	NS	18%	.65 (.91-.98)
RVESV (ml)	**Baseline**	70 ±15	71 ±17	NS	18%	.84 (.61-.93)
	Peak exercise	55 ±1	57 ±17	NS	19%	.70 (.54-.92)
RVESVI (ml/m^2)	**Baseline**	37 ±7	37 ±6	NS	16%	.80 (.40-.90)
	Peak exercise	34 ±6	35 ±8	NS	18%	.60 (.59-.95)
RVEF (%)	**Baseline**	48 ±5	48 ±6	NS	5%	50 (.21-.69)
	Peak exercise	56 ±6	55 ±7	NS	9%	.74 (.40-.91)
RVSV (ml/m^2)	**Baseline**	64 ±16	63 ±16	NS	25%	.86 (.66-.94)
	Peak exercise	66 ±16	65 ±16	NS	30%	.50 (.40-.96)
RVSVI (ml/m^2)	**Baseline**	34 ±8	33 ±9	NS	19%	.87 (.70-.95)
	Peak exercise	36 ±8	35 ±9	NS	20%	.59 (.50-.96)
TAPSI (mm)	**Baseline**	12 ±4	12 ±4	NS	16%	.93 (.84-.97)
	Peak exercise	18 ±6	17 ±5	NS	22%	.97 (.93-.99)
FAC (%)	**Baseline**	40 ±6	39 ±3	NS	7%	.48 (.30-.79)
	Peak exercise	49 ±8	48 ±6	NS	16%	.73 (.32-.89)

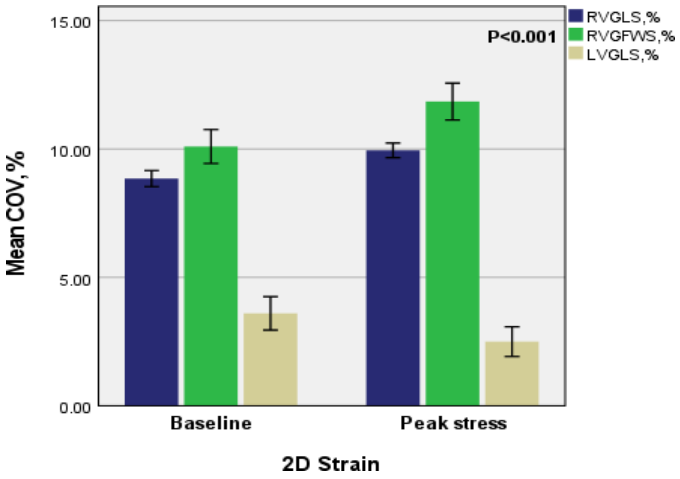
RVEDV=right ventricular end diastolic volume; RVEDVI=indexed right ventricular end diastolic volume; RVESV=right ventricular end systolic volume; RVESVI=indexed right ventricular end systolic volume; RVEF=right ventricular ejection fraction; RVSV=right ventricular stroke volume; RVSVI=indexed right ventricle stroke volume; TAPSE=tricuspid annular plane systolic excursion; FAC=fractional area change. * P value of the difference between scan 1 and scan 2 measurements; NS=non-significant.

Table 4. 8. Test-retest reproducibility of 4DLV volume parameters.

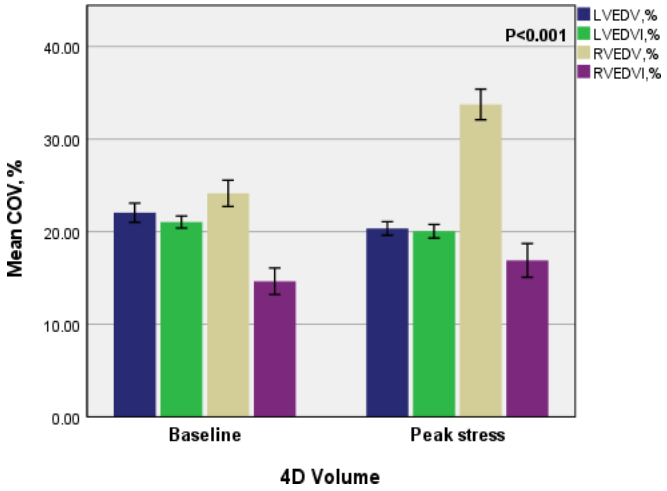
4D LV parameters reproducibility		Scan 1 N=20	Scan 2 N=20	P value*	COV	ICC, 95%CI
LVEDV (ml)	**Baseline**	109±13	110±13	NS	22 %	.88 (.71-.95)
	Peak exercise	96±16	96±15	NS	20%	.74 (.38-.90)
LVEDVI (ml/m2)	**Baseline**	59±8	59±8	NS	21%	.93 (.84-.97)
	Peak exercise	52±9	52±9	NS	20%	.78 (.45-.91)
LVESV (ml)	**Baseline**	45±8	46±7	NS	25%	.90 (.73-.95)
	Peak exercise	30±9	31±7	NS	22%	.77 (.42-.90)
LVESVI (ml/m^2)	**Baseline**	25±4	26±9	NS	27%	.82 (.44-.87)
	Peak exercise	16±4	16±4	NS	14%	.80 (.50-.92)
LVEF (%)	**Baseline**	58±4	57±4	NS	14%	.84 (.59-.93)
	Peak exercise	68±5	68±4	NS	16%	.82 (.55-.92)
LVSV (ml/m^2)	**Baseline**	63±8	64±9	NS	20%	.81 (.51-.92)
	Peak exercise	65±10	66±10	NS	22%	.65 (.20-.86)
LVSVI (ml/m^2)	**Baseline**	33±5	33±6	NS	20%	.85 (.32-.89)
	Peak exercise	35±7	35±7	NS	27%	.82 (.55-.93)
CO (l/m)	**Baseline**	4±.8	4±.9	NS	.5%	.62 (.40-.85)
	Peak exercise	8±1	8±1	NS	.8%	.48 (.29-.79)

LVEDV=left ventricular end diastolic volume; LVEDVI=indexed left ventricular end diastolic volume; LVESV=left ventricular end systolic volume; LVESVI=indexed left ventricular end systolic volume; LVEF=left ventricular ejection fraction; LVSV=left ventricular stroke volume; LVSVI=indexed left ventricular stroke volume; CO=cardiac output. * P value of the difference between scan 1 and scan 2 measurements; NS=non-significant.

a)



b)



c)

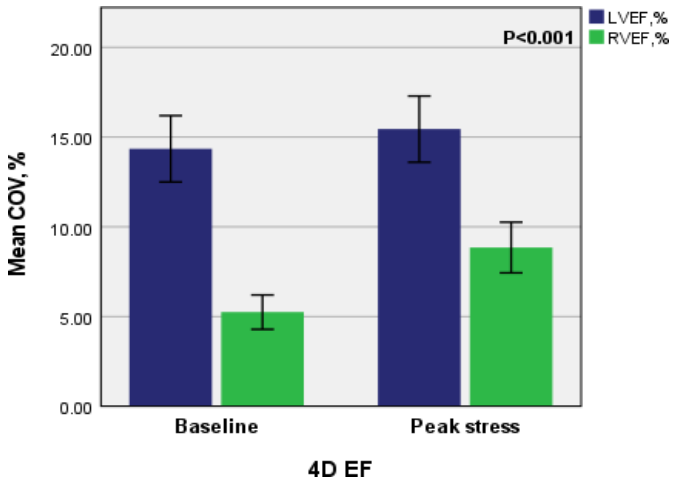


Figure 4. 1. Bar chart for (a) 2DRV and LV strain COV (mean ± SD), (b) 4DLV and RV volume COV (mean ± SD), and biventricular 4DEF COV (mean ± SD). p<0.001 indicate the difference of COV between baseline and at peak stress for each parameter.

4.5 Discussion

In this chapter we have demonstrated the following, measures of RV function from 2D and 4D echo can be performed with a good level of agreement between operators and a good reproducibility. There is a tendency form simple measures (single point identification, e.g., TAPSE and S') to outperform area measures, while semi-automated measures outperformed manual measures, suggesting that the future direction is through a computer directed approach to reduce variability. The coefficient of variability (COV) is very low for cardiopulmonary exercise testing. The COV was good for most measures but became noticeably worse for volume measures during stress for both right and left ventricles. Interestingly not for EF, suggesting variability measures were consistent in systole and diastole.

Stress echocardiography is challenging, and it is more difficult when assessing patients with complex conditions like patients with repaired TOF. Ideally, there should be an agreed standard protocol for stress echocardiography targeting specific parameters in each patient group. The test should be simple, reproducible and feasible in order to be considered into routine clinical care. There is a complete lack of consensus concerning the specific diagnostic of stress echocardiography protocol, and in identifying which echo parameter has the best agreement and COV that should be implemented into clinical practice in adult patients with repaired TOF. The use of 2D echo to undertake complex measures in complex patients is challenging, but using 4D measures under stress conditions is more so. We predicted that the coefficient of variability would be high, perhaps too high, to render these measures of value. However, in terms of agreement and COV, the semiautomated 2D strain to measure LV and RV strain were found to have the best reliability at baseline and during stress echocardiography with least COV (<10%), followed by 4DLV volume and function analyses (<27%), and the highest variability was observed with 4DRV volume and function analyses despite good reliability (COV, 35%).

To the best of our knowledge, this is the first study to test the reproducibility of stress echocardiography and CPET with all 2D functional, 2DSTE and 4D in patients with repaired TOF. For 2D measures, inter and intra observer variability assessment showed more reproducibility for 2D conventional longitudinal function points measures than area measures both at baseline and at peak stress. For 2D semi-automated strain measure, excellent inter and intra observer reproducibility were observed at baseline and at peak stress that were higher than 2D conventional measures. When assessing agreements between measures, the least variability was observed also in the semi-automated strain measures. Very few studies have evaluated RV during stress echocardiography in patients with repaired TOF (Ait-Ali et al., 2014, Bhatt et al., 2019b, Kingsley et al., 2018, O'Meagher et al., 2014), and LV (Davlouros et al., 2002, Fernandes et al., 2012, Ghai et al., 2002). However, inter and /or intraobserver reproducibility of measurements were reported in only one of these reports who have shown higher reproducibility for 2D RV functional measures (Ait-Ali et al., 2014).

Our test-retest reproducibility analysis for CPET showed an excellent reproducibility with minimal inter test variance. 20 repeated CPET studies demonstrated an excellent agreement for all measures of peak VO_2 (peak absolute, relative and predicted). Using CPET in patients with repaired TOF lacks standardisation of testing and a dedicated modified protocol (Constantine et al., 2021, Alborikan et al., 2020). Our excellent agreement of all CPET measures is a very important finding specifically for the subsequent sub-studies analyses in this thesis, which makes us more confident about the CPET data that are presented in each chapter. Agreement of our CPET results is in line with previous studies in heart failure patients (Cohen-Solal et al., 1991, Sinclair et al., 2009), but once again there is no data available in patients with repaired TOF.

Assessment of 4D during stress echocardiography is novel and challenging that requires a very experienced operator. Our reproducibility results of semi-automated 4DLV and 4DRV parameters at baseline including volume, indexed volume, functional derived parameters ranged from good to excellent in both inter and intra-observer reproducibility assessments. During exercise, both were less reproducible, but when compared between the two, 4DLV parameters were more reproducible than 4DRV parameters, with ICC ranged from good to excellent, while ICC ranged from fair to good for 4D RV volume parameters. Interestingly, this is supported by inter test observer variance analyses which showed good agreement between all 4D measures with highest test variance observed for 4DRV parameters (COV, 35%), and 4DLV parameters (COV, 27%). The observer related variability was expected due to the complex geometry of RV chamber and the difficulties of 4DRV and LV analyses; however, our results are surprisingly better than what we have predicted when establishing the study protocols.

The excellent reproducibility of semiautomated strain parameters and functional parameters may further encourage the use of stress echocardiography in this group of patients. Since we found a considerable variability for 4D semiautomated parameters for both RV and LV despite good reproducibility, our results imply the need for some caution in using them especially during the stress at this time. This significant variability during exercise has been reported for 2D strain and volume measures in healthy individuals depending on the vendor or software platform (Farsalinos et al., 2015, Nelson et al., 2012), but 4D validity studies in patients with repaired TOF are lacking. We confidently state that the best measures to assess during stress echocardiography in this patient group with excellent reproducibility both at rest and at peak stress are the 2D semiautomated strain measures including RVGLS, RVGFWS and LVGLS, followed by 2D functional measures for RV and LV including RVS', TAPSE, and LVS', but not for the area measures. Finally, 4D semiautomated measures can be assessed with acceptable reproducibility, however during stress echocardiography it deteriorates and extra caution should be taken. It may be that technological developments such as increased processing power leading to higher frame rates for smaller voxel size (higher temporal and spatial resolution) as well as the use of echocardiographic contrast agents may lead to improvements.

Inter and intra observer validity assessments of stress echocardiography have never been assessed and there is no recommended strategy for proper assessment of RV and LV during stress echocardiography in the current guidelines in patients with repaired TOF (Picano et al., 2009). Our data enable us to make recommendation to undertake stress echocardiography in adult patient with repaired TOF. First, 2D semiautomated functional measures are the most reproducible measures with minimal degree of variance in both inert and intra observer variability. Second, CPET has an excellent agreement of all measures with very low test-retest variability. Third, 2D longitudinal functional measures are more reproducible than 2D area measures. Fourth, 4D volumetric parameters for both RV and LV are less reproducible during stress with worse coefficient of variability would expect for the 4DRV measures. This study has major clinical implications with the excellent inter-study reproducibility of CPET shown and with the overall echocardiographic reproducibility ranged from good to excellent which make the test clinically valid for sequential assessment in this patient group. We are therefore confident of being able to validate our results in the following sub-studies in this thesis.

4.6 Study limitations

The main limitations of this study were the relatively small number of patients selected for the reproducibility study and all analyses were performed by experienced observers. The chance of higher variability increases with less experienced operators especially for the advanced echocardiographic techniques. The time frame between two tests for test-retest reproducibility is considered long period however, our study gathered data from clinically stable TOF patients and the results represent the most reproducible studies to date.

4.7 Conclusions

Evaluation of biventricular function, volume and strain parameters by 2D and 4D echo during stress echocardiography in adult patients with repaired TOF can be performed with acceptable reproducibility. The variability of CPET parameters is very low. Variability is at highest for 4D semiautomated volume measures despite good reproducibility, while lowest variability was observed for 2D semiautomated measures of myocardial deformation.

Chapter 5 Right and left ventricular structural, functional characteristics, volumes, mechanics and myocardial augmentation during exercise; how do they predict exercise capacity in patients with Tetralogy of Fallot and pulmonary regurgitation

5.1 Abstract

Background: Identifying exercise determinants in patients with repaired Tetralogy of Fallot (rTOF) has been always a controversial topic. We sought to investigate exercise performance in addition to identify the best exercise predictors in this population.

Methods: We prospectively recruited 100 patients, 60 repaired TOF patients with severe PR (SPR), and 40 repaired TOF patients matched control with no or mild PR. All 100 patients underwent cardiopulmonary exercise testing with echocardiography. All analyses were performed at baseline and at peak stress. RV contractile reserve (CR) was defined by the change in tricuspid lateral annular peak systolic velocity (ΔRVS'), and change in RV fractional area change (ΔFAC). LV CR was defined by the change in Doppler-derived left ventricular systolic function (ΔLVS'), and change in LV global longitudinal strain (ΔLVGLS).

Results: There was no significant difference in exercise performance observed between the SPR and control groups with similar peak absolute oxygen consumption VO_2 (ml/min) (1695±627 vs 1744±521, ml/min, $p>0.05$). At baseline, overall reduced RV systolic function was observed in the control and normal LV systolic function was observed in both groups. During exercise, lower RV CR was observed in the SPR group by ΔRVS' (41±28 vs 48±20 %, $p<0.05$); and ΔFAC (20±15 vs 23±16, %, $p<0.05$), while it was greater for LV CR by ΔLVS' (67±34 vs 61±28 %, $p<0.05$). Change in ΔLVGLS was the same (15±17 vs 16±15, %, $p>0.05$) in both groups. There were no associations observed between objective exercise measures with the degree of PR at rest and during exercise. Augmentation of LVGLS and FAC were shown independent associations with peak VO_2 ($r=.55$, $r=.45$, $p<0.05$).

Conclusions: There was no difference in exercise limitation between patients who do and do not have PR. Marked reduction in exercise capacity in this population is more dependent upon the ability of left and right ventricles to augment the longitudinal function rather than to severity of PR.

5.2 Introduction

Chronic pulmonary regurgitation (PR) remains the most common haemodynamic complication in adult patients with repaired TOF despite advances in surgical strategies (Gerrah et al., 2015, Ternestedt et al., 2001). Chronic PR leads to right ventricular (RV) dilatation and dysfunction, and progressive limitation in functional capacity, however the exact echocardiography haemodynamic factor behind impaired exercise capacity and how it contributes to reduced exercise capacity are a complex topic in patients with repaired TOF (Khairy et al., 2010, Wijesekera et al., 2016, Brown et al., 2009). There is a lack of understanding in this population in how to incorporate the objective exercise measures into management protocols in this population. Furthermore, there is scant data to support the use of echocardiography and cardiopulmonary exercise testing (CPET) to better understand biventricular response during exercise with inconsistent results about the haemodynamic determinants of exercise capacity in adult survivors of TOF (Meierhofer et al., 2017, Yap et al., 2013, Samman et al., 2008). We therefore aimed to (1) evaluate exercise performance and induced exercise echocardiographic haemodynamic changes in adult patients with repaired TOF, (2) to establish the expected range of biventricular contractile reserve, and (3) to identify the best exercise predictors of progressive exercise limitations in this population.

5.3 Methods

5.3.1 Main hypothesis and objectives

We aimed to develop a new understanding of the impact of severe pulmonary regurgitation on cardiac structure, functional performance and to what extent they are related by describing resting and stress induced echocardiographic physiological changes. We hypothesised that combined CPET and echocardiography could reveal the exact cause of functional limitations in this population. This hypothesis is the primary hypothesis of the project.

In order to do this, we asked the following questions

1) To compare structural and functional characteristics in patients with repaired Fallot with and without severe PR.
2) To describe right ventricular (RV) and left ventricular (LV) augmentation during stress in 2D and 4D echocardiography.
3) To describe the influence of pulmonary regurgitation (PR) on exercise tolerance in adult patients with repaired TOF.
4) To create a model to identify the best predictors of exercise performance in this population.

5.3.2 Study cohort

100 adult patients with repaired TOF were prospectively evaluated, 60 patients with severe pulmonary regurgitation (SPR) were compared to 40 repaired TOF patients with mild or no PR as a control group. Details of the recruitment strategy as well as inclusion and exclusion criteria are as described before (section 3.1 and 3.3).

5.3.3 Echocardiography analysis

Details of 2D, speckle tracking echocardiography (STE), 4D imaging acquisition, and stress echocardiography protocol are described in general method chapter (section 3.6).

5.3.4 Cardiopulmonary exercise testing (CPET)

Protocols were individually determined according to the patient ability with work rate of the test was ranged between 15 to 20w, increased every minute until voluntary exhaustion. The full CPET protocol for all 100 cases was previously described in general method chapter (section 3.7). Peak absolute VO_2 (ml/min) was used as the main outcome variable for the primary end point as it has been previously shown to be a strong predictor of mortality in patients with repaired TOF (Babu-Narayan et al., 2014), however we expanded our correlation analyses with weighted peak VO_2 (ml/kg/min) for further objective assessment.

5.3.5 Statistical analyses

All data were tested for normality using the Kolmogorov-Smirnov statistical test. To test the normality of the data subjectively, Q-Q plots were assessed. To test the hypothesis, the Shapiro-Wilks test was performed for all continuous variables. Continuous variables were presented as mean and standard deviations (SD). Student's paired t tests were performed for all parameters. Pearson correlation coefficients (r) were used to determine relationships between haemodynamic echocardiographic response and exercise parameters. Correlation coefficients between .1 and .3 were considered low, between .31 and .5 were considered moderate and those over .5 were considered high. All variables that were significant at univariate correlation analysis were entered into one multivariable regression analysis in all patients to adjust for presence of severe pulmonary regurgitation. Details of the regression model were described as corresponding R, R^2, standardised beta (regression coefficient), and p value. A p value of $<.05$ was considered as statistically significant. All statistical analyses were performed using IBM SPSS statistics version 27 (IBMCorp, London, United Kingdom).

5.4 Results

5.4.1 Baseline characteristics, TOF population (100 patients)

60 adult patients with repaired TOF, symptomatically stable but with severe pulmonary regurgitation (SPR group) were compared to 40 repaired TOF patients with mild (63%), or without pulmonary regurgitation (37%) as a control group. Mean age was (35±13 vs 33±11, years, $p<0.05$). 31 patients with SPR (52%) were male, and 29 patients (48%) were female, while 19 patients (48%) were male, and 21 patients (52%) were female in the control group. QRS duration was longer in the SPR group (153±20 vs 150±22, msec, $p<0.05$).

In the SPR, 49 patients (82%) underwent primary repair under 10 years of age. 9 patients (15%) had their primary repair more than 10 years old of age, and two patients had late repairs at age of 23 and 50 years of age. 9 patients (15%) had either surgical PVR or RV-PA conduit replacement as a second surgery for subsequent severe pulmonary regurgitation in 7 patients, while 2 patients for subsequent infundibular stenosis. 51 patients (85%) had primary surgery with complete repair with transannular patch (TAP). The remaining 9 patients (15%) had their complete repair with either Dacron VSD patch closure with infundibulectomy (2 patients), pulmonary valvotomy with infundibular resection (2 patients), Gor Tex VSD patch closure (one patient), right ventriculotomy (one patient), RV-PA conduit (2 patients) or infundibular patch (one patient).

In the control group, 35 patients (88%) underwent primary repair under 10 years of age, 4 patients (10%) more than 10 years of age and one patient had late repair at age of 44 years of age. 21 patients (53%) had surgical PVR as a second surgery to correct subsequent severe PR, while one patient had percutaneous pulmonary valve implantation (PPVI). 27 patients (68%) had the primary complete repair with transannular patch. 13 patients (22%) had had their complete repair with either Dacron VSD patch closure with infundibulectomy (one patient), RV to PA conduit (5 patients), pulmonary valvotomy (4 patients), Teflon VSD patch closure (one patient), pulmonary banding with VSD repair infundibulectomy (one patient), or Gore Tex VSD patch closure with infundibular resection (one patient) (Table 5.1). The only significant differences between the SPR and control cohorts were gender where marginally more males in SPR group, and previous TAP (85 vs 68, %, respectively $p<0.05$).

Table 5. 1. General characteristics of the study population (SPR and control groups).

Baseline characteristics	SPR Group Mean ±SD	Control Group Mean ± SD	P value
Age (yrs)	35 ±13	33±11	**<0.05**
Sex			
Male	31 (52%)	19 (48%)	**<0.05**
Female	29 (48%)	21(52%)	**<0.05**
Height (cm)	166±11	169±8	NS
Weight (kg)	71±16	73±14	NS
BMI (Kg/m^2)	25±5	26±5	NS
ORS duration (ms)	153±20	150±22	NS
Type of surgery			
TAP	51 (85%)	27 (68%)	**<0.05**
Other RVOT intervention	9 (15%)	13 (32%)	**<0.05**

BMI=body mass index; TAP= transannular patch. * Bold values indicate significant level (p<.05); NS=non-significant.

5.4.2 Baseline echocardiographic parameters

5.4.2.1 RV structure, function and 4D volumetric parameters

2D/M-mode/4D analyses

Larger RV size was observed in the SPR group (mean basal RV diameter was 5±.3 vs 4.1±.6, cm, p<0.05). 93% of the population had moderate to severe RV dilatation in the SPR group, while 48% had mild or moderate RV dilatation in the controls, when compared to normal range (>4.2 cm abnormal, (Lang et al., 2015), Table 5.2). There was normal RV longitudinal systolic function by FAC in both groups (43±6 vs 41±5, %, p>0.05), with 12% of the SPR group was below the normal range while in 20% in the controls (>35% in normal individual, (Lang et al., 2015), Table 5.2).

Larger 4D RV volumes in systole and diastole were also observed in the SPR group (179±51 vs 128±30, ml, p<0.05); (88±31 vs 67±21, ml, p<0.05). 73% of the SPR group had RV volume above the normal range, compared with 28% in the controls (normal reference value of 92-147 and 36-67, ml for RVEDV and RVESV respectively, (Maffessanti et al., 2013)) (Figure 5.1). 4D RVEF was normal in both groups (51±7 vs 48± 8, %, p<0.05) (Table 5.2).

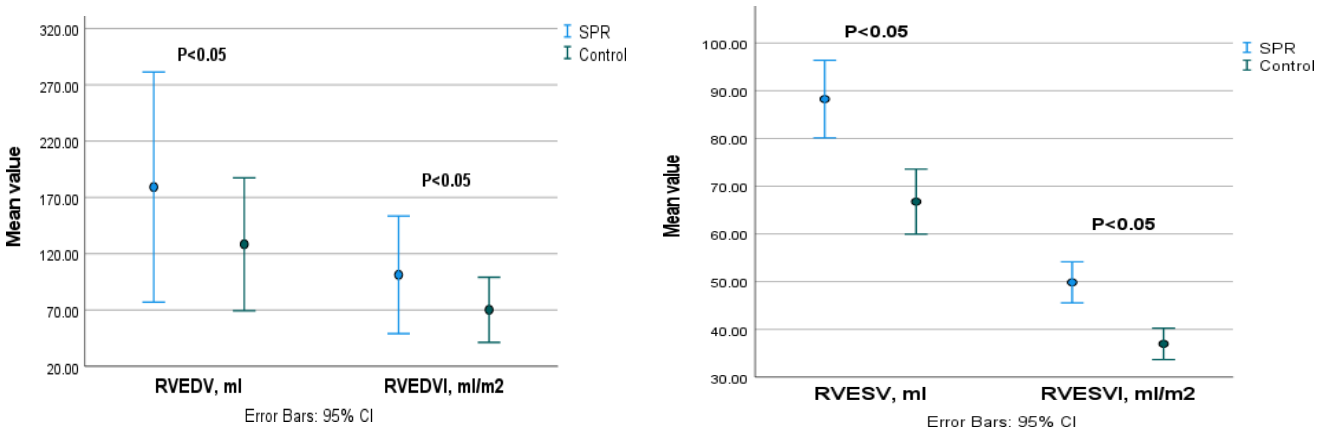


Figure 5. 1. Baseline difference of RVEDV and RVESV with indexed values between groups.

Table 5. 2. Baseline RV structural and functional measures in SPR and control groups.

Baseline RV parameters	SPR Group Mean ± SD	Control Group Mean ± SD	P value
2D Structure and function			
RVD Mid (cm)	4±.5	3.4±.5	**<0.05**
RVD Basal (cm)	5±.3	4.1±.6	**<0.05**
RVEDA (cm^2)	28±6	22±6	**<0.05**
RVESA (cm^2)	16±4	13±4	**<0.05**
FAC (%)	43±6	41±5	NS
TAPSE (mm)	17±3	14±4	**<0.05**
RVS' (cm/s)	8±2	6±2	**<0.05**
RA (cm^2)	19±5	17±4	**<0.05**
Pulmonary Regurgitation			
PR PG (mmHg)	22±8	-	-
PR PHT (ms)	84±22	-	-
PR index	.4±.1	-	-
PACT (ms)	147±27	126±21	**<0.05**
DSTVI	1.2±.33	--	
TR (mmHg)	23±10	18±9	**<0.05**
PASp (mmHg)	35±7	32±8	**<0.05**
4D Volume and function			
RVEDV (ml)	179±51	128±30	**<0.05**
RVEDVI (ml/m2)	101±26	70±14	**<0.05**
RVESV (ml)	88±31	67±21	**<0.05**
RVESVI (ml/m^2)	50±16	37±10	**<0.05**
RVEF (%)	51±7	48±8	**<0.05**
RVSV (ml/m^2)	91±27	62±15	**<0.05**
RVSVI (ml/m^2)	51±14	34±8	**<0.05**
4D TAPSI (mm)	17±3	13±5	**<0.05**
4D FAC (%)	44±6	42±6	NS

RVD mid=right ventricular mid-size; RVD base=right ventricular basal-size; RVEDA=right ventricular end diastolic area; RVESA=right ventricular end systolic area; FAC=fractional area change; TAPSE=tricuspid annular plane systolic excursion; RVS'=right ventricular systolic velocity; RA=right atrium; PR PG=pulmonary regurgitation pressure gradient; PR PHT=pulmonary regurgitation pressure half time; PR index=severe pulmonary regurgitation index; PACT=pulmonary acceleration time; DSTVI=the ratio of diastolic and systolic time-velocity integrals; TR=tricuspid regurgitation; PASp=pulmonary artery systolic pressure; RVEDV=right ventricular end diastolic volume; RVEDVI=indexed right ventricular end diastolic volume; RVESV=right ventricular end systolic volume; RVESVI=indexed right ventricular end systolic volume; RVEF=right ventricular ejection fraction; RVSV=right ventricular stroke volume; RVSVI=indexed right ventricle stroke volume. Bold values indicate significant level ($p<0.05$); NS=non-significant.

5.4.2.2 LV structure, function and 4D volumetric parameters

2D/M-mode/4D analyses

Normal left ventricular size was observed in 90% of SPR and in 83% of the controls. LV dimensions in systole and diastole were similar (LVEDd was 38±6 vs 39±8, p>0.05 and LVESd 26±5 vs 27±6, mm, p>0.05), while 2DLVEF (58±5 vs 59±5, %, p>0.05) was also the same. LV diastolic function showed a trend towards higher filling pressure (E/e') and lower long axis systolic excursion in the control group (17±2 vs 15±3, mm, p<0.05), while graded diastolic dysfunction was mildly impaired (grade I), with similar normal filling pressure in both groups (Table 5.3).

For 4D variables, normal LV end diastolic and end systolic volume were observed with no difference between groups: 110±29 vs 105±13, ml, p>0.05 and; 46±14 vs 44±8, ml, p>0.05 for LVEDV and LVESV in the SPR and control group, respectively. 4D LVSV was smaller in the control group (64±16 vs 55±9, ml, p<0.05), however there was no significant difference in 4D LVEF between the two groups (59±4 vs 58±4, %, p>0.05). The summary of baseline 4D RV/LV functional and volume parameters is shown in table 5.2 and 5.3.

Table 5. 3. Baseline LV structural and functional measures in the SPR and control groups.

Baseline LV parameters	SPR Group Mean ± SD	Control Group Mean ± SD	P value
2D Structure and function			
LVEDD (mm)	38±6	39±8	NS
LVESDD (mm)	26±5	27±6	NS
LVEF (%)	58±5	59±5	NS
MAPSE (mm)	17±2	15±3	**<0.05**
LVS' Average (cm/s)	7±3	7±6	NS
E/A	1.5±.4	1.3±.3	NS
E' average (cm/s)	8±2	6±2	**<0.05**
E/e'	10±5	13±4	**<0.05**
4D Volume and function			
LVEDV (ml)	110±29	105 ±13	NS
LVEDVI (ml/m^2)	67±20	58±8	**<0.05**
LVESV (ml)	46±14	44±8	NS
LVESVI (ml/m^2)	29±13	24±4	**<0.05**
LVEF (%)	59±4	58±4	NS
LV SV (ml)	64±16	55±9	**<0.05**
LVSVI (ml/m^2)	39±13	33±5	**<0.05**
CO (l/m)	5±1	4±.7	NS

LVEDD=left ventricular end diastolic diameter; ; LVESD=left ventricular end systolic diameter; LVEF=left ventricular ejection fraction; MAPSE=mitral annular systolic excursion; ; LVS'=left ventricular systolic velocity; E/A=**r**atio between E-wave and A-wave; E' average= average of septal and lateral early mitral inflow velocity**;** E/e'=ratio between early mitral inflow velocity and mitral annular early diastolic velocity; LVEDV=left ventricular end diastolic volume; LVEDVI= indexed left ventricular end diastolic volume; LVESV=left ventricular end systolic volume; LVESVI=indexed left ventricular end systolic volume; LVEF= left ventricular ejection fraction; LVSV=left ventricular stroke volume; LVSVI=indexed left ventricular stroke volume; CO=cardiac output. Bold values indicate significant level ($p<0.05$); NS=non-significant.

5.4.3 Baseline myocardial longitudinal mechanics analyses

5.4.3.1 RV myocardial strain parameters measured by 2DSTE

Strain values were generally lower (better function) in the SPR group than in the control group. In the SPR group, mean RV global strain (RVGLS) was -17±3 %, with 47% demonstrating a RVGLS >-17%, while in the control group was -15±3%, with 25% demonstrating a RVGLS >-17%. Mean global RV free wall strain (RVGFWS) was -19±3, with 58% demonstrating a RVGFWS > -19%, while in the control group was -17±3% with 25 % demonstrating a RVGLS >-17% (Figure 5.2, Table 5.4). For both RVGLS and RVGFWS in the control group, 75% demonstrated mean values below the normal range (> -20% RVGLS, >-19%, RVGFWS, (Park et al., 2018)).

5.4.3.2 RV longitudinal function parameters measured by 2D echocardiography

RV systolic longitudinal function measured by 2D TAPSE and RV free wall S' by TDI was impaired in both groups with more reduced values in the control (17±3 vs 14±4, mm, $p<0.05$); (8±2 vs 6±2, cm/s, $p<0.05$) for TAPSE and RVS' in the SPR and control groups, respectively. For TAPSE, 45% of SPR group was below the normal range, while 68% in the controls (>18 mm in normal individual, (Lang et al., 2015)). For RVS', 73% of SPR group was below the normal range, while 90% in the controls (>10 cm/s in normal individual, (Lang et al., 2015)). Similar to 2D measurements, there was a statistically significant difference in systolic functional parameters measured by 4D TAPSE, 4D RVEF with more lower values observed in the controls (17±3 vs 13±5, mm, $p<0.05$; 51±7 vs 48±8, %, $p<0.05$, Table 5.2).

5.4.3.3 Left ventricle strain mechanics parameters measured by 2DSTE

In the SPR group, a LV global strain mean value of -15±2, % was observed, with 95% demonstrating a GLS <-20%. LV global strain in the control group was not significantly different of -15±3, %, with a GLS< -20% in all participants (>-20% in normal individual, (Wang et al., 2020), Figure 5.2). Baseline difference of RV/LV myocardial strain parameters for both groups is presented in table 5.4.

5.4.3.4 LV longitudinal function parameters measured by 2D echocardiography

LV longitudinal systolic function (LVS') measured by TDI was impaired in both groups with no significant difference (7±3 vs 7±6, cm/s, p>0.05). 93% of SPR demonstrating an impaired LVS' <10 cm/s while all of control group had reduced values. MAPSE measured by M- mode was normal in both groups (17±2 vs 15±3, mm, p<0.05) (Table 5.3).

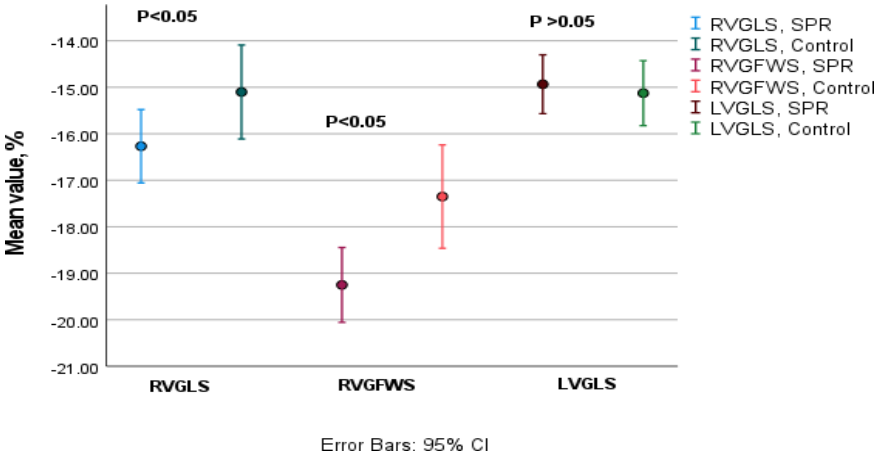


Figure 5. 2. Baseline RV/LV longitudinal strain difference between the SPR and control groups**.**

Table 5. 4. Baseline myocardial longitudinal strain mechanics difference between the SPR and control groups**.**

Myocardial Mechanics	SPR Group Mean ± SD	Control Group Mean ± SD	P value
Right Ventricle			
RVGLS (%)	-17±3	-15±3	**<0.05**
RVGFWS (%)	-19±3	-17±3	**<0.05**
Left ventricle			
LS, A4C (%)	-14±3	-14±3	NS
LS, A2C (%)	-16±3	-15±3	NS
LS, A3C (%)	-15±3	-16±3	NS
LVGLS (%)	-15±2	-15±3	NS

RVGLS=right ventricular global longitudinal strain; RVGFWS=right ventricular global free wall stain; LS A4C=apical 4 chamber longitudinal strain; LS A2C=apical 2 chamber longitudinal strain; LS A3C=apical 3 chamber longitudinal strain; LVGLS=left ventricular global longitudinal strain. Bold values indicate significant level ($p<0.05$); NS=non-significant.

5.4.4 Right ventricular contractile reserve in adult patients with repaired TOF - bigger volume less RV contractile reserve?

The individual changes in RV size and functional parameters during stress is presented in table 5.5 and 5.6. RV functional contractile reserve was defined by the change in tricuspid annular plane systolic excursion (ΔTAPSE), change in RV fractional area change (ΔFAC), change in Doppler-derived tricuspid lateral annular peak systolic velocity (ΔRVS'), and the change in 4DRV function (Δ4DRVEF). RV volumetric contractile reserve was defined by the change in 4DRV volumetric parameters with the indexed values (ΔRVEDV, ΔRVEDVI, ΔRVESV, ΔRVESVI, ΔRVSV and ΔRVSVI). RV myocardial strain contractile reserve was defined by the change in RV global strain (ΔRVGLS) and the change in RV global free wall strain (ΔRVGFWS). In the exploratory 4D analyses, 4DRV changes on stress were evaluated. The reproducibility of these measures is described in detail in chapter 4.

In the SPR group, amongst the functional parameters, RVS' increased by 41±28%, TAPSE by 39±28%, FAC by 20±15% and 4DRVEF by 12±12%. In the control group, RVS' increased by 48±20%, TAPSE by 42±28%, FAC by 23±16%, and 4DRVEF increased by 19±19%. When compared to the controls, greater right ventricular systolic contractile reserve was observed in the control group by ΔRVS' 41±28 vs 48±20, %, $p<0.05$; ΔTAPSI 39±28 vs 42±28, %, $p<0.05$; ΔFAC 20±15 vs 23±16, %, $p<0.05$, and Δ4DRVEF 12±12 vs 19±19, %, $p<0.05$ in the SPR and control group, respectively (Table 5.5,5.6, Figure 5.3).

Mirroring functional changes, better RV volume contractile reserve was observed in the control. In the SPR group, 4D RVEDV was reduced by -13±13%, 4D RVESV by- 22±17%, and 4D RVSV reduced by -2±17%. In the control, 4D RVEDV was reduced by -15±13%, 4D RVESV by-24±19%, and 4D RVSV increased by 7±20 % (Table 5.5,5.6, Figure 5.4).

For RV strain parameters, there were significant differences in all RV strain parameters between baseline and low exercise intensity in the global and global free wall RV strain with no difference in the peak values between the SPR and control group (Table 5.7, Figure 5.5). RVGLS in the SPR group increased by 16±20%, and RVGFWS by 7±13%. In the control group, RVGLS demonstrated higher augmentation with 20±20 % of change and RVGFWS by 13±18% (Table 5.8).

When compared to the control, higher augmentation of RV strain parameters was observed in the control (16±20 vs 20±20, %, $p<0.05$), and (7±13 vs 13±18, %, $p<0.05$), for RVGLS and RVGFWS in the SPR and control group, respectively (Table 5.8). There were no significant changes in PR indices during exercise in the SPR group (Table 5.9). Summary of changes during stress in RV function, myocardial strain parameters and contractile reserve parameters in entire study population are shown in table 5.5, 5.6, 5.7, and 5.8.

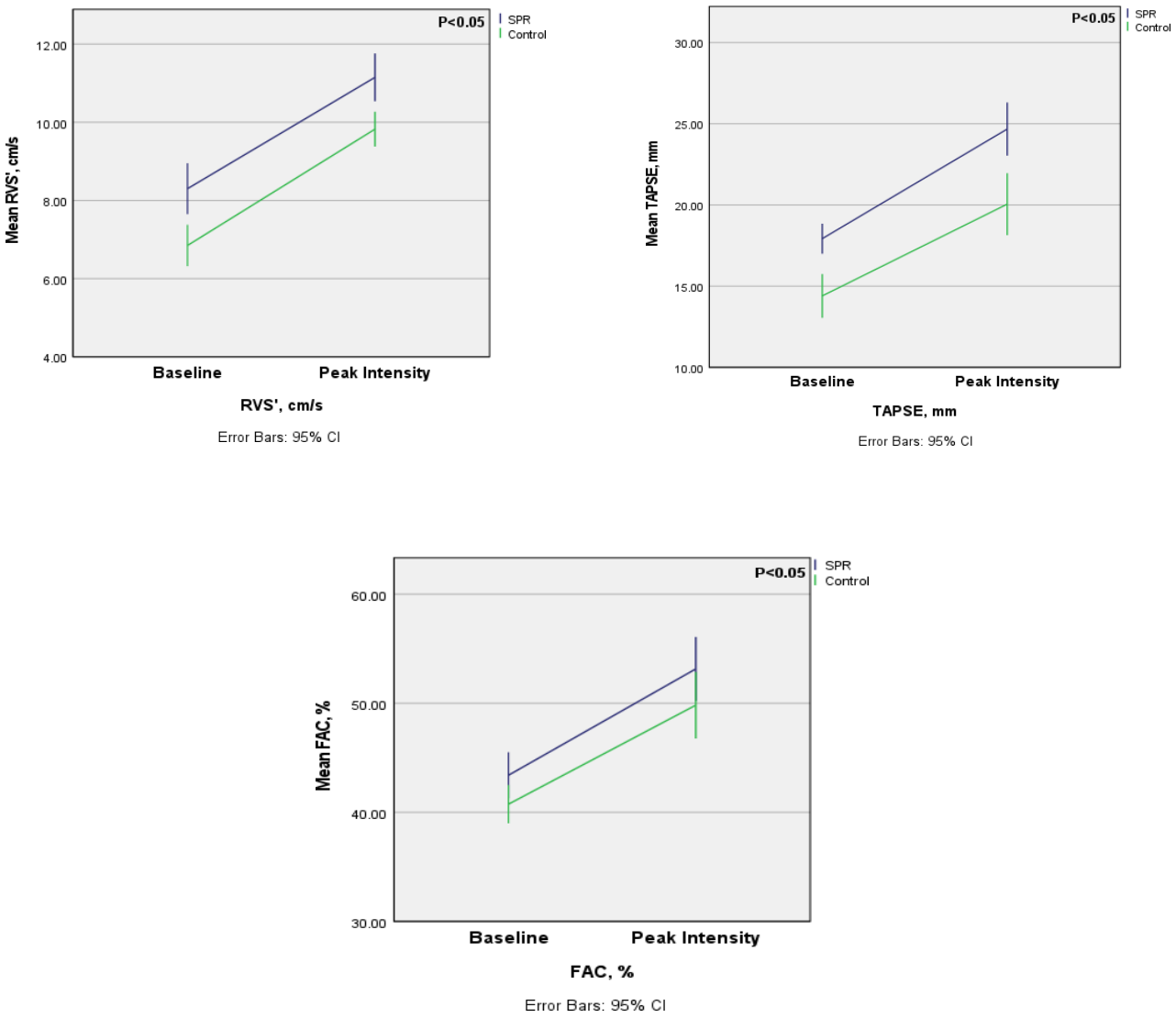


Figure 5. 3. 2D RV functional parameters change difference between rest and peak stress in both groups.

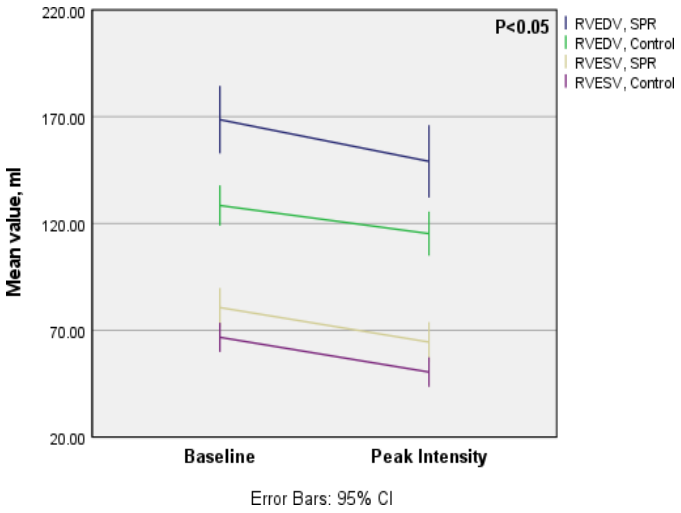


Figure 5. 4. RV Volume change difference between baseline and peak stress in both groups.

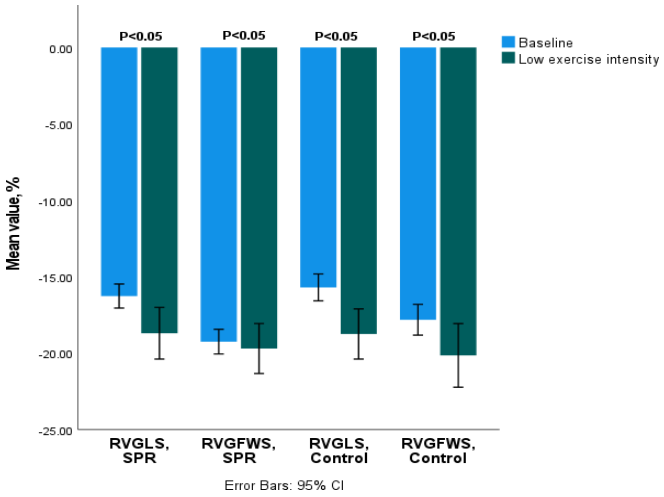


Figure 5. 5. RV strain parameters change difference between baseline and low exercise intensity in both groups.

Table 5. 5. 2D/4D RV structural, functional and volumetric parameters difference during exercise in the SPR and control groups.

Right Ventricle parameters 2D Analyses		**Baseline**	**Peak intensity**	**P value Baseline vs peak**	**P value SPR vs Control at peak stress**
RVD mid (cm)	**SPR**	4±.5	3.9±.6	**<0.05**	**<0.05**
	Control	3.4±.5	3.3 ±.5	NS	
RVD basal (cm)	**SPR**	5±.3	4.5±.6	**<0.05**	**<0.05**
	Control	4.1±.6	3.9±.5	**<0.05**	
TAPSE (mm**)**	**SPR**	17±3	24±5	**<0.05**	**<0.05**
	Control	14±4	20 ±6	**<0.05**	
FAC (%)	**SPR**	43±6	52 ±9	**<0.05**	**<0.05**
	Control	41±5	49±9	**<0.05**	
RVS' (cm/s)	**SPR**	8± 2	11±2	**<0.05**	**<0.05**
	Control	6±2	9±2	**<0.05**	
TR (mmHg)	**SPR**	23±10	39 ±16	**<0.05**	**<0.05**
	Control	18±9	34±11	**<0.05**	
4D Analyses					
RVEDV (ml)	**SPR**	179±51	157 ±51	**<0.05**	**<0.05**
	Control	128±30	115±32	**<0.05**	
RVEDVI (ml/m2)	**SPR**	101±26	88±26	**<0.05**	**<0.05**
	Control	70±14	63±15	**<0.05**	
RVESV (ml)	**SPR**	88±31	69 ±29	**<0.05**	**<0.05**
	Control	67±21	29 ±9	**<0.05**	
RVESVI (ml/m^2)	**SPR**	50±16	39±15	**<0.05**	**<0.05**
	Control	37±10	28±10	**<0.05**	
RVEF (%)	**SPR**	51±7	57±8	**<0.05**	NS
	Control	48±8	57±9	**<0.05**	
RVSV (ml)	**SPR**	91±27	88±28	NS	**<0.05**
	Control	62±15	64±16	NS	
RVSVI (ml/m^2)	**SPR**	51±14	50±14	NS	**<0.05**
	Control	34±8	35 ±8	NS	
4D TAPSI (mm)	**SPR**	17±3	23±5	**<0.05**	**<0.05**
	Control	13±5	19 ±7	**<0.05**	
4D FAC (%)	**SPR**	44±6	53±10	**<0.05**	**<0.05**
	Control	42±6	48±10	**<0.05**	

RV mid=right ventricular mid-size; RV base=right ventricular basal-size; TAPSE=tricuspid annular plane systolic excursion; FAC=fractional area change; RVS'=right ventricular systolic velocity; TR=tricuspid regurgitation; RVEDV=right ventricular end diastolic volume; RVEDVI= indexed right ventricular end diastolic volume; RVESV=right ventricular end systolic volume; RVESVI=indexed right ventricular end systolic volume; RVEF=right ventricular ejection fraction; RVSV=right ventricular stroke volume; RVSVI= indexed right ventricle stroke volume. Bold values indicate significant level ($p<0.05$); NS=non-significant.

Table 5. 6. Functional and volumetric contractile reserve parameters in both groups.

% Change from rest to RER >1 Functional and Volumetric parameters	SPR Group Mean ± SD	Control Group Mean ± SD	P value
Right Ventricle			
2D			
ΔFAC (%)	20±15	23±16	**<0.05**
ΔTAPSE (%)	39±28	42±28	**<0.05**
ΔRVS' (%)	41±28	48±20	**<0.05**
4D			
ΔRVEDV (%)	-13±13	-15±13	**<0.05**
ΔRVEDVI (%)	-13 ±10	-15±13	**<0.05**
Δ RVESV (%)	-22±17	-24±19	**<0.05**
ΔRVESVI (%)	-22±18	-29±19	**<0.05**
ΔRVEF (%)	12±12	19±19	**<0.05**
ΔRVSV (%)	-2±17	7±20	**<0.05**
ΔRVSVI (%)	-2±7	7±20	**<0.05**
Δ4D TAPSI (%)	31±24	36±18	**<0.05**
Δ4D FAC (%)	21±15	22±17	NS
Left ventricle			
2D			
ΔLVEF (%)	26±9	24±6	**<0.05**
ΔMAPSE (%)	37±17	33±21	**<0.05**
ΔLVS' average (%)	67±34	61±28	**<0.05**
ΔLVEDV (%)	-16±14	-13±8	**<0.05**
Δ LVEDVI (%)	-19±17	-13±9	**<0.05**
Δ LVESV (%)	-38±18	-35±13	**<0.05**
Δ LVESVI (ml/m^2)	-40±22	-34±13	**<0.05**
Δ4D LVEF (%)	20±9	18±6	NS
ΔLVSV (ml)	3±11	-2±2	**<0.05**
ΔLVSVI (ml/m^2)	3±14	-4±8	**<0.05**
ΔCO	84±44	62±48	**<0.05**

ΔFAC=contractile reserve of fractional area change; ΔTAPSE=contractile reserve of tricuspid annular plane systolic excursion; ΔRVS'=contractile reserve of right ventricular systolic velocity; ΔRVEDV=contractile reserve of right ventricular end diastolic volume; ΔRVEDVI=contractile reserve of indexed right ventricular end diastolic volume; ΔRVESV= contractile reserve of right ventricular end systolic volume; ΔRVESVI=contractile reserve of indexed right ventricular end systolic volume; ΔRVEF=contractile reserve of right ventricle ejection fraction; ΔRVSV= contractile reserve of right ventricular stroke volume; ΔRVSVI= contractile reserve of indexed right ventricle stroke volume; ΔLVEF=contractile reserve of left ventricular ejection fraction; ΔMAPSE=contractile reserve of mitral annular systolic excursion; ΔLVS'=contractile reserve of left ventricular systolic velocity; ΔLVEDV=contractile reserve of left ventricular end diastolic volume; ΔLVEDVI=contractile reserve of indexed left ventricular end diastolic volume; ΔLVESV=contractile reserve of left ventricular end systolic volume; ΔLVESVI=contractile reserve of indexed left ventricular end systolic volume; ΔLVSV=contractile reserve of left ventricular stroke volume; ΔLVSVI=contractile reserve of indexed left ventricular stroke volume; ΔCO=contractile reserve of cardiac output**.** Bold values indicate significant level ($p<0.05$); NS=non-significant.

Table 5. 7. Longitudinal strain mechanics changes during exercise in the SPR and control groups.

Myocardial strain parameters		Baseline	Low Intensity	P value Baseline vs low intensity	P value Cases vs control at low intensity
RVGLS (%)	**SPR**	-17±3	-19±4	**<0.05**	NS
	Control	-15±3	-18±4	**<0.05**	
RVGFWS (%)	**SPR**	-19±3	-20±4	**<0.05**	NS
	Control	-17±3	-19±5	**<0.05**	
LS, A4C (%)	**SPR**	-14±3	-16±3	**<0.05**	NS
	Control	-14±3	-17±3	**<0.05**	
LS, A2C (%)	**SPR**	-16±3	-18±4	**<0.05**	NS
	Control	-15±3	-18±4	**<0.05**	
LS, A3C (%)	**SPR**	-15±3	-17±4	**<0.05**	NS
	Control	-16±3	-17±4	**<0.05**	
LVGLS (%)	**SPR**	-15±2	-17±3	**<0.05**	NS
	Control	-15±3	-17±2	**<0.05**	

RVGLS=right ventricular global longitudinal strain; RVGFWS=right ventricular global free wall stain; LS A4C=apical 4 chamber longitudinal strain; LS A2C=apical 2 chamber longitudinal strain; LS A3C=apical 3 chamber longitudinal strain; LVGLS=left ventricular global longitudinal strain. Bold values indicate significant level ($p<0.05$); NS=non-significant.

Table 5. 8. Longitudinal strain contractile reserve parameters in both groups.

% Change from rest to RER >1 Myocardial Mechanics	SPR Group Mean ± SD	Control Group Mean ± SD	P value
Right Ventricle			
ΔRVGLS (%)	16±20	20±20	**<0.05**
ΔRVGFWS (%)	7±13	13±18	**<0.05**
Left ventricle			
ΔLVGLS (%)	15±17	16±15	NS

ΔRVGLS=contractile reserve of right ventricular global longitudinal strain; ΔRVGFWS=contractile reserve of right ventricular global free wall stain; ΔLVGLS=contractile reserve of left ventricular global longitudinal strain. Bold values indicate significant level (p<0.05); NS=non-significant.

Table 5. 9. Severe PR parameters during exercise in the SPR group.

Pulmonary regurgitation n=60	Baseline	Peak intensity	P value Baseline vs Peak
PR PG (mmHg)	22±8	26±7	**<0.05**
PR PHT (ms)	84±21	84±21	**<0.05**
PR index	.4±.1	.3±.1	**<0.05**
PACT (ms)	147±27	77 ± 25	**<0.05**
DSTVI	1.2±.3	1.1±.5	NS

PRPG=pulmonary regurgitation pressure gradient; PR PHT=pulmonary regurgitation pressure half time; PR index= severe pulmonary regurgitation index; PACT=pulmonary acceleration time; DSTVI=the ratio of diastolic and systolic time-velocity integrals. Bold values indicate significant level (p<0.05); NS=non-significant.

5.4.5 Left ventricle contractile reserve in adult patients with repaired TOF- less RV volume better LV contractile reserve?

There were significant differences in all structural, functional and volumetric parameters between baseline and peak stress (Table 5.10). LV functional contractile reserve was defined by the change in 2D LVEF (ΔLVEF), change in mitral annular plane excursion (ΔMAPSE) and the change in Doppler- derived left ventricular systolic function (ΔLVS'). LV volumetric contractile reserve was defined by the change in 4DLV volumetric parameters with the indexed values (ΔLVEDV, ΔLVESV, ΔLVEDVI, ΔLVESVI, ΔLVSV, ΔLVSVI). LV myocardial strain contractile reserve was defined by the change in LV global longitudinal strain (ΔLVGLS).

In the SPR group, amongst the functional parameters, 2D left ventricular systolic function measured by 2D LVEF biplane method increased by 26±9%, LVS' by 67±34% and MAPSE by 37±17%. In the control group, 2D LVEF increased by 24±6%, LVS' by 61±28% and MAPSE by 33±21%. Lower left ventricular longitudinal systolic contractile reserve was observed in the control group compared to the SPR group by all metric; ΔLVS', ΔLVEF, ΔMAPSE (67±34 vs 61±28, %, $p<0.05$); (26±9 vs 24±6, %, $p<0.05$); (37±17 vs 33±21, %, $p<0.05$) (Table 5.6, Figure 5.6, a).

For 4DLV volume parameters both the SPR and control subjects had significant changes between baseline and peak stress except the stroke volume in the control (Table 5.10). 4D LVEDV was reduced by -16±14%, LVESV by -38±18% and LVSV increased by 3±11% in the SPR group, whilst worse LV volume contractile reserve observed in the control group characterised by -13±8% reduction in 4D LVEDV, -35±13% reduction in 4D LVESV and -2±8% reduction in 4D LVSV (Table 5.6, Figure 5.6, b).

For LV strain parameters, there were significant differences in LVGLS between baseline and low exercise intensity with no difference in the peak values between severe pulmonary regurgitation and control groups (Table 5.7, Figure 5.8). For LVGLS contractile reserve, similar ΔLVGLS values were observed in both groups (15±17 vs 16±15, %, p>0.05, Table 5.8).

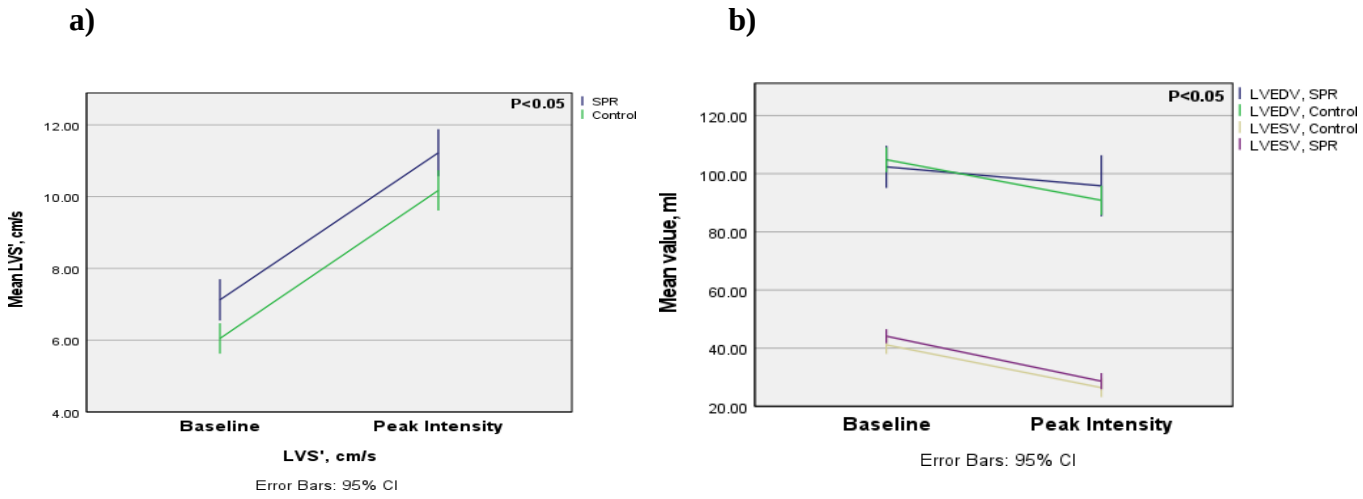


Figure 5. 6. (a) LV longitudinal systolic augmentation (LVS') change difference between rest and peak exercise in both groups. (b) LV volume change difference between rest and peak stress in both groups.

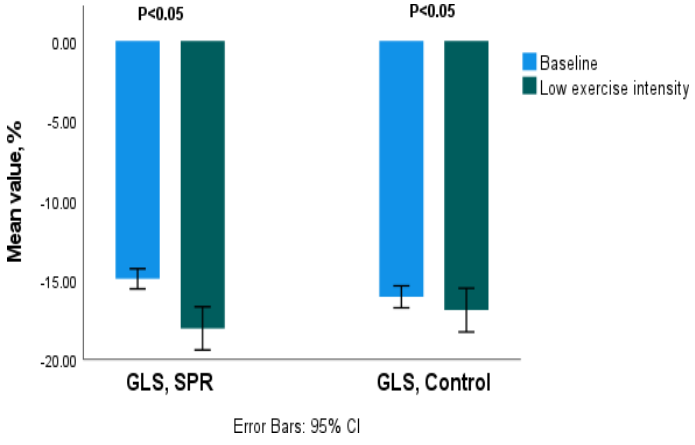


Figure 5. 7. LVGLS change difference between baseline and low exercise intensity in both groups.

Table 5. 10. 2D/4D LV structural, functional and volumetric parameters in the SPR and control groups.

Left Ventricle parameters 2D Analyses		**Baseline**	**Peak intensity**	**P value Baseline vs peak**	**P value Cases vs control at peak stress**
LVEDD (mm)	**SPR**	38±6	36±6	**<0.05**	NS
	Control	39±8	38±7	NS	
LVESD (mm)	**SPR**	26±5	21±5	**<0.05**	NS
	Control	27±6	22±4	**<0.05**	
LVEF (%)	**SPR**	58±5	73±9	**<0.05**	NS
	Control	59±5	71 ±7	**<0.05**	
MAPSE (mm)	**SPR**	17±2	23±3	**<0.05**	**<0.05**
	Control	15±3	20 ±5	**<0.05**	
LVS' average (cm/s)	**SPR**	7±3	12±2	**<0.05**	**<0.05**
	Control	7±6	10±2	**<0.05**	
E/A average (cm/s)	**SPR**	1.5±.4	1.2±.3	**<0.05**	NS
	Control	1.3±.3	1.1±.1	**<0.05**	
E' average	**SPR**	8±2	13±2	**<0.05**	NS
	Control	6±2	12±2	**<0.05**	
E/e'	**SPR**	10±5	10±3	NS	NS
	Control	13±4	11± 3	**<0.05**	
4D Analyses					
LVEDV (ml**)**	**SPR**	110±29	93 ±30	**<0.05**	NS
	Control	105±13	91±16	**<0.05**	
LVEDVI (ml/m2)	**SPR**	67±20	53±15	**<0.05**	NS
	Control	58±8	49±8	**<0.05**	
LVESV (ml)	**SPR**	46±14	29±15	**<0.05**	NS
	Control	44±8	29±9	**<0.05**	
LVESVI (ml/m^2)	**SPR**	29±13	16±8	**<0.05**	NS
	Control	24±4	16± 5	**<0.05**	
LVEF (%)	**SPR**	59±4	70±7	**<0.05**	NS
	Control	58±4	69±6	**<0.05**	
LVSV (ml)	**SPR**	64±16	70 ±18	**<0.05**	NS
	Control	61±9	62±10	NS	
LVSVI (ml/m^2)	**SPR**	39±13	37 ±9	**<0.05**	NS
	Control	33±5	34±6	NS	
CO (l/min)	**SPR**	5±1	8±3	**<0.05**	NS
	Control	4±.6	7±2	**<0.05**	

LVEDD=left ventricular end diastolic diameter; LVESD=left ventricular end systolic diameter; LVEF=left ventricular ejection fraction; MAPSE=mitral annular systolic excursion; ; LVS'=left ventricular systolic velocity; E/A=ratio between E-wave and A-wave; E' average=average of septal and lateral early mitral inflow velocity**;** E/e'=ratio between early mitral inflow velocity and mitral annular early diastolic velocity; LVEDV=left ventricular end diastolic volume; LVEDVI=indexed left ventricular end diastolic volume; LVESV=left ventricular end systolic volume; LVESVI=indexed left ventricular end systolic volume; LVEF=left ventricular ejection fraction; LVSV=left ventricular stroke volume; LVSVI=indexed left ventricular stroke volume; CO=cardiac output. Bold values indicate significant level ($p<0.05$); NS=non-significant.

5.4.6 Cardiopulmonary exercise testing (CPET) performance in adult patients with repaired TOF

All subjects successfully completed cardiopulmonary exercise testing using an upright bicycle with echocardiography. The average protocol was used 15w, with minimum 5w and maximum 20w protocol. The average exercise time was 10±2 minutes in the SPR group and 9±1 minutes in the control group with similar maximal workload achieved (150±55 vs 151±49, w, p>0.05). A respiratory exchange ratio (RER) of >1.01 was achieved in 97 patients, with a mean value of 1.2±.1 in both groups.

Cardiopulmonary exercise test showed no difference between two groups in the mean absolute value of oxygen consumption (1695±627 vs 1744±521, ml/min, p>0.05), in the mean corrected value 24±7 vs 25±, ml/kg/min, p>0.05), and in the mean peak percent of predicted (75±17 vs 76±17, %, p>0.05) in the SPR and control group, respectively. Mean peak percent of predicted VO_2 ranged from 30 to 117 % in the SPR group while it ranged from 47 to 118 % in the control group. 48 patients (80%) fell below the normal range of peak predicted VO_2 in the SPR group, while 28 patients (70%) in the control group, with no difference also in between (67 ±14 vs 68 ±11, %, p>0.05).

Out of 60 patients in the SPR group, 12 patients (20%) had normal exercise capacity (maximum predicted VO_2 >85%). Borderline exercise intolerance was found in 5 patients (8%) (when max VO_2 81–85% of predicted). Mild exercise intolerance was found in 26 patients (43%) (when max VO_2 65–80% of predicted). Moderate exercise intolerance was found in 15 patients (25%) (when max VO_2 40 - <65% of predicted). Severe exercise intolerance was found in 2 patients (when max VO_2 <40 % of predicted). Out of 40 patients in the control group, 12 patients (30%) had normal exercise capacity (maximum predicted VO_2>85%). Borderline exercise intolerance was found in 4 patients (10%). Mild exercise intolerance was found in 15 patients (38%). Moderate exercise intolerance was found in 9 patients (23%).

Against a range of submaximal effort parameters showed also no difference between two groups in mean Oxygen Uptake Efficiency Slope (OUES) (1885±722 vs 1869±543, ml/min/l/min, p>0.05), in mean predicted OUES (73±20 vs 74±18, %, p>0.05), and in VE/VCO_2 slope (26±4 vs 25±5, p>0.05). OUES was reported below the normal range (when OUES <80%) in 42 patients (70%) in the SPR group and in 30 patients (75%) in the control group. Ventilatory efficiency was measured using V_E/VCO_2 slope which was high (when slope >30, (Clark et al., 1995)) in 16 patients (27%) in the SPR group, and in 11 patients (28%) in the control group.

Among all of exercise parameters, similar exercise performance was observed between the severe pulmonary regurgitation group and control group with no or mild pulmonary regurgitation in exercise duration (10±2vs 9±1, min, p>0.05), maximal effort RER (1.2±.1 vs 1.2±.1, p>0.05), and in peak predicted VO_2 (75±17 vs 76±17, %, p>0.05), and in absolute and relative VO_2 (Table 5.11). The termination of exercise was either due to leg fatigue, shortness of breath or general fatigue. Detailed baseline cardiopulmonary exercise performance data and the difference between groups are summarised in table 5.11.

Table 5. 11. Cardiopulmonary exercise performance of the SPR and control groups.

CPET parameters	SPR Group Mean± SD	Control Group Mean ± SD	P value
Max effort measures			
Exercise duration (min)	10±2	9±1	NS
Exercise protocol (w)	15	15	NS
Peak oxygen uptake VO_2 (ml/min)	1695±627	1744±521	NS
Peak oxygen consumption VO_2 (ml/kg/min)	24±7	25±7	NS
Peak percent of predicted VO_2 (%)	75±17	76±17	NS
Impaired peak percent of predicted VO_2 (%)	67 ±14	68 ±11	NS
Peak RER	1.2±.1	1.2±.1	NS
Peak HR (beat/min)	150±20	155±19	NS
Predicted HR (%)	81±10	84±10	NS
SBP at Peak (mmHg)	192±33	185±26	NS
Peak Workload (W)	150±55	151±49	NS
Peak Workload predicted (%)	90±29	88±23	NS
O_2 Saturation (%)	97±1	98±1	NS
Peak O_2 Pulse (ml/beat)	11±4	11±3	NS
Predicted O_2 Pulse (%)	91±18	92±19	NS
Peak VE/VCO_2	31±5	30±5	NS
Sub max measures			
VE/VCO_2 at AT	28 ±3	28±5	NS
VE/VCO_2 slope	26±4	25±5	NS
OUES (ml/min/l/min)	1885±722	1869±543	NS
Predicted OUES (%)	73±20	74±18	NS

VO_2=peak absolute maximum oxygen uptake in absolute (ml/min), relative (ml/kg/min), percent of predicted (%); RER=respiratory exchange ratio; HR=heart rate; SBP=systolic blood pressure; O_2 pulse=oxygen uptake divided by heart rate peak and predicted; VE/VCO_2=th**e** relationship between minute ventilation and carbon dioxide production at peak, at anaerobic threshold, and slope; OUES=Oxygen uptake efficiency slope peak and predicted. NS=non-significant.

5.4.7 Determinants of exercise capacity in patients with repaired Tetralogy of Fallot

To identify potential determinants of exercise capacity and to test the association between CPET parameters and different echocardiographic parameters at rest and at peak exercise, univariate correlation analyses using Pearson's correlation coefficient (r) were performed. Univariate correlation analyses were also constructed for the right and left ventricle contractile reserve parameters which were defined by the percentage of change of functional, volumetric and mechanical strain parameters between baseline and peak stress. For this part of the analysis cases and controls were considered together and the presence of severe PR where required (0/1) was entered into the models as a factor.

A multivariable regression analysis model was constructed with absolute VO_2 (ml/min) for the primary end point analysis (contractile reserve parameters). Weight corrected VO_2 (ml/Kg/min) was also analysed for the secondary analysis (resting and peak stress echocardiographic parameters).

5.4.7.1 Correlation of right ventricular parameters with exercise capacity parameters

No right ventricular size parameters were correlated with exercise capacity measures at rest and at peak exercise. For RV function, right ventricular systolic function at baseline measured by 2D FAC was correlated with absolute peak VO_2 (ml/min) ($r=.37$, $p<0.05$). Interestingly RVS' was not significant for the primary end point but was for weight corrected VO_2 ($r=0.30$, $p<0.05$). At peak stress, right ventricular systolic function measured by 2DFAC and 2DTAPSE was correlated with absolute VO_2 ($r=.39$, $p<0.05$); ($r=.27$, $p<0.001$), respectively (Table 5.12).

For 4DRV volume at baseline, only RVSV was correlated with an absolute VO_2 (ml/min) (r=.39, p<0.05). At peak stress, none of the 4DRV parameters were correlated with exercise measures (Table 5.12). For RV contractile reserve, only change in FAC between baseline and peak stress was correlated with an absolute VO_2 (ml/min) (r= .36, p<0.05). Summary of correlation between exercise measures and RV function and volume is presented in table 5.12.

Table 5. 12. Univariate analysis of right ventricular parameters in the entire population.

Right Ventricle parameters 2D analyses n=100	**VO_2(ml/min)**		**VO_2(ml/Kg/min)**		**Contractile reserve Δ**	
	Baseline	**Peak stress**	**Baseline**	**Peak stress**	**VO_2(ml/min)**	**VO_2(ml/Kg/ min)**
RVD mid (cm)	-.36	.01	-.09	-.04	.22	.10
RVD basal (cm)	.20	-.04	.22	.19	.01	.04
TAPSE (mm**)**	-0.16	**.27****	-.18	**.29***	.20	.16
FAC (%)	**.37***	**.39***	**.38***	**.40***	**.36***	.20
RVS' (cm/s)	.04	-.00	**.30***	.11	.10	.04
4D Analyses						
RVEDV (ml)	.11	-.05	.12	.02	.18	.16
RVEDVI (ml/m2)	.11	-.00	.16	.06	.06	.00
RVESV (ml)	.05	-.15	.09	-.07	.01	.02
RVESVI (ml/m^2)	.08	-.12	.14	-.04	.07	.01
RVEF (%)	-55	.23	-.04	.25	.11	.06
RVSV (ml)	**.39***	.05	.22	.11	.02	.11
RVSVI (ml/m^2)	.09	.09	.15	.15	.10	.03
4D TAPSI (mm)	.02	.04	.07	-.03	.20	.10
4D FAC (%)	.02	.21	.19	**.26***	.21	.17

RVD mid=right ventricular mid-size; RVD base=right ventricular basal-size; TAPSE=tricuspid annular plane systolic excursion; FAC=fractional area change; RVS'=right ventricular systolic velocity; RVEDV=right ventricular end diastolic volume; RVEDVI=indexed right ventricular end diastolic volume; RVESV=right ventricular end systolic volume; RVESVI=indexed right ventricular end systolic volume; RVEF=right ventricular ejection fraction; RVSV=right ventricular stroke volume; RVSVI=indexed right ventricle stroke volume.*p <0 .05, ** p<0.001, Δ=change between baseline and peak stress. All values in table represent r values.

5.4.7.2 Correlation of pulmonary regurgitation in the SPR group with exercise capacity parameters

None of the pulmonary regurgitation parameters including PR PG, PHT, PR index and DSTVI were correlated with maximum oxygen consumption parameters at baseline and at peak stress. Likewise, none of the PR contractile reserve parameters were correlated with maximum oxygen uptake (Table 5.13).

Table 5. 13. Univariate analysis of pulmonary regurgitation parameters in the entire population.

Pulmonary regurgitation n=100*	**VO2(ml/min)**		**VO_2 (ml/Kg/min)**		**Contractile reserve Δ**	
	Baseline	**Peak stress**	**Baseline**	**Peak stress**	**VO_2(ml/min)**	**VO_2(ml/Kg/min)**
PR PG (mmHg)	.22	.34	.10	.20	.07	.00
PHT (ms)	.33	.11	.21	.33	-03	-10
DSTVI	.04	.10	.13	.16	-.07	-.04
PR Index	.03	.13	.22	.19	-.01	-.09

PR PG=pulmonary regurgitation pressure gradient; PRPHT=pulmonary regurgitation pressure half time; DSTVI=the ratio of diastolic and systolic time-velocity integrals; PR index=severe pulmonary regurgitation index. Δ=change between baseline and peak stress. * All values in table represent r values.

5.4.7.3 Correlation of left ventricular parameters with exercise capacity parameters

The only baseline LV structural or functional parameters to be associated with peak VO_2 were E/e' (r= -0.32, $p<0.05$) and resting stroke volume (r = 0.27, $p<0.05$). Interestingly, LV systolic measures at peak exertion augmentation by TDI (LVS' average) (r=.50, $p<0.05$) or 2DLVEF was associated with corrected VO_2 (r=.35, $p<0.05$) (Table 5.14).

For LV contractile reserve, identified by 2DLVEF and LVS' were correlated with an absolute VO_2 (ml/min) (r= .49, r=.35 $p<0.05$) (Table 5.14).

Table 5. 14. Univariate analysis of left ventricular parameters in the entire population.

Left ventricle parameters n=100*	**VO_2 (ml/min)**		**VO_2 (ml/Kg/ min)**		**Contractile reserve Δ**	
2D Analyses	**Baseline**	**Peak stress**	**Baseline**	**Peak stress**	**VO2(ml/min)**	**VO2(ml/Kg/ min)**
2DLVEF	.09	.02	.01	**.35***	**.49***	.25
LVS' average, cm/s	.12	.30	.20	**.50***	**.35***	.20
MAPSE, mm	.04	.10	.05	.11	.06	.04
E/e'	**-.32***	-.10	-16	-.13	.10	.11
E/A	.03	.17	.02	.16	.02	.08
4D Analyses						
LVEDV, ml	.16	.15	.09	.13	.15	.07
LVEDVI, ml/m^2	.17	.11	.12	.01	.11	.09
LVESV, ml	-.15	-.06	.07	-.03	.05	.05
LVESVI, ml/m^2	.11	.03	.10	.05	.06	.04
LVSV, ml	.12	.03	.04	.06	.00	.05
LVSVI, ml/m^2	**.27***	.17	**.29***	.16	.10	.13
CO, ml	.13	.08	.06	-.04	.19	.16
LVEF %	.01	.02	.08	-.01	.20	.22

LVEF=left ventricular ejection fraction; LVS'=left ventricular systolic velocity; MAPSE=mitral annular systolic excursion; E/e'=ratio between early mitral inflow velocity and mitral annular early diastolic velocity; E/A=ratio between E-wave and A-wave**;** LVEDV=left ventricular end diastolic volume; LVEDVI=indexed left ventricular end diastolic volume; LVESV=left ventricular end systolic volume; LVESVI=indexed left ventricular end systolic volume; LVSV=left ventricular stroke volume; LVSVI=indexed left ventricular stroke volume; CO=cardiac output.*p< 0.05. Δ=change between baseline and peak stress. * All values in table represent r values.

5.4.7.4 Correlation of right ventricular strain parameters with exercise capacity parameters

At rest, RV global free wall strain showed strong correlation with peak absolute VO_2 (r=.46, p<0.001). At low exercise intensity, all RV strain parameters including RVGLS and RVGFWS had a significant correlation with corrected VO_2 (r=.36, r=.38, p<0.05), but this relationship was not observed for peak absolute VO_2 (the primary end point) (Table 5.15).

For RV strain contractile reserve, identified by RVGLS was correlated with an absolute VO_2 (ml/min) (r=.36, p<0.05) (Table 5.15).

5.4.7.5 Correlation of left ventricular strain parameters with exercise capacity parameters

LVGLS did not show a statistical correlation with peak absolute VO_2 at baseline although the result was stronger during exercise (r=.30, p<0.05) (Table 5.15). For LV strain contractile reserve, the relationship was stronger with peak absolute VO_2 (r= .47, p<0.001) (Table 5.15).

Table 5. 15. Univariate analysis of right and left ventricular strain parameters in the entire population.

Mechanical parameters n=100*		**VO_2(ml/min)**		**VO_2(ml/Kg/ min)**		**Contractile reserve Δ**	
		Baseline	**Low exercise**	**Baseline**	**Low exercise**	**VO2(ml/min)**	**Vo2(ml/Kg/ min)**
RVGLS (%)	**Cases**	.24	.17	.23	**.36***	**.36***	.20
RVGFWS (%)	**Cases**	**.46****	.25	**.44****	**.38***	.11	.17
LVGLS (%)	**Cases**	.20	**.30***	**.44***	**.55****	**.47****	.22

RVGLS=right ventricular global longitudinal strain; RVGFWS=right ventricular global free wall stain; LVGLS=left ventricular global longitudinal strain. *p< 0.05, ** p<0.001. Δ=change between baseline and peak stress. All values in table represent r values.

5.4.8 Predictive modelling of objective exercise capacity

A multivariable regression analysis model was constructed with VO_2 (ml/min) for the primary end points (contractile reserve parameters), and for the secondary end points with weight corrected VO_2 (ml/Kg/min) for resting and peak exercise potential predictors. All analyses were performed for all 100 patients. The presence of severe PR was recorded as present or absent, which did not change the overall correlation in both models.

Multivariable regression model for the potential predictors of contractile reserve showed that change in left ventricular longitudinal strain throughout exercise (ΔLVGLS) (r=-.55, p<0.05), and RV systolic contractile reserve by Δ2D FAC (r=.45, p<0.05) were found to be the most important predictors of peak oxygen consumption (VO_2, ml/min) (overall R^2= .48, p<0.001, Table 5.16, Figure 5.8).

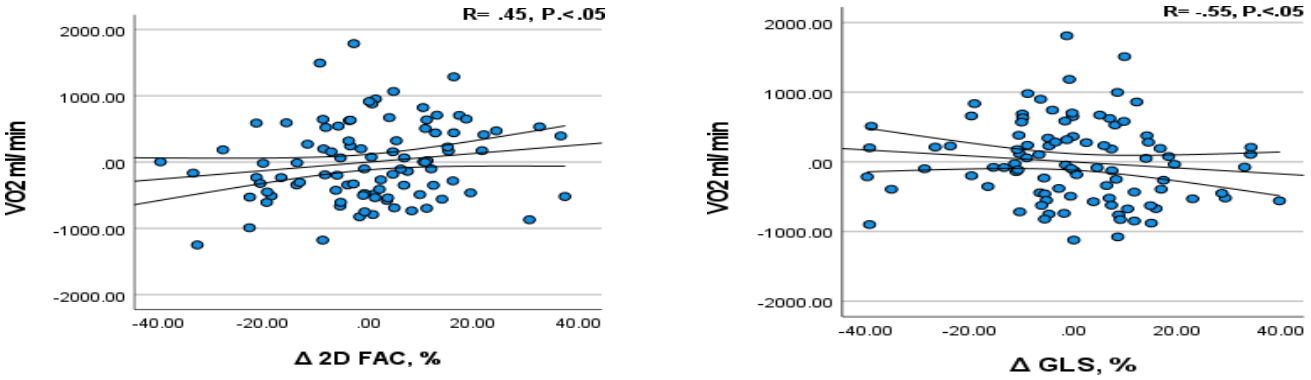


Figure 5. 8. Scatter plots of significant RV/LV contractile reserve predictors of VO_2 (ml/min) (Δ2D FAC and ΔLVGLS, R^2=.48, p< 0.001) (primary end points).

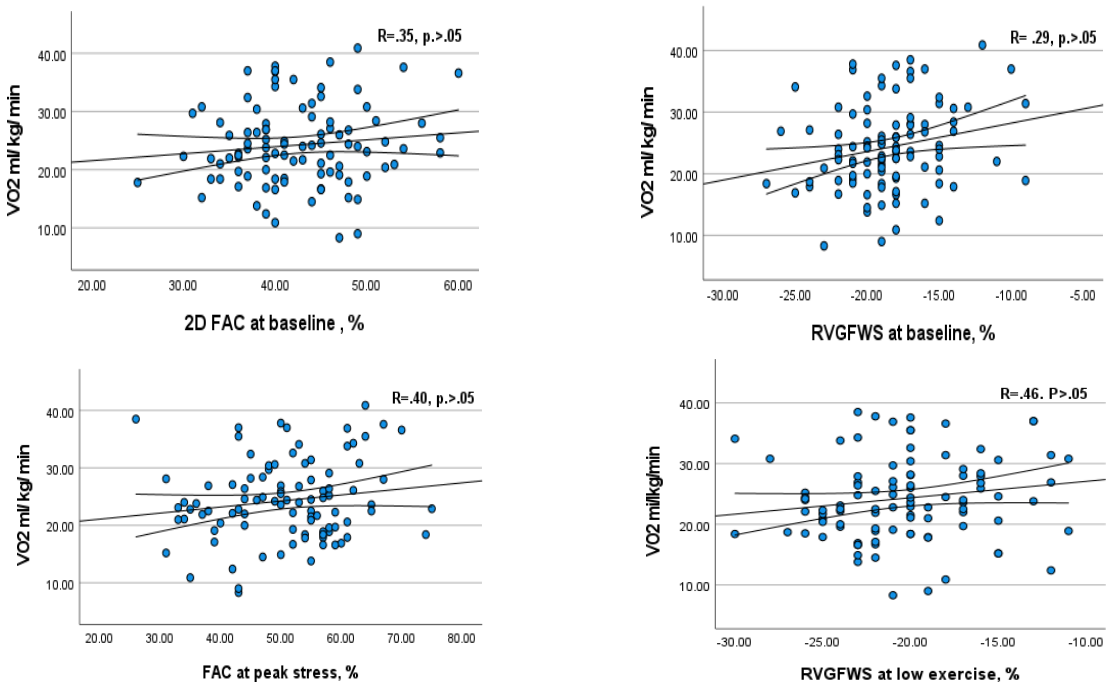
Table 5. 16. Multivariable linear regression for % of change of exercise predictors in the entire population.

Multivariable prediction model 1 (VO_2, ml/min) PR (0/1) R^2= .48, p<0.001 n=100	**Coefficients Beta**	**R**	**P value**	**R^2**
Pulmonary regurgitation	-.16	.27	NS	.10
Δ2D FAC	.47	**.45**	**<0.05**	.35
ΔLVGLS	-40	**-.55**	**<0.05**	.40

ΔFAC=contractile reserve of fractional area change; ΔLVGLS=contractile reserve of left ventricular global longitudinal strain. Bold values indicate significant level (p<0.05).

A secondary analysis focused around corrected VO_2 max produced a subtly different but allied range of independent associations. In this analysis only left ventricular systolic augmentation (LVS', average) (r=.55, p<0.05,), and LVGLS at low exercise intensity (r=.60, p<0.05, Figure 5.9) were predictive, while all RV parameters were no longer significant (overall R^2=.38, p<0.05, Table 5.17, Figure 5.9). In both analyses the presence of severe PR was treated as a factor and recorded as present or absent, in neither model did it change the overall relationship.

a) RV parameters



b) LV parameters

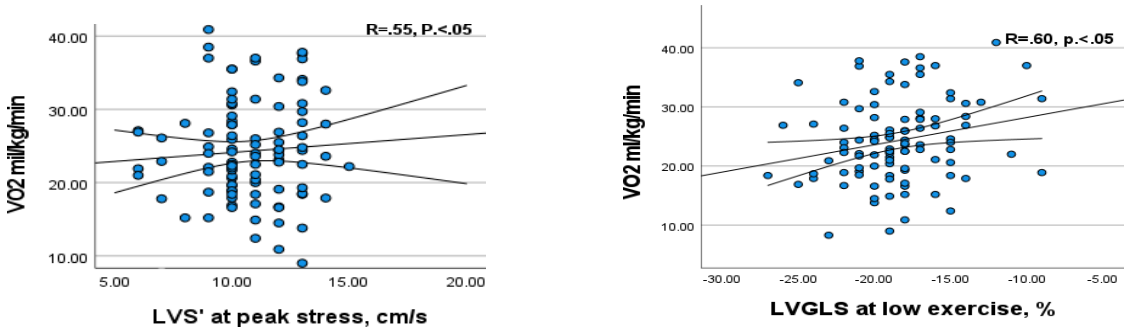


Figure 5. 9. Scatter plots of the (a) RV parameters (not predictive) and (b) LV best predictors of peak VO_2 (ml/Kg/min, overall R^2 = .38, p<0.05).

Table 5. 17. Multivariable linear regression of resting and exercise predictors (secondary end points).

Multivariable prediction model 2 (VO_2, ml/kg/min), PR (0/1) R^2= .38, p<0.05 n=100	**Coefficients Beta**	**R**	**P value**	**R^2**
Pulmonary regurgitation	.11	.22	NS	.10
2D FAC (%) **at rest**	.33	.35	NS	.22
RVGFWS (%) **at rest**	.36	.29	NS	.20
LVS' (cm/s) **at peak**	**.38**	**.55**	**<0.05**	**.40**
2D FAC (%) **at peak**	.29	.40	NS	.30
RVGFWS (%) **at low exercise intensity**	.38	.46	NS	.33
LVGLS (%) **at low exercise intensity**	**.44**	**.60**	**<0.05**	**.46**

FAC=fractional area change; RVGFWS=right ventricular global free wall stain; LVS'=left ventricular systolic velocity; LVGLS=left ventricular global longitudinal strain. Bold values indicate significant level (p<0.05).

5.5 Discussion

The relationship between myocardial structure, function and pulmonary regurgitation and exercise ability is incompletely understood in repaired Tetralogy of Fallot (rTOF). This study provides new insights into the how ventricular volumes, biventricular systolic function and myocardial deformation predict VO_2 during cardiopulmonary exercise testing (CPET). This is the first study in adult survivors of TOF comparing CPET, standard and advanced echocardiographic techniques with the description of functional and myocardial augmentation, RV/LV volumes in the presence of severe PR during stress echocardiography. We prospectively studied 100 adult patients with (rTOF), 60 patients with severe pulmonary regurgitation (SPR group) and 40 patients with mild or no pulmonary regurgitation as a control group. The groups were similar other than a slight difference in age and the frequency of TAP surgery. We found that there is marked reduction in exercise capacity in both groups against predicted values but not a difference between those with and without PR. This is a key finding as it questions the use of PR severity as an indicator for symptomatic treatment of rTOF patients with exercise limitation. It also begs the question which structural and functional adaptions are related to exercise limitation. The degree of functional limitations in our results was in keeping with most previous studies (Meierhofer et al., 2017, Bhatt et al., 2019a, Yang et al., 2015, Müller et al., 2015, Buys et al., 2011, Kipps et al., 2011, Samman et al., 2008, Izbicki et al., 2008, Hock et al., 2019), and with a recent systematic review that showed an overall mild exercise intolerance (when max VO_2 65–80% of predicted), with peak predicted VO_2 of 68 ± 2.8 (Alborikan et al., 2020).

As expected right ventricular myocardial volumes were larger in severe PR group and impaired resting RV longitudinal function was observed in both groups. Left ventricular volumes and ejection fraction were largely normal in both groups but with similar impairment in LVGLS. There are several reports which documented impaired resting RV functional parameters, and LV strain mechanics in patients with repaired TOF (Scherptong et al., 2009, Bernard et al., 2014, Menting et al., 2015). During exercise, although both groups had significant if attenuated augmentation between rest and peak exercise in all functional parameters, patients with RV dilation, higher RV volumes and severe pulmonary regurgitation demonstrated worse RV contractile reserve by all longitudinal systolic functional measures (ΔFAC, ΔTAPSE, ΔRVS', and in ΔRV strain (ΔRVGLS and ΔRVGFWS)). This can be explained by the complex dynamic interactions between PR and the presence of severe volume overload which prevent further RV contractile augmentation provoked by stress in these patients. This has been previously reported (Kingsley et al., 2018, Bhatt et al., 2019a). Furthermore, the marked reduction in contractile reserve in the global RV function, RVGLS and in RVGFWS during exercise was comparable with few reports in patients with repaired TOF (Takayasu et al., 2011, Weidemann et al., 2002, Roche et al., 2015, Yazaki et al., 2020). However, in contradistinction we observed better LV contractile reserve in the SPR group measured by longitudinal systolic velocities and ejection fraction by ΔLVEF, ΔLVS'. There are no previous data examining LV contractile reserve during stress in patients with repaired TOF. However, compared with very few investigations in heart failure with normal ejection fraction, the values obtained in our population, both in the SPR and control groups, appear reduced (25 vs 15% increases in GLS, and 89 vs 67 % increase in LVS') (van Zalen et al., 2015, Wang et al., 2014).

This balance between differential contractile reserve may go some way to explain our key finding that in our cohort despite the presence of RV dilatation and severe PR, there was no difference in exercise performance. This result is in keeping with some, but not all, previous studies which have similarly failed to show such associations having used cardiac magnetic resonance (Menon et al., 2012, Meadows et al., 2007, Geva et al., 2004, Luo et al., 2017). Other studies showed associations, and were generally older investigations with smaller cohort size (Giardini et al., 2006, Roest et al., 2002, Freling et al., 2014, Helbing et al., 1996, Marx et al., 1988). Our findings suggest a complex interplay between volume load imposed by severe PR and the ultimate failure of compensatory mechanisms that lead to biventricular dysfunction and exercise intolerance. Perhaps there may be other factors in patients with severe PR that contribute to such discrepancies, such as progressive RV dilation or LV dysfunction that could affect functional augmentation during exercise and be more reflective of the effect of pulmonary regurgitation on exercise capacity.

In attempting to define which echocardiographic measurements would predict VO_2 we found that for RV parameters at rest, only functional parameters including FAC, RVS' and RVGFWS were associated with exercise capacity. Importantly RV volume did not influence exercise capacity in our cohort. Measured during stress, this signal from functional measures was amplified with TAPSE, FAC and RVGFWS, which all correlated with peak oxygen uptake. In addition, LV longitudinal augmentation parameters were also becoming more important suggesting a role for left side systolic impairment in the impaired exercise capacity. In our prediction model, our result demonstrates that augmentation of LV longitudinal strain and the augmentation of right ventricular function (FAC) were the only independent predictors of exercise limitations in this population ($r=.55$, $r=.45$, $p<0.05$). There is a paucity of data in this area however very few publications have shown that longitudinal function in general is better predictor of exercise performance than global right or left ventricular function or volume (Alghamdi et al., 2013, Menon et al., 2012). In the analysis of contractile reserve our reduced RV and LV contractile reserve between baseline and peak stress was independent from the degree of pulmonary insufficiency. These findings suggest that the degree of exercise limitations in this population has no relation to the right-side imposed changes by pulmonary valve pathology, changed in RV volume or size, but rather to the ability of the RV and LV to augment function in response to exercise.

The use of symptoms as therapeutic end points to guide intervention may sometimes not accurate predictors of cardiopulmonary exercise capacity. The ESC, ACC/AHA have published guidelines for PVR, which place weight on the appearance of symptoms and known risk factors, such as RV dilatation and dysfunction, impaired functional capacity, and the degree of PR. Much emphasis has been placed on RV volume with considering RVEDVI of 150 ml/m^2 the threshold for recommendation of PVR (Baumgartner et al., 2021, Warnes et al., 2008). However, we like others have failed to show any relationship between RV volume parameters and severity of pulmonary regurgitation with exercise capacity. RV contractile reserve was important in our study and while previous studies have primarily concentrated on the association between significant PR, RV size, volume and dysfunction with exercise intolerance, we have also demonstrated the critical role in LV augmentation in defining the exercise potential. LV parameters are likely unaffected by the presence or absence of PR and so call into question some of the assumptions underpinning the target of PR intervention to improve symptoms. It may be timing of pulmonary surgical intervention in these patients before irreversible RV/LV contractile impairment, as opposed to volume changes, may be a more appropriate target. Developing new markers in this population integrating multiple risk factors including CPET and LV parameters for optimal decision making in asymptomatic patients with repaired TOF is clinically indicated.

There are significant gaps in the literature regarding understanding of myocardial augmentation and its relationship with exercise performance in this population. Our results address these by detailed description of resting structural and functional characteristics, myocardial deformation throughout exercise, providing for the first-time ranges for contractile reserve augmentation and identifying the best predictors of exercise performance in this population. We have demonstrated that there is a marked reduction in exercise capacity in this population independent from RV size, volume and the severity of pulmonary insufficiency. This confirmed by the inability to detect any relationship between range of RV augmentation parameters and pulmonary regurgitation. We found that it was heavily dependent on the ability of LV and RV to augment longitudinal function during exercise by LVS', RV FAC and LVGLS.

5.6 Study limitations

This study was limited by the echocardiographic assessment of right ventricular function, volume and the degree of pulmonary regurgitation, which is less precise and reproducible than by cardiac magnetic resonance. The population may not be representative of the entire spectrum of patients with repaired TOF, in particular our population does not represent the result of more recent surgical approaches that could influence our results. Nevertheless, our interesting results provide new insights into the evaluation of adult survivors of TOF who are asymptomatic with severe pulmonary regurgitation. Finally, we specifically did not address the issue of long-term RV remodelling, fibrosis and arrhythmic potential which may represent an alternative reason to undertake intervention to reduced PR.

5.7 Conclusions

There is no difference in maximum exercise capacity / oxygen uptake, between patients with repaired TOF who do and do not have severe PR. Despite larger right ventricles in those with PR it is the functional parameters and most specifically the ability to augment the right ventricle and the longitudinal function of the left ventricle which most closely relate to exercise ability. Given that indications for surgery or percutaneous intervention in this population are largely based around either symptoms and or RV dilatation, and in the absence of randomised evidence, this call into question the basis for current guidelines.

Chapter 6 Complex myocardial mechanics in adult patients with repaired TOF- A novel study assessing myocardial work and mechanical dispersion at rest and during exercise

6.1 Abstract

Background: The aims of this study were to investigate the LV myocardial work (MW) and biventricular mechanical dispersion (MD) response during stress echocardiography and to what extent they can predict exercise capacity in repaired TOF patients.

Methods: We analysed MW and MD for 100 adult patients with repaired TOF, 60 with severe pulmonary regurgitation (SPR), and 40 patients with negligible PR (NPR). MW was derived as the area of pressure strain loop using speckle tracking echocardiography and blood pressure. MD was derived as the SD of time Q/R wave on ECG to peak longitudinal strain and expressed in millisecond. All analyses were performed at baseline and at low exercise intensity (when RER during cardiopulmonary exercise testing between .85 to .95).

Results: Reduced MW was observed in the entire population with an overall mean global work efficiency (MWE) was 85±7%. Overall mean value of global work index (GWI) was 1198±312, mmHg%, 1701±303 mmHg% for global constructive work (GCW), and 293±194 mmHg% for global wasted work (GWW). The SPR group had lower MWE, lower GCW and higher GWW. During exercise, overall MWE decreased by -2±10%, GWI increased by 36±43%, GCW increased by 68±40%, GWW increased by 120±110%. Mean value of RV MD in the entire population was 46±18 ms, while 64±11 ms for LV MD. Mechanical dispersion was greater in the NPR group compared to those in the SPR group during exercise. We found that biventricular MD, constructive and wasted MW were closely associated with peak oxygen uptake, however only contractile reserve of myocardial wasted work (**Δ**GWW) was a strong predictor of exercise capacity ($r=-.50$, $p<0.05$).

Conclusions: In patients with repaired TOF, LV MW indices are reduced and biventricular MD are pronounced. Augmentation of myocardial efficiency parameters and timing were associated with objective exercise ability, suggesting that they are potential determinants of cardiopulmonary capability.

6.2 Introduction

Invasive assessment to measure LV pressure-volume relationship is the gold standard to determine LV stroke volume (Voorhees and Han, 2015), and myocardial energetic efficiency. The recent assessment of left ventricular myocardial work analysis is a non-invasive echocardiographic tool to determine LV stroke volume named 'LV myocardial work' (MW), which based on systolic blood pressure and speckle-tracking echocardiography (LV global longitudinal strain) (LVGLS) during systole and isovolumetric relaxation. It has been validated against invasive measurements and it is considered a less loading dependent than LV ejection fraction (LVEF) or GLS that could provide an integrate information on LV active systolic and diastolic myocardial work (Russell et al., 2012, Hubert et al., 2018). This reflects cardiac contractility more reliably and this is important in patients with repaired TOF who have a chronic loading condition. To date, there is no data in the literature about MW assessment in patients with repaired TOF.

Mechanical dispersion (MD) is a novel approach that can measure inhomogeneous biventricular contraction and could predict arrhythmic events in different cardiac diseases (Haugaa et al., 2010a). Prolonged mechanical dispersion has been associated with poor outcomes in patients with post myocardial infarction (Haugaa et al., 2013), severe aortic stenosis (Klaeboe et al., 2017), and in different types of cardiomyopathies (Haugaa et al., 2012, Sarvari et al., 2011). Although adult patients with repaired TOF are known to have abnormalities in RV contractile timing, mechanical dispersion has not been systematically evaluated (Thambo et al., 2004).

Since these methods are still investigative and there is little available literature in congenital heart disease, we aimed to (1) establish an expected range of LV myocardial work and biventricular mechanical dispersion in adult patients with repaired TOF, (2) assess the change of MW and MD during exercise and to (3) what extent these measures can predict exercise capacity in this population.

6.3 Methods

6.3.1 Main hypothesis and objectives

We aimed to study LV myocardial work and biventricular dispersion in adult patients with repaired TOF for further explanation of impaired myocardial function and to what extent they are related to reduced exercise capacity.

6.3.2 Myocardial work analysis

All LV myocardial work analyses have been performed by one observer (Sahar Alborikan, S.A). LV MW is an advanced method to measure LV function, which based on blood pressure and global longitudinal strain from the same software used to measure GLS by speckle tracking echocardiography. Figure 6.1 describes the main components to measure LV MW. The software calculates automatically four main parameters including global constructive work (GCW), global wasted work (GWW), global work index (GWI), and global work efficiency (GWE) as mean of the respective segmental values.

Constructive work describes the net effect generating from positive work (myocardial shortening) performed during systole plus negative work (lengthening) performed during isovolumetric relaxation which contributes to the LV ejection. Wasted work describes the net effect generating from negative work (lengthening) performed during systole plus positive work (shortening) performed during isovolumetric relaxation which does not contribute to LV ejection. The software further provides a GWI, i.e., total work performed which equals the=area of the pressure-strain loop, and GWE =GCW/GCW=GWW (Russell et al., 2012).

Figure 6.2 simplifies the process of analysing MW in one patient from our study group. Aortic valve opening and closure were determined as the first step using continuous wave Doppler, then GLS was calculated using automated functional imaging (AFI), and the last step was manually entering systolic and diastolic blood pressure. LV apical four, three, and two chamber views were analysed off-line to calculate GLS at baseline and during exercise echo using integrated software (AFI, EchoPac, Version 202, GE). Systolic and diastolic blood pressure values were entered manually for all patients, and the software automatically calculated constructive and wasted work parameters. All MW assessment was performed at baseline and at low exercise intensity (when RER between .85 to .95) for all 100 patients. Adequate LV image quality was a challenging factor in our patients especially during exercise with higher heart rates, however, we verified time points during exercise (low exercise intensity) for adequate LV imaging minimising any effect on loop area calculation in MW assessment. Figure 6.3 shows an example of normal MW net results in one patient from a patient with mild pulmonary regurgitation.

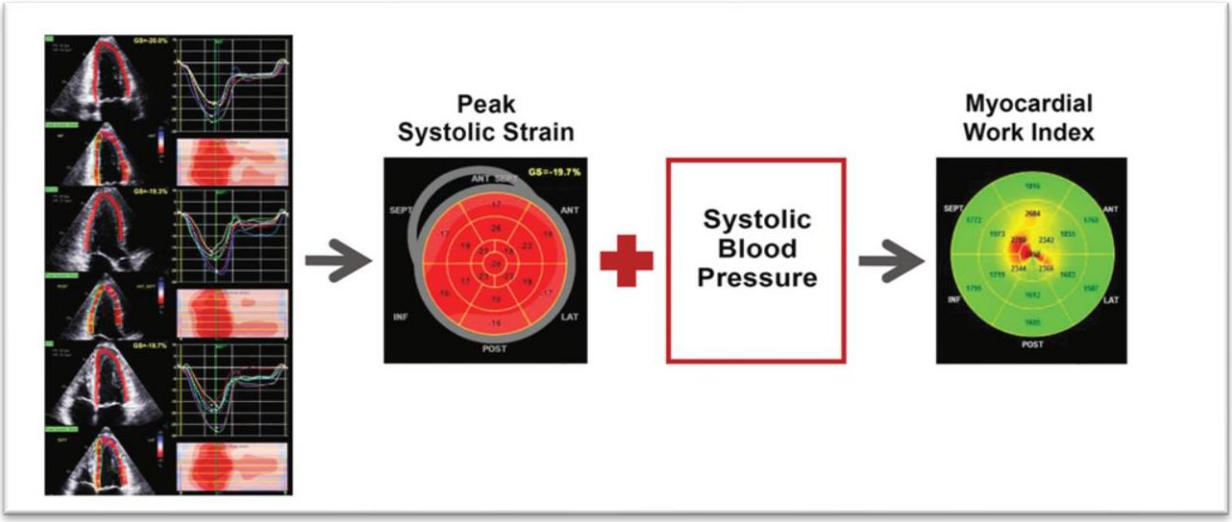
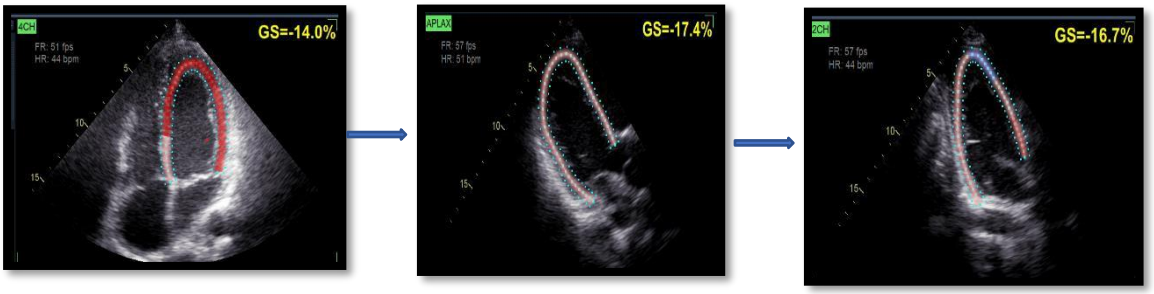


Figure 6. 1. A combination of peak global longitudinal strain (left panel, apical 2,3, and 4 chamber views). Combination of bull's eye plot of peak systolic strain and non-invasive systolic blood pressure as a surrogate for systemic left ventricular pressures. Myocardial work-based bull's eye is calculated from a complex algorithm within the ultrasound machine software (GE Healthcare) (Sengupta et al., 2020).

a)



b) **c)**

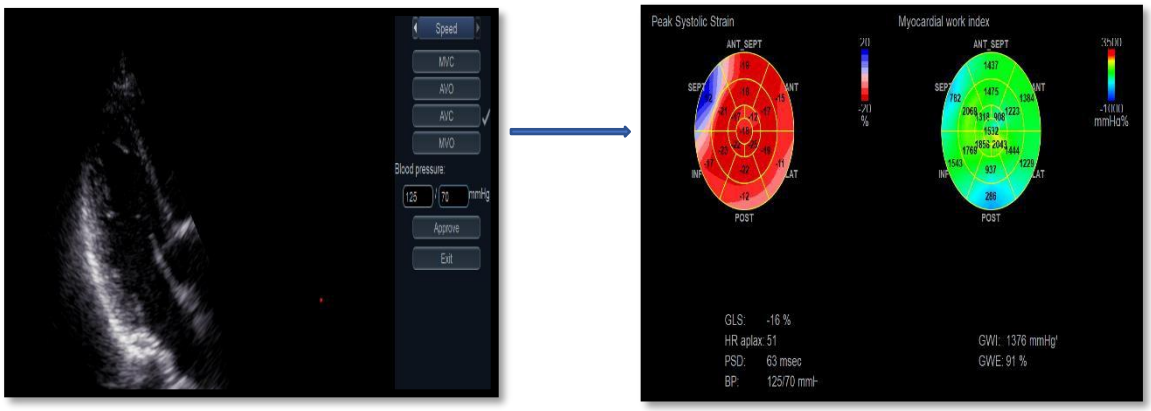


Figure 6. 2. Determination of LV myocardial work. a) the process of measuring GLS from apical 4,3 and 2 chambers. b) systolic and diastolic blood pressure levels have to be entered for MW assessment. c) MW end results (adapted from study cases).

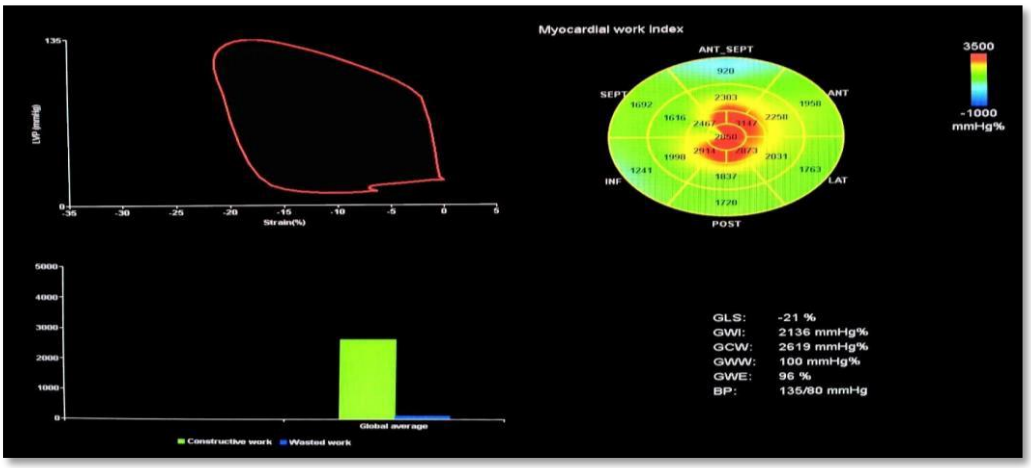


Figure 6. 3. An example of normal LV MW results analysis; global constructive work (GCW), global wasted work (GWW), global work index (GWI), and global work efficiency (GWE) (adapted from study cases).

6.3.3 Myocardial dispersion analysis

Myocardial dispersion and contraction duration were calculated from GLS using semiautomated AFI imaging. Myocardial contraction duration (CD) was measured as the time from the onset of QRS to maximum myocardial shortening. Mechanical dispersion (MD) was calculated as SD of the time from onset of QRS to peak strain in all segments using STE automated function. RV and LV MD were measured as the SD of time Q/R wave on ECG to peak longitudinal strain and expressed in millisecond in 6 RV and 16 LV segments (measure of the contraction heterogeneity) (Haugaa et al., 2010a). All myocardial dispersion assessment was performed at baseline and at low exercise intensity (when RER between .85 to .95) for all 100 patients. LV Mechanical dispersion was more challenging than RV in the presence of severe PR in the SPR group which affect the endocardial tracing and increased the needs for manual adjustment especially for the apical two chamber view. Figure 6.4. simplifies the process of MD and CD measurements in two patients from study cohorts.

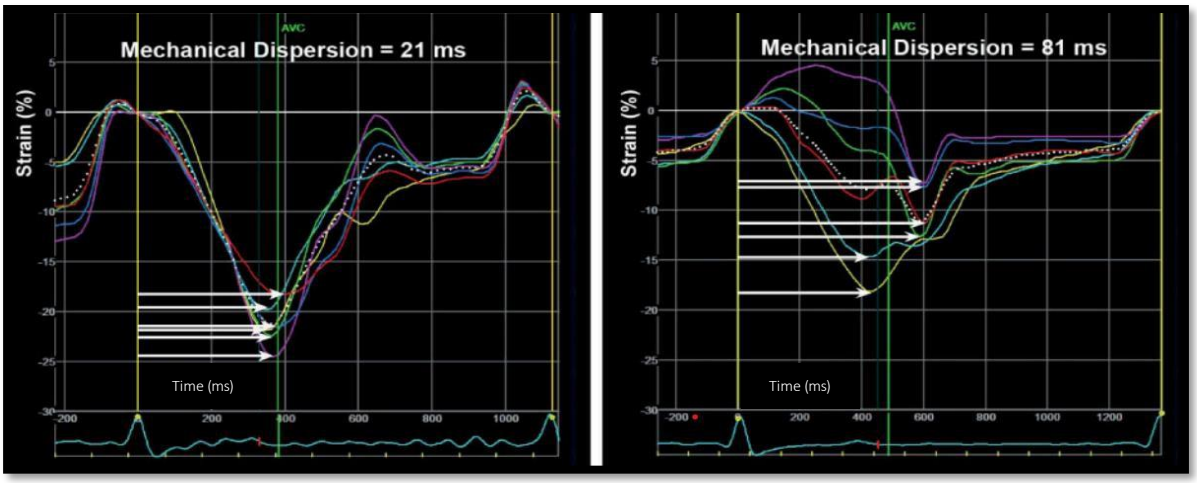


Figure 6. 4. LVGLS strain curves in two subjects with normal (left), and pathological (right) mechanical dispersion from patient with mild PR (left), and in patient with severe PR (right). Yellow vertical lines indicate the onset of R on ECG. White horizontal arrows indicate contraction duration (CD) per strain segment (time to peak negative strain). Mechanical dispersion was measured from SD of CD of all segments (adapted from study cases).

6.3.4 Statistical analysis

Continuous data were presented as mean ±SD. The mean differences between myocardial work and dispersion at baseline and during stress between the two groups were evaluated with Student's paired t test. Pearson correlation coefficients (r) were used to determine relationships between myocardial work and dispersion with exercise parameters. All variables that were significant at univariable correlation analysis were entered into one multivariable regression analysis in all patients with adjustment for presence of severe pulmonary regurgitation. Details of the regression model were described as corresponding R, R^2 and standardised beta (regression coefficient). A p value of <0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS statistics version 28.

6.4 Results

6.4.1 Baseline characteristics, TOF population (100 patients)

100 adult patients (60 adult patients with repaired TOF and severe pulmonary regurgitation, SPR group) and 40 repaired TOF patients with mild or negligible PR (NPR). Relevant differences between SPR and NPR are highlighted in table 6.1**.** Reduced peak VO_2 was observed in both groups with no significant difference (75±17 vs 76±17, %, p>0.05). The results from this chapter are drawn from the same population that was described in chapter 5. Detailed baseline characteristics, surgical history and exercise performance are as described before (section 5.3.1 and 5.3.6).

Table 6. 1. Baseline characteristics of population.

Baseline Characteristics	**SPR Group Mean ± SD**	**NPR Group Mean ± SD**	**P value**
Age (yrs)	35 ±13	33±11	**<0.05**
Gender **Male** **Female**	 31 (52%) 29 (48%)	 19 (48%) 21(52%)	**<0.05**
Height (cm)	166±11	169±8	NS
Weight (kg)	71±16	73±14	NS
BMI (Kg/m2)	25±5	26±5	NS
ORS duration (ms)	153±20	150±22	NS
SBP (mmHg)	124±16	123±12	NS
DBP (mmHg)	73±10	75±11	NS
Peak percent of predicted VO_2 (%)	75±17	76±17	NS

SBP=systolic blood pressure; DBP=diastolic blood pressure; mmHg=millimeter of mercury. Bold values indicate significant level (p<0.05); NS=non-significant.

6.4.2 LV myocardial work

Overall, the mean value of MWE in the entire population was 85±7%, but ranged from 56 to 98%. 80% of the population had an abnormal MWE (normal MWE >91%, (Manganaro et al., 2019)). Overall mean value of GWI was 1198±312, mmHg%, 1701±303 mmHg% for GCW, and 293±194 mmHg% for GWW.

Comparing MWE between patients with and without PR, those with SPR were abnormal in 83% and those with negligible PR (NPR), 89% of patients had an abnormal MWE (83±7 vs 86±7, %, $p<0.05$). Comparing the two groups at baseline, those with SPR had lower MWE (83±7 vs 86 ±7, %, $p<0.05$), lower GCW (1680±297 vs 1800 ±316, mmHg%, $p<0.05$) and a higher GWW (326±214 vs 244±145, mmHg%, $p<0.05$) (Table 6.2).

During exercise, overall MWE decreased by -2±10%, GWI increased by 36±43%, GCW increased by 68±40%, GWW increased by 120±110% (Table 6.3). Comparing SPR with NPR, an opposite effect to the resting differences was observed with lower GWI and GCW in the NPR (1418±531 vs 1605±516, mmHg%, $p<0.05$), and (2341±671 vs 2689±646, mmHg%, $p<0.05$). More myocardial wasted work was still observed in the SPR group during exercise (582±337 vs 402±151, mmHg%, $p<0.05$). There was no significant difference in GWE between groups during exercise (82±7 vs 83±6, %, $p>0.05$), in the SPR and NPR groups, respectively (Table 6.4, Figure 6.5). Analysing contractile reserve showed lower augmentation in the NPR, by **Δ**GWI (28±34 vs 41±48 vs, %, $p<0.05$), **Δ**GCW (60±45 vs 70±39, %, $p<0.05$), and by **Δ**GWE (-4±7 vs -1±12, %, $p<0.05$) (Table 6.3).

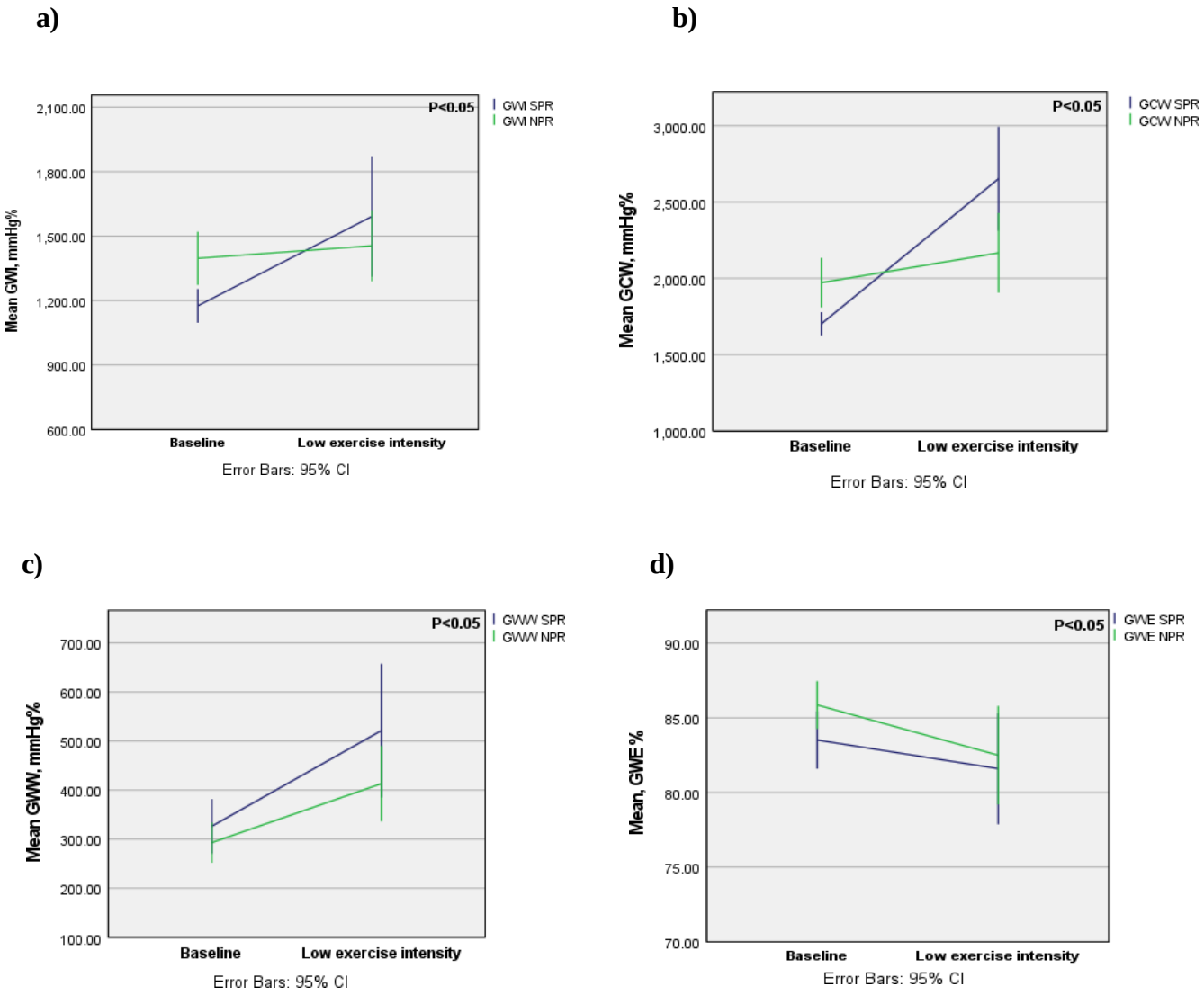


Figure 6. 5. Difference of myocardial work performance during exercise in both groups. (a) Myocardial Global Work Index, (b) Myocardial Global Constructive Work, (c) Global Wasted Work, (d) Global Work Efficiency.

Table 6. 2. Baseline myocardial work and mechanical dispersion parameters in the entire population and the difference between groups.

Baseline Analysis	Overall Mean ±SD n=100	SPR Group Mean ±SD n=60	NPR Group Mean ± SD n=40	P value SPR vs NPR
GWI (mmHg%)	1198±312	1176±306	1231±322	**<0.05**
GCW (mmHg%)	1701±303	1680±297	1800 ±316	**<0.05**
GWW (mmHg %)	293±194	326±214	244±145	**<0.05**
GWE (%)	85±7	83±7	86±7	**<0.05**
LV CD (ms)	354±42	351±43	352±52	NS
RV CD (ms)	369±44	365±44	375±43	**<0.05**
LV MD (ms)	64±11	43±12	42±9	NS
RV MD (ms)	46±18	44±16	48±19	**<0.05**

GWI=global work index; GCW=global constructive work; GWW= global wasted work; GWE=global work efficiency; LV CD= left ventricular contraction duration; RV CD=right ventricular contraction duration; LV MD=left ventricular mechanical dispersion; RV MD= right ventricular mechanical dispersion. Bold values indicate significant level ($p<0.05$); NS= non-significant.

Table 6. 3. Difference of myocardial work and mechanical dispersion contractile reserve parameters in the entire population and the difference between groups.

% Change from rest to RER >1 Myocardial Work	Overall Mean ± S.D n=100	SPR Group Mean ± S.D n=60	NPR Group Mean ± S.D n=40	P value SPR vs NPR
ΔGWI (%)	36±43	41±48	28±34	**<0.05**
ΔGCW (%)	68±40	70±39	60±45	**<0.05**
ΔGWW (%)	120±110	127±100	108±103	**<0.05**
ΔGWE (%)	-2±10	-1±12	-4±7	**<0.05**
ΔLV MD	-13±11	-13±11	-12±10	NS
ΔRV MD	-18±14	-18±7	-13±10	**<0.05**

ΔGWI=contractile reserve of global work index; ΔGCW=contractile reserve of global constructive work; ΔGWW=contractile reserve of global wasted work; ΔGWE=contractile reserve of global work efficiency; ΔLV MD=contractile reserve of left ventricular mechanical dispersion; ΔRV MD=contractile reserve of right ventricular mechanical dispersion. Bold values indicate significant level ($p<0.05$); NS=non-significant; Δ=change between baseline and peak stress.

Table 6. 4. LV myocardial work change difference during exercise in the entire population and the difference between groups.

LV Myocardial Work Parameters		Baseline	Low intensity	P value Baseline vs low intensity	P value SPR vs NPR At low intensity
GWI (mmHg%)	**Overall**	1198±312	1584±521	**<0.05**	**<0.05**
	SPR	1176±306	1605±516	**<0.05**	
	NPS	1212±322	1418±531	**<0.05**	
GCW (mmHg%)	**Overall**	1701±303	2550±670	**<0.05**	**<0.05**
	SPR	1680±297	2689±646	**<0.05**	
	NPS	1700 ±316	2341±671	**<0.05**	
GWW (mmHg %)	**Overall**	293±194	511±291	**<0.05**	**<0.05**
	SPR	326±214	582±337	**<0.05**	
	NPS	244±145	402±151	**<0.05**	
GWE (%)	**Overall**	85±7	82±7	**<0.05**	NS
	SPR	83±7	82±7	**<0.05**	
	NPS	86 ±7	83±6	**<0.05**	

GWI=global work index; GCW=global constructive work; GWW= global wasted work; GWE=global work efficiency. NS= non-significant. Bold values indicate significant level ($p<0.05$); NS=non-significant.

6.4.3 Biventricular mechanical dispersion

Overall, the mean value of RV MD in the entire population was 46±18 ms, while overall mean RV CD was 369±44 ms. 93% of the entire population had prolonged RV MD (RV MD abnormal >26 ms, (Leren et al., 2017)). Overall mean value of LV MD was 64±11 ms, while overall LV CD was 354±42 ms. 93% of the entire population had prolonged LV MD (LV MD abnormal when >31 ms, (Verdugo-Marchese et al., 2020),Table 6.2). Comparing RV MD between patients with and without PR, those with SPR were abnormal in 93% and those with negligible PR (NPR), 92% of patients had abnormal RV MD. LV MD was abnormal in 93% for those with SPR, and in all patients (100%) with NPR.

During exercise, RV MD decreased by -18±14%, LVMD decreased by -13±11% (Table 6.3). Comparing SPR with NPR, longer RV mechanical dispersion was observed in the NPR group (48±19 vs 44±16, ms, $p<0.05$), and during exercise (42±9 vs 36±17, ms, $p<0.05$). For LV MD, similar overall mean mechanical dispersion was observed in both groups at baseline (43±12 vs 42±9, ms, $p>.05$) (Table 6.5). Likewise, longer LV dispersion was found in the NPR during exercise (43±9 vs 41±14, ms, $p<0.05$) (Table 6.5, Figure 6.6). Analysing contractile reserve showed similar change in LV dispersion (ΔLV MD) (-13±11vs -12±10, %, $p>0.05$), and less reduction in ΔRV MD in the NPR group (-13±9 vs -18±7, %, $p<0.05$) (Table 6.3).

Likewise, for RV contraction duration, longer contraction duration was observed in the NPR group at baseline (375±43 vs 365±44, ms, $p<0.05$), and during exercise (317±47 vs 291±40, ms, $p<0.05$), (Table 6.5). For LV CD, overall mean contraction duration was the same in both groups at baseline (351±43 vs 352±52, ms, $p>0.05$). Longer LV CD in the NPR during exercise was observed (306±35 vs 300±43, ms, $p<0.05$) (Table 6.5, Figure 6.7).

a) **b)**

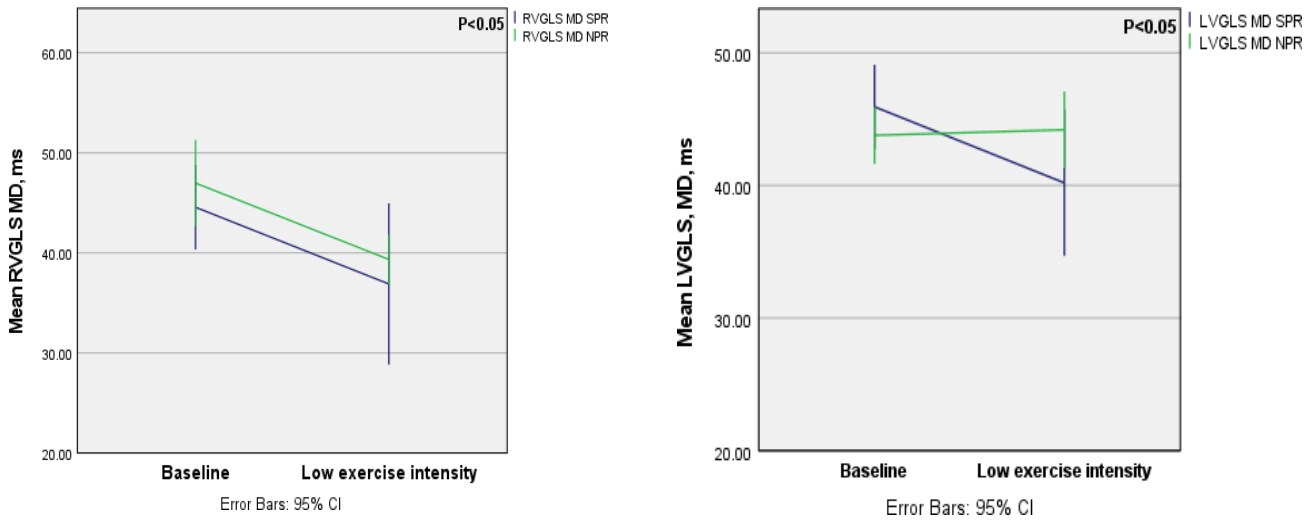


Figure 6. 6. Difference of mechanical dispersion in both groups. (a) Right ventricle global longitudinal strain dispersion. (b) Left ventricle global longitudinal strain dispersion.

a) **b)**

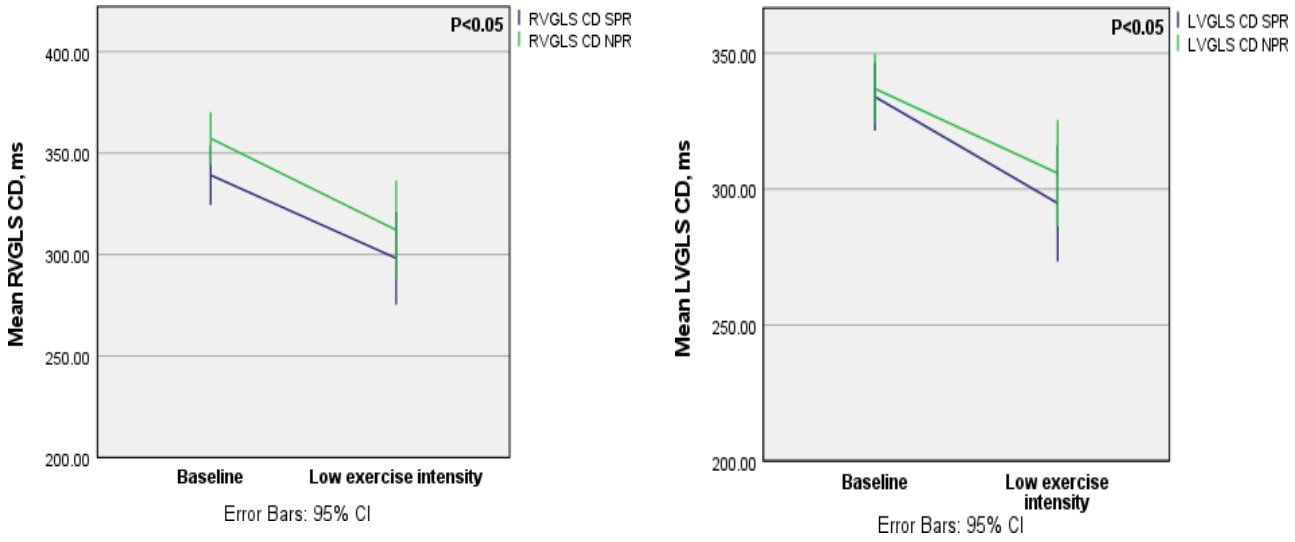


Figure 6. 7. Difference of contraction duration in both groups. (a) Right ventricle global longitudinal strain CD; (b) Left ventricle global longitudinal strain CD.

Table 6. 5. Difference of mechanical dispersion and contraction duration parameters during exercise in the entire population and the difference between groups.

Myocardial Dispersion		Baseline	Low Intensity	P value Baseline vs peak	P value SPR vs NPR at low intensity
LVGLS CD (ms)	**Overall**	353±42	303±40	**<0.05**	**<0.05**
	SPR	351±43	300±43	**<0.05**	
	NPS	352±52	306±35	**<0.05**	
RVGLS CD (ms)	**Overall**	369±43	301±44	**<0.05**	**<0.05**
	SPR	365±44	291±40	**<0.05**	
	NPS	375±43	317±47	**<0.05**	
LVGLS MD (ms)	**Overall**	45±11	42±11	**<0.05**	**<0.05**
	SPR	43±12	41±14	NS	
	NPS	42±9	43±9	NS	
RVGLS MD (ms)	**Overall**	46±18	39±14	**<0.05**	**<0.05**
	SPR	44±16	36±17	**<0.05**	
	NPS	48±19	42±9	**<0.05**	

LV CD=left ventricular contraction duration; RV CD=right ventricular contraction duration; LV MD=left ventricular mechanical dispersion; RV MD=right ventricular mechanical dispersion. Bold values indicate significant level ($p<0.05$); NS=non-significant.

6.4.4 Association with maximum oxygen uptake (VO_2)

Univariate correlation analysis showed significant associations between myocardial work contractile reserve parameters (ΔGWW and ΔGWE) and peak predicted VO_2 (%) (r=.41, r=.33, $p<0.001$). Myocardial dispersion parameters showed significant correlations between RV MD and LV MD with predicted VO_2 during exercise (r=.47, r=.36, $p<0.001$, Table 6.6).

A multi regression model with peak predicted VO_2 (%) was constructed which showed that only ΔGWW was the most significant predictor of functional capacity (overall $R^2=.30$, $p<0.05$). All analyses were performed for all 100 patients. The presence of severe PR was recorded as present or absent, which did not change the overall correlation in both models (Table 6.7, Figure 6.8).

Table 6. 6. Univariate correlation analysis of LV MW and biventricular MD parameters in the entire population (n=100).

Myocardial dispersion *	Predicted VO_2 (%)		Contractile reserve (%)
n=100	**Baseline**	**Peak Stress**	**Predicted VO_2 (%)**
GWI (mmHg%)	.03	.07	.27
GCW (mmHg%)	.21	.02	.13
GWW (mmHg %)	-.04	-.08	**.41**
GWE (%)	.07	.07	**.33**
LVMD (ms)	.16	**.36**	.10
RV MD (ms)	.22	**.47**	.22

GWI=global work index; GCW=global constructive work; GWW=global wasted work; GWE=global work efficiency. LV MD=left ventricular mechanical dispersion; RV MD=right ventricular mechanical dispersion. Bold values indicate significant level ($p<0.001$). * All values in table represent r values.

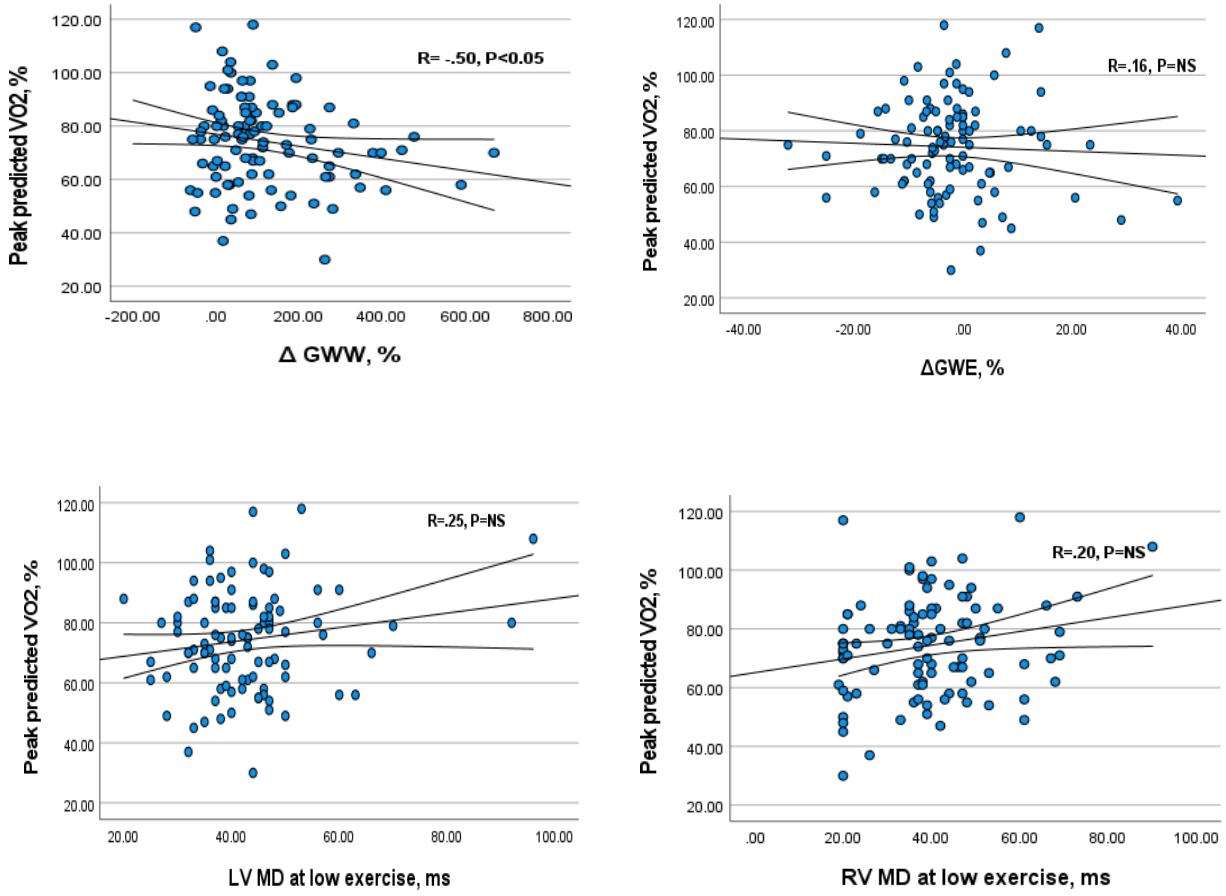


Figure 6. 8. Scatter plots of LV MW and biventricular MD predictors (only ΔGWW) of exercise capacity (R^2=.30, $p< 0.05$).

Table 6. 7. Multivariable linear regression of potential exercise predictors (MW and MD parameters) in the entire population (n=100).

Multivariable prediction model (VO_2, %) R^2= .30, p<0.05, PR (0/1) n=100	**Coefficients Beta**	**R**	**P value**	**R^2**
Pulmonary regurgitation n=100	-.20	.22	NS	.10
ΔGWW (%)	.42	**-.50**	**<0.05**	.30
ΔGWE (%)	.58	.16	NS	.07
LV MD (ms) at low exercise	.11	.25	NS	.18
RV MD (ms) at low exercise	.20	.20	NS	.10

ΔGWW=contractile reserve of global wasted work; ΔGWE=contractile reserve of global work efficiency; LV MD=left ventricular mechanical dispersion; RV MD=right ventricular mechanical dispersion. Δ=change between baseline and peak stress. Bold values indicate significant level (p<0.05); NS= non-significant.

6.5 Discussion

Myocardial work is a promising tool for evaluation of heart failure progression that may further improve the evaluation of myocardial function (Hedwig et al., 2021). It is a novel set of echocardiographic techniques that overcome load dependency of LV ejection fraction and LV strain. Evaluation of MW allows to differentiate myocardial constructive from wasted work components, therefore it is important in patients with repaired TOF as it will offer new insights into cardiac strain mechanics which can provide a new understanding of the pathophysiology of disease progression in the LV and RV. Myocardial work has been already validated in heart failure and coronary artery disease patients (Qin et al., 2021), and normal referenced values of myocardial work indices have been identified (Truong et al., 2021, Manganaro et al., 2019). In this study we have evaluated myocardial work performance in patients with repaired TOF for the first time at rest and during stress echocardiography. Our MW results are comparable to the largest multicentred European study for normal reference range (Manganaro et al., 2019), and to a recent report over a wide age range population (Morbach et al., 2020). Our results showed an impairment in all MW indices including GWI, GCW, GWW and GWE at baseline and during stress echocardiography in the entire repaired TOF population.

When comparing between groups in our cohort at baseline, there were significant differences of myocardial work indices with lower LV myocardial performance and higher wasted myocardial work (GWW) were observed in patients with severe PR than those who do not have loading condition. Interestingly, during exercise, an opposite effect to the resting differences was observed in those with negligible PR with more negative myocardial work efficiency. This mirrors our former key results of worse LV functional, volume and strain contractile reserve (CR) parameters in patients with negligible PR in the main chapter findings, while greater LV CR was observed in the SPR group with worse RV CR. This suggests that the presence of severe PR contributes to low RV CR that compensated by normalising LV CR response (less bad) during exercise, with the opposite mechanism observed in patients with no PR. This balance between CR of MW and differential functional CR may explain further the key finding in this thesis that despite the presence of severe PR and RV dilation, there was no difference in exercise performance between patients with and without severe PR.

Our novel LV MW and dispersion results in these patients are markedly abnormal in the entire repaired TOF population and associated with objective exercise measures irrespective of the severity of PR. This is very important finding as it has been shown recently that there is a link between MW indices and LV myocardial fibrosis, and sudden cardiac death in hypertrophic and dilated cardiomyopathy patients (Gonçalves et al., 2021, Cui et al., 2021). Myocardial fibrosis in patients with repaired TOF is associated with an increased risk of mortality, arrhythmias, and sudden cardiac death (Babu-Narayan et al., 2006, Chen et al., 2016). Furthermore, myocardial fibrosis leads to a decreased ability of myocardial movement and deformation, and has been shown to be associated with exercise intolerance and arrhythmias in this population with potential important clinical value in evaluating prognosis (Broberg et al., 2016). Diffuse biventricular fibrosis contributes to the lack of biventricular improvement following pulmonary valve replacement (PVR) in this population (Chen et al., 2016). Our findings of markedly reduced LV MW and pronounced LV mechanical dispersion together support our key finding in the main chapter in which the degree of exercise intolerance has no relation to severe PR, but rather to the ability of biventricular function to augment longitudinal function. This emphasises the importance of ongoing LV adverse remodelling in asymptomatic patients with repaired TOF who might be at higher risk of ongoing fibrosis which leads to progressive exercise intolerance, and this would encourage the assessment of MW to disclose unknown ongoing fibrosis at earlier stages and improve the risk assessment for better cardiac function recovery post PVR.

Mechanical dispersion in patients with repaired TOF is often disregarded when biventricular strain analysis is performed. Effective myocardial work assessment for both ventricles should be coupled to the electrical conduction and mechanical motion. In repaired TOF patients, it is known that they are at higher risk of arrythmias (van der Ven et al., 2019). The risk stratification of sudden cardiac death in the current guidelines is mostly based on QRS duration >180 ms, severity of PR and older age at repair (Baumgartner et al., 2021). However, this risk stratification is insufficient and a better method measuring detailed myocardial dyssynchrony is indicated. The upper normal limit for mechanical dispersion has been reported to be 35 msec (Leren et al., 2015). Our study results demonstrated that asymptomatic patients with repaired TOF had prolonged RV and LV mechanical dispersion regardless of the presence of severe PR with more pronounced RV mechanical dispersion in patients with no PR. During exercise, an interesting finding was observed, biventricular mechanical dispersion was longer in this group,

suggesting a more inhomogeneous LV and RV myocardial contraction in response to exercise. This is an important finding as an increased mechanical dispersion in addition to lower MW performance during exercise may identify which part of myocardium is at risk of arrhythmias and sudden cardiac disease in different pathologies (Haugaa et al., 2010b, Haugaa et al., 2013). Therefore, a combination of mechanical dispersion, global strain, and MW may improve selection of patients with repaired TOF into risk assessments for surgical reintervention.

Peak strain dispersion identifies whether the peak strain time for LV and RV is consistent or not, and this is used to identify early systolic dysfunction (Haugaa et al., 2013). Our results showed that heterogeneous contraction of LV and RV is present in asymptomatic patients with repaired TOF with higher RV and LV degree of heterogeneity at baseline and during stress echocardiography. We found that RV and LV MD were associated with peak oxygen uptake, suggesting the ability of mechanical dispersion to predict functional limitations in this population. To date, RV mechanical dispersion in patients with repaired TOF has not been reported as a marker of ventricular arrhythmias (Baumgartner et al., 2021). RV mechanical dispersion is possibly reflecting the ongoing myocardial fibrosis in this population which might directly link to higher risk of ventricular arrhythmias (Leren et al., 2017). In our regression analysis, we found a significant correlation between biventricular MD, LV MW indices with peak predicted VO_2. Contractile reserve described by wasted myocardial work (**Δ**GWW) was independently associated with functional limitations. This is a new finding in the repaired TOF population, but such correlation was observed in few studies in patients with heart failure (Hedwig et al., 2021), and in patients with amyloidosis (Park et al., 2018).

This study provides several interesting findings in myocardial work and mechanical dispersion assessment in patients with repaired TOF. First, MW including GWI, GCW and GWE were reduced in patients with repaired TOF at baseline with more negative work efficiency was observed during exercise and higher wasted myocardial work (GWW). Second, their augmentation during exercise were related to impaired exercise capacity. Third, biventricular mechanical dispersion was pronounced in all population during exercise and it was related to limited exercise capacity. Fourth, GWW might be more sensitive marker for the evaluation of myocardial work impairment in these patients. This study forms the first evaluation of combined novel MW assessment and mechanical dispersion in adult patients with repaired

TOF, providing detailed evaluation of expected range of MW performance during exercise echocardiography and proposing the best MW parameters that can predict impaired exercise capacity.

6.6 Study limitations

The main limitations of this study are the single-centre design, and the comparison needs healthy subjects to be assessed in the same way for proper validation. Assessment of MW and MD is challenging during stress echocardiography at higher heart rates that could lead to inaccurate MW and dispersion measures. However, we were aware of this issue and have chosen specific exercise time points (low exercise intensity) for strain related images to minimise technical errors at higher heart rates. larger multicentred studies with assessment of myocardial fibrosis are required to confirm our results. We presented the expected range of LV MW and biventricular dispersion at baseline and during exercise for the first time in adult patients with repaired TOF.

6.7 Conclusions

In adult patients with repaired TOF, LV myocardial work parameters are impaired and biventricular mechanical dispersion is pronounced. Severe PR tends to cause a more adverse myocardial work profile with lower MW indices and higher GWW. Conversely there is greater augmentation of MW indices in those with severe PR. Augmentation of myocardial efficiency parameters were associated (with moderate statistical strength) with objective exercise ability suggesting that not just the extent, but also the timing with respect to the events of the cardiac cycle are important determinants of cardiopulmonary capability.

Chapter 7 Contribution of LV longitudinal systolic and myocardial strain functional augmentation to exercise intolerance in adult survivors of TOF- A secondary analysis from the main findings

7.1 Abstract

Background: The trajectory of left ventricle (LV) longitudinal systolic function slopes described by the relation of systolic augmentation (LVS') and global longitudinal strain (GLS) to maximum oxygen uptake (VO_2) and their contribution to exercise intolerance are novel in patients with repaired TOF.

Methods: We analysed incremental systolic function slopes for 100 adult patients with repaired TOF, 60 with severe pulmonary regurgitation (SPR)-loaded group, and 40 repaired TOF patients with no PR-control unloaded group. Systolic efficiency slope (SES) was calculated by individual subject regression slopes for S' and work rate (WR) with VO_2. Myocardial efficiency slope (MES) was calculated by individual subject regression slopes for GLS and WR with VO_2.

Results: Reduced peak corrected VO_2 (ml/kg/min) was observed in both groups with no significant difference (24±7 vs 25±7, ml/kg/min, $p>0.05$). There were significant differences in SES slopes between the SPR and control groups. Higher SES slopes were observed in the unloaded control group with the increments of VO_2 and work rate (4±5.1 vs 3±4.8, $p<0.05$), and (35±24 vs 29±41, $p<0.05$), for S'/VO_2 and S'/WR. Likewise in MES slopes (-8 ±-3 vs -4.1±-1, $p<0.05$), and (-53±-32 vs -43±-22, $p<0.05$), for GLS/VO_2 and GLS/WR. There were significant associations between SES-VO_2/S', MES-VO_2/GLS slopes and VO_2, but it was superior with SES-VO_2/S' ($r=.38$, $r=.29$ $p<0.001$), however this was not observed with work rate.

Conclusions: We have developed new mechanical slopes that described systolic longitudinal function augmentation per unit VO_2 for the first time in patients with repaired TOF. The systolic efficiency slope was a powerful predictor of exercise limitations. We confirmed that the relationship between systolic augmentation is a function of contractility rather than just attained work rate in this population.

7.2 Introduction

Results from the main study presented in chapter 5 have demonstrated the importance of left ventricle (LV) and right ventricle (RV) contractile reserve during cardio pulmonary exercise testing (CPET) in adult patients with repaired TOF. We showed that relationships between all LV and RV functional parameters were higher at peak stress than at rest in this population. We found that LV longitudinal function was a stronger predictor of exercise capacity than RV longitudinal function although the importance of RV contractile reserve by the global index (ΔFAC) was observed but to lesser extent. The relationship between exercise measures of left ventricle function and exercise capacity has been rarely investigated in congenital heart disease including Tetralogy of Fallot patients, and this was shown in our systematic review in chapter 2 (Yang et al., 2015, Samman et al., 2008, Yap et al., 2013). The shape of the LV systolic augmentation curve and the relative contribution to exercise capacity during exercise echocardiography are rarely described in healthy individuals (van Zalen et al., 2021), and this is a novel concept in patients with repaired TOF. There are significant gaps in our understanding of the mechanistic relationship between LV functional contractile reserve parameters, including LV systolic augmentation and LV global longitudinal strain, which we have demonstrated to be important predictors of exercise capacity in adult survivors with TOF.

We therefore undertook this secondary analysis for further assessment of LV functional parameters to gain mechanistic insight into the relationship between LV systolic augmentation to the incremental rise in oxygen uptake (VO_2) during CPET.

7.3 Methods

7.3.1 Main hypothesis and objectives

We aimed; 1) to determine whether the regressive slope relationship between left ventricular longitudinal systolic function (LVS') and VO_2 augmentation (S'/VO_2), and (2) the regressive slope of left ventricular global longitudinal strain (GLS) and VO_2 augmentation (GLS/VO_2) could predict peak corrected oxygen uptake (VO_2) (ml/kg/min) in patients with repaired TOF. We also aimed (3) to determine the ability of longitudinal systolic augmentation with increments in work rate (S'/WR, GLS/WR) in exercise capacity prediction. By evaluation of LV incremental augmentation slopes as a function of either physical augmentation to understand better whether the previously described relationships between function on exercise were simply a function of additional work undertaken in healthier individuals or related to a difference in the contractility energetic relationship.

7.3.2 Cardiopulmonary exercise testing (CPET)

Protocols were individually determined according to the patient ability with work rate of the test was ranged between 15 to 20w. All 100 participants successfully completed CPET. The detailed CPET protocol and patients' performance are provided in the general methods and main chapters (chapters 3 and 5, section 3.7 and 5.3.6). We have chosen peak corrected VO_2 (ml/kg/min) for this secondary analysis as it was shown in chapter 5 that both LVS' and GLS were highly predictive of weighted VO_2 in multivariable regression models.

7.3.3 2D Echocardiography

Left ventricular systolic function (LVS') (average of the septal and lateral wall velocity) was derived from colour-derived tissue Doppler imaging (TDI). LVS' was defined as the highest velocity during systole after iso volumetric contraction. Global longitudinal strain (GLS) was derived from speckle tracking echocardiography (STE) using automated function imaging (AFI). AFI automatically generates GLS from the average peak strain from apical three, two and four-chamber views. All measurements were performed at baseline and at peak stress for all 100 patients. The detailed echocardiographic assessment of LV function and stress echocardiography protocol are described in the general methods chapter (chapter 3, section 3.6).

7.3.4 Mechanical augmentation slopes calculation during CPET

a) Systolic efficiency slope (SES)

We calculated individual subject regression slopes for VO_2 and S' (SES). This represents the change in S' lines augmentation associated with a 1 unit increase in VO_2, and therefore a measure of myocardial efficiency. The ratio of LVS' to VO_2 in the form of the regression parameters of S'/VO_2 was calculated for all 100 patients. For each patient, prespecified points of S' and VO_2 were identified at different exercise time points for slope determination; at baseline, at low exercise (between 3 and 5 minutes) and at peak exercise, guided by the respiratory exchange ratio (RER) value (low exercise when RER was between .85-.95, peak exercise when RER>1.1). A similar technique was used to calculate individuals S' to incremental work rate (WR/S') during exercise. All prespecified S' three points were linked to corresponding VO_2 with the equation (Slope=VO_2/S'), and with WR (Slope=WR/S'). The mean functional and work rate SES slopes were calculated for all 100 patients to establish whether the amount of VO_2 and WR increased for every incremental unit of systolic longitudinal function could predict exercise capacity in this population.

b) Myocardial efficiency slope (MES)

We expanded the technique further to develop new slope describing myocardial longitudinal strain augmentation by GLS, for which we have used the term MES. For each patient, prespecified points of GLS and VO_2 were identified at different exercise time points for slope determination; at baseline, at low exercise (between 3 and 5 minutes) and at peak exercise, guided by the respiratory exchange ratio (RER) value (low exercise when RER was between .85-.95, peak exercise when RER>1.1). A similar technique was used to calculate individuals GLS to incremental work rate (WR/GLS) during exercise. All prespecified GLS three points were linked to corresponding VO_2 with the equation (Slope=VO_2/GLS), and with WR (Slope= WR/GLS). The mean functional and work rate MES slopes were calculated for all 100 patients to establish whether the amount of VO_2 and WR increased for every incremental unit of longitudinal strain function could predict exercise capacity in this population.

7.3.5 Statistical analysis

Continuous data were presented as mean ±SD. All data were tested for normality using the Kolmogorov-Smirnov statistical test. Slope relationships between S', GLS and VO_2 were compared between groups using Student's paired t test for independent parameters. Pearson correlation coefficients (r) were used to determine relationships between new developing slopes (SES and MES) and exercise parameters. All significant correlations in univariate analyses were entered into a multivariable regression model to determine the best predictor of peak weighted VO_2 (ml/kg/min). The statistical analysis was performed using IBM SPSS statistics version 28. A p value of <0.05 was considered statistically significant.

7.4 Results

7.4.1 Baseline characteristics, TOF population (100 patients)

The results from this chapter were drawn from the same population that was described in chapter 5. From adult patients with repaired TOF (n=100), 60 with severe pulmonary regurgitation (SPR-loaded group) were compared to 40 patients with repaired TOF with mild or no PR as a control-unloaded group. Relevant differences between the SPR and control are highlighted in table 7.1. Reduced peak corrected VO_2 (ml/kg/min) was observed in both groups with no significant difference (24±7 vs 25±7, ml/kg/min, p>0.05) (Table 7.1). A respiratory exchange ratio (RER) of > 1.01 was achieved in 97 patients, with a mean value of 1.2±.1 in both groups. Detailed baseline characteristics, surgical history and exercise performance are provided in chapter 5, section 5.3.1 and 5.3.6.

Table 7. 1. Baseline characteristics of the study population (n=100).

Baseline Characteristics (n=100)	**SPR Group Mean ± SD**	**Control Group Mean ± SD**	**P value**
Age (yrs)	35 ±13	33±11	**<0.05**
Gender **Male** **Female**	 31 (52%) 29 (48%)	 19 (48%) 21(52%)	**<0.05**
Height (cm)	166±11	169±8	NS
Weight (kg)	71±16	73±14	NS
BMI (Kg/m2)	25±5	26±5	NS
Peak oxygen consumption VO_2 (ml/kg/min)	24±7	25±7	NS
Peak Workload (W)	150±55	151±49	NS
Peak RER	1.2±.1	1.2±.1	NS

BMI= body mass index; RER= respiratory exchange ratio. Bold values indicate significant level (p<.05); NS=non-significant.

7.4.2 Left ventricular systolic and myocardial strain augmentation slopes

7.4.2.1 Systolic efficiency slope (SES)-(VO_2/ S', and WR/ S' slope)

Overall mean slope of VO_2/S' in the entire population was 3.5±2, and the mean slope of WR/S' was 33±20. In comparison between groups, there were significant differences in SES slopes between loaded-SPR and the unloaded control group (Table 7.2). Higher SES slopes were observed in the unloaded control group with VO_2 and with work rate (4±5.1 vs 3±4.8, $p<0.05$), and (35±24 vs 29±41, $p<0.05$), for VO_2/S' and WR/S' in the control and SPR groups, respectively (Figure 7.1, a and b).

7.4.2.2 Myocardial efficiency slope (MES) -VO_2/ GLS, and -WR/ GLS

Overall mean slope of VO_2/GLS in the entire population was -5±-3, and the mean slope of WR/ GLS was -48±-20. In comparison between groups, there were significant differences in MES slopes between loaded-SPR and the unloaded control group (Table 7.2). Higher MES slopes were observed in the unloaded control group with VO_2 and with work rate (-8±-3 vs -4.1±-1, $p<0.05$), and (-53±-32 vs -43±-22, $p<0.05$), for VO_2/ GLS and WR/ GLS (Figure 7.1, c and d).

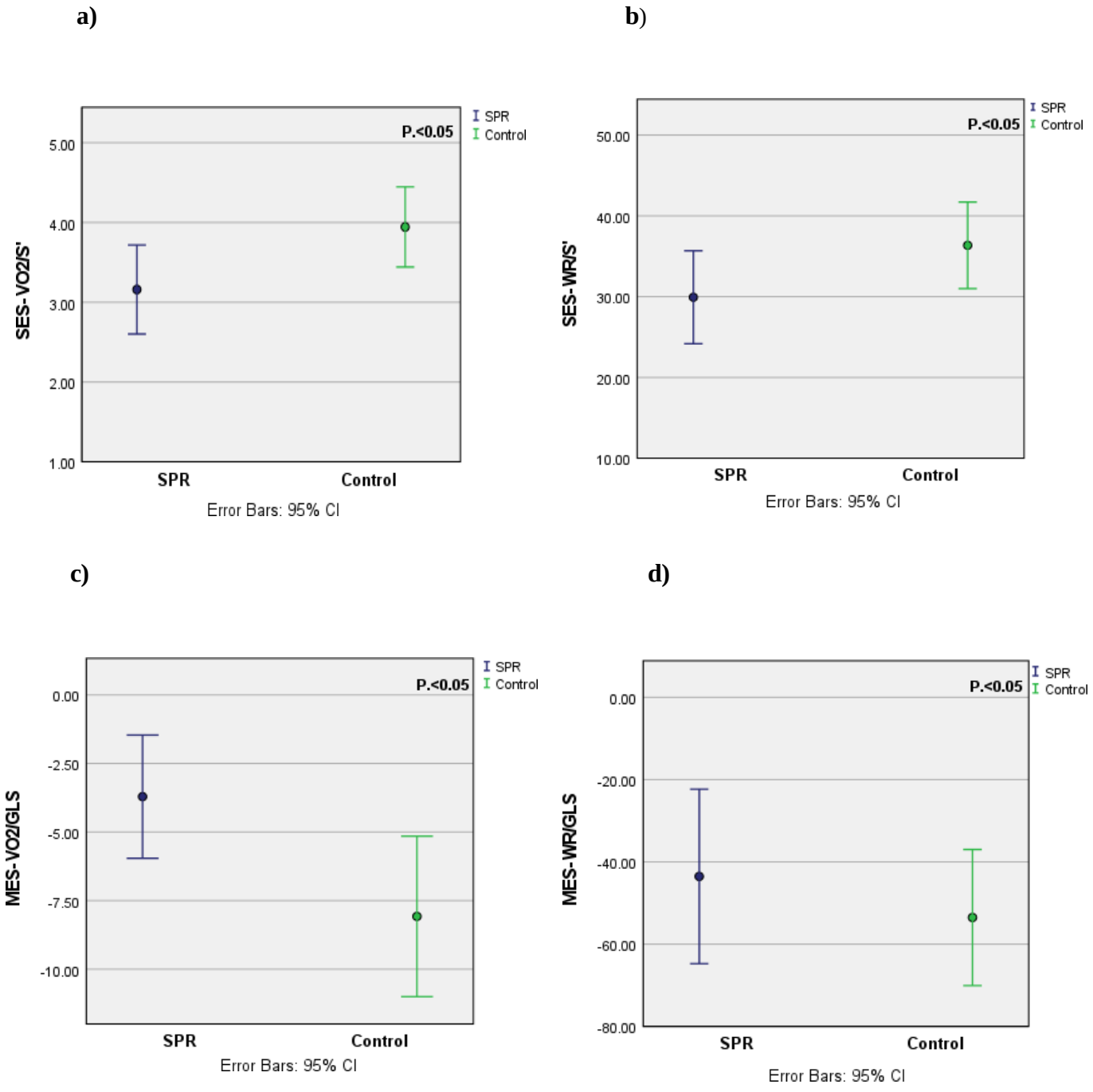


Figure 7. 1. Mechanical and workload incremental slopes difference between two groups; a) VO_2/S', b) WR/S', c) VO_2/ GLS, and d) WR/ GLS.

Table 7. 2. Myocardial augmentation slopes in the entire population (n=100), and the difference between groups.

Myocardial augmentation slopes SES, MES		Mean Slope	P value SPR vs control
***VO_2 /S'**	Total (n=100)	3.5 ±2	**<0.05**
	SPR (n=60)	3 ±4.8	
	Control (n=40)	4 ±5.1	
WR/S'	Total (n=100)	33±20	**<0.05**
	SPR (n=60)	29 ±41	
	Control (n=40)	35 ±24	
***VO_2/GLS**	Total (n=100)	-5±-3	**<0.05**
	SPR (n=60)	-4.1±-1	
	Control (n=40)	-8 ±-3	
WR/GLS	Total (n=100)	-48±20	**<0.05**
	SPR (n=60)	-43 ±-22	
	Control (n=40)	-53 ±-32	

VO_2/S'=average slope of systolic longitudinal function to oxygen uptake; WR/S'=average slope of systolic longitudinal function to work rate; VO_2/GLS=average slope of global longitudinal stain function to oxygen uptake; WR/GLS=average slope of global longitudinal stain function to work rate. *Peak corrected VO_2 (ml/kg/min). Bold values indicate significance level of $p<0.05$.

7.4.2 Association between systolic and myocardial strain augmentation slopes and corrected VO_2 (ml/kg/min) -The VO_2/ S', VO_2/GLS and peak VO_2 relationships

Univariate correlation analysis demonstrated significant associations between VO_2/S', VO_2/GLS slopes with VO_2, but it was superior with VO_2/S' (r=.38, r=.29, $p<0.001$) (Table 7.3). LV contractility by LVS' and myocardial strain augmentation (GLS) increased with work rate, however, slopes describing longitudinal and mechanical aspects of systolic function during exercise (WR/S' and WR/GLS slopes) did not show any significant correlation with VO_2 (Table 7.3).

Table 7. 3. Univariate analysis of longitudinal systolic function and myocardial strain slopes in the entire population (n=100).

Myocardial augmentation slopes n=100	VO2(ml/Kg/ min)
VO_2/S'	**.38****
WR/S'	.07
VO_2/GLS	**-.29***
WR/GLS	-.10

VO_2/S'=average slope of systolic longitudinal function to oxygen uptake; WR/S'=average slope of systolic longitudinal function to work rate; VO_2/GLS=average slope of global longitudinal stain function to oxygen uptake; WR/GLS=average slope of global longitudinal stain function to work rate. *Peak corrected VO_2 (ml/kg/min). *Bold values indicate significance level of p<0.05, ** bold values indicate significance level of p<0.001. All values in table represent r values.

A multivariable regression model was constructed for all 100 repaired TOF patients to determine which aspect of LV systolic augmentation was able to predict peak corrected VO_2. The presence of severe PR was recorded as present or absent, which did not change the overall correlation in both models. When combining the effect of both systolic and longitudinal strain slopes in one model, end results showed that SES slope (for systolic functional augmentation) was a stronger predictor than in univariate analysis and MES slope (for myocardial strain augmentation) (r=.70, r= -.43, p<0.05) (Table 7.4, Figure 7.2). The overall model fit was R^2 = .55, p<0.001.

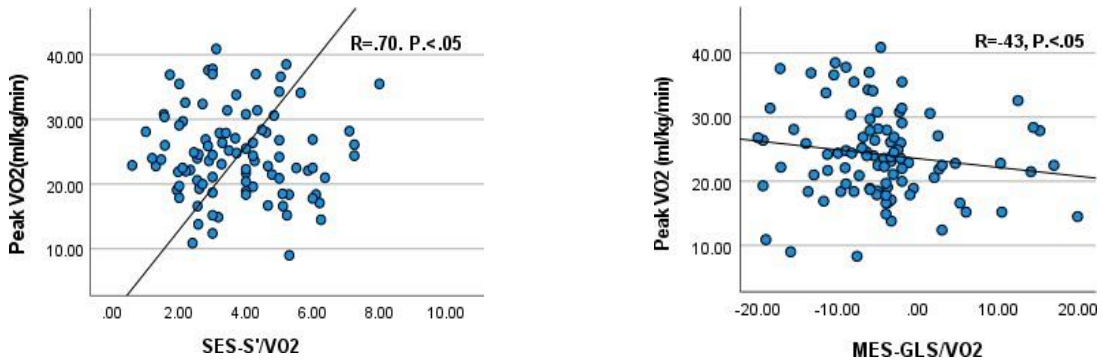


Figure 7. 2. Scatter plots of systolic efficiency slope (SES)-S-VO_2 relationship (left panel). S'=systolic velocity; VO_2 peak oxygen consumption (ml/kg/min). Myocardial efficiency slope (MES)-GLS-VO_2 relationship (right panel). GLS=global longitudinal strain; VO_2=peak oxygen consumption (ml/kg/min).

Table 7. 4. Multi regression model of myocardial longitudinal augmentation slopes in the entire population (n=100).

Myocardial augmentation slopes-VO_2 (ml/kg/min) * R^2=.55, p<0.001 n=100	**Coefficients Beta**	**R**	**R^2**	**P value**
Pulmonary regurgitation	.18	.27	.10	NS
SES-S'/VO_2	**.61**	**.70**	**.40**	**<0.05**
MES-GLS/ VO_2	**-.38**	**-.43**	**-.20**	**<0.05**

S'/VO_2=average slope of systolic longitudinal function to oxygen uptake**;** GLS/VO_2=average slope of global longitudinal stain. * Outcome variable, VO_2 peak (ml/kg/min), bold values indicate significance level of p<0.05; NS=non-significant.

7.5 Discussion

There is very limited data on the relationship between myocardial augmentation and exercise capacity in cardiovascular disease and none in adult patients with repaired TOF (van Zalen et al., 2015, McIntosh et al., 2013). Chapter 5 showed a detailed functional description of myocardial response of left ventricular longitudinal function during stress echocardiography highlighting the importance of contribution of LV longitudinal systolic function described by systolic augmentation (LVS'), and global longitudinal strain (GLS) at rest with more contribution observed at peak stress to functional capacity in this population. GLS is a more advanced method to measure LV function which is feasible during stress echocardiography and it is a prognostic tool of long-term prognosis in cardiovascular mortality (Biering-Sørensen et al., 2017). It has clear advantage of being angle independent, however, GLS requires higher frame rates that might not be high enough to track increased heart rates at peak stress (Sicari et al., 2008). To date, very few reports have evaluated its incremental prognosis during exercise echocardiography (Wang et al., 2014, van Zalen et al., 2015, McIntosh et al., 2013).

The LV velocity measure and strain are different measures of systolic performance that cannot be interchangeable. S' measures the initiation and development, predominantly in the earlier stages of systole, and although not formally time bound, it is an indirect estimation of myocardial acceleration. GLS is a measure of displacement at end systole and is therefore a summation of all myocardial motion during systolic contraction. In our study, we selected S' and GLS as our principle long axis evaluation parameters due to their known reproducibility during exercise (Rubis et al., 2009). The close relationship between LVS' and VO_2 that was shown previously was also evident in a range of pathologies (McIntosh et al., 2013, van Zalen et al., 2015). However, the trajectory of LVS' and GLS slopes and their incremental prognostic values are not known yet in adult patients with repaired TOF. This sub study was constructed to evaluate the systolic myocardial augmentation response with corresponding VO_2 to exercise for the first time in adult survivors of TOF.

Evaluation of myocardial deformation during stress echocardiography including LVGLS and LVS' is novel and the association we demonstrated between LV longitudinal systolic function with peak VO_2 suggests that GLS and LVS' are both important parameters. Both parameters augment with exercise (particularly for LVS' augmentation increases without plateau) meaning that the S' reserve and VO_2 relationship may relate solely to exercise ability and exercise time, rather than describing a function of the myocardium. The systolic efficiency slope (SES, S'/VO_2), circumvents this risk by examining not solely the absolute augmentation of systolic function, but the augmentation per unit VO_2. In healthy adults this could predict exercise capacity (van Zalen et al., 2021), applying this to our cohort, we have again found a significant relationship between SES and peak VO_2 which was shown to be a powerful predictor of exercise capacity in this population. When compared to a study on healthy individuals, our results showed less overall mean S'/VO_2 slope (3.5 vs 4.5), which may suggest that any slope less than 4.5 is considered abnormal (van Zalen et al., 2021).

As we have demonstrated that GLS is more predictive during exercise than S' and we have extended the methodology further to describe myocardial augmentation during exercise between GLS and VO_2, and have named this myocardial efficiency slope (MES). The MES describes for the first time the regressive relationship between the slope of GLS to VO_2 (GLS/VO_2) that could predict peak VO_2 (the amount of GLS augmentation required to increase VO_2 by a single unit), which was also a significant predictor in our population, but relatively weaker than SES. Thus, by two measures of longitudinal function we have demonstrated that it is the incremental increase in VO_2 for each unit of myocardial augmentation which then predicts a significant proportion of the peak oxygen uptake. There is nothing in the current literature for our MES slopes to be compared with, but our result clearly describes the strong relationship between myocardial longitudinal function by GLS and peak VO_2.

Comparing the slopes in our cohort between loaded and unloaded groups, higher LV slopes augmentation by LVS' and GLS was observed in the unloaded-control group. This suggests that the magnitude of contribution of LV systolic augmentation to exercise capacity in this population is higher in patients who do not have severe pulmonary regurgitation. However, in the prediction model, myocardial longitudinal slopes were predictive of exercise capacity irrespective of severity of pulmonary regurgitation, which means that the degree of severity of pulmonary regurgitation does not affect the strength of the relationship. Consequently, there must be other factors that contribute to such discrepancies between groups, such as degree of RV dysfunction or LV dysfunction that could affect functional augmentation during exercise echocardiography. This is mechanistically complex in this population but does suggest strongly that exercise capacity has no relation to pulmonary regurgitation, but rather to ability of LV and RV to augment longitudinal function during exercise.

The new slopes, SES and MES do not require a prespecified maximal exercise or heart rate which is a key advantage in our population who are known to not always reach maximal effort. This suggests that SES and MES could form the basis for clinical tools which are suitable methods for assessing disease progression in adult survivors of TOF. The slope between S' and VO_2 in our results was more predictive than the slope between GLS and VO_2, but these unique relationships mirror our previous findings of the importance of LV longitudinal function in explaining impaired exercise tolerance in patients with repaired TOF. This secondary analysis demonstrates greater insights and stronger evidence into longitudinal ventricular mechanisms and contribution to exercise intolerance in this population.

7.6 Study limitations

Main limitations of this study were the technical challenges imposed by recording TDI and STE during exercise at different stages. TDI is known to have major limitations, such as angle dependency and the requirement of high frame rate acquisition which is more challenging during stress, but despite this, it was shown that measurement of S' during stress is reliable (Rubis et al., 2009). The GLS acquisition during stress echocardiography might not be accurate at higher heart rates, however, we recorded our GLS at low exercise intensity to maintain accepted ranges of frame rate. Despite these limitations, our findings introduce novel longitudinal and mechanical incremental slopes that can provide additional information on functional limitations in patients with repaired TOF.

7.7 Conclusions

In this chapter we have described for the first-time parameters that incorporate contraction parameters and energetic parameters in a single slope measure and show that these slopes predict VO_2 in patients with repaired TOF. As these slopes and not the similar slopes with WR predict VO_2 it strongly suggests that our previous findings of augmenting VO_2 are a function of systolic augmentation of contractility rather than just attained work rate.

Chapter 8 Normal right ventricular augmentation during stress echocardiography- A comparative study

8.1 Abstract

Background: The assessment of right ventricular (RV) contractile reserve (CR) during exercise echocardiography in healthy subjects is not well described. The objectives of this study were to describe normal RV CR in healthy cases and to compare CR to patients with defined RV compromise because of previous repair of TOF.

Methods: 40 healthy individuals with satisfactory RV windows were randomly selected and retrospectively analysed from previously published study (marathon study). These cases were compared to 100 adult patients with repaired TOF (rTOF). We defined RV CR by the change in tricuspid lateral annular systolic velocity (ΔRVS'), change in tricuspid annular plane systolic excursion (ΔTAPSE), and change in fractional area change (ΔFAC). All parameters were evaluated at baseline and at peak stress.

Results: During exercise, RVS' was increased by 60±20%, followed by TAPSE 48±15%, and the lowest with FAC by 32±10%. These ranges were significantly higher than in TOF population. RV CR was greater in males than females for all RV functional measures (34±10 vs 28±10, %, $p<0.05$); (50±10 vs 44±11, %, $p<0.05$); (61±12 vs 52±11, %, $p<0.05$), for ΔFAC, ΔTAPSE, and ΔRVS', respectively. There was no association between RV CR and functional capacity parameters in healthy individuals, whereas in patients with repaired TOF there was a significant association with peak absolute VO_2 (ml/min) ($r=.36$, $p<0.001$, with ΔFAC).

Conclusions: We have presented normal values for RV CR parameters in healthy cases during stress echocardiography which were markedly reduced in patients with repaired TOF. RV CR is an important determinant of exercise capacity in patients with abnormal RV function but not in normal subjects.

8.2 Introduction

The normal right ventricle (RV) has the ability to augment systolic function during exercise that can be described during stress echocardiography. Maladaptation of this ability may determine symptoms (La Gerche and Claessen, 2015). RV contractile reserve is defined as the response of the RV to stress that may be an important prognostic factor, however the best parameter for its evaluation remains to be determined (Kass et al., 1987, Pergola et al., 2021). Measuring RV contractile reserve during stress echocardiography is more challenging but may be more informative than assessing RV systolic function at rest. RV contractile reserve in normal individuals remains poorly described with a range of measurable parameters and no clear definitions of normality. Target parameters include the FAC (from 2D imaging), TAPSE (from M-mode), and tricuspid annular systolic velocity RVS' (from tissue Doppler), all of which describe different facets of myocardial contraction.

Resting echocardiographic parameters have limited ability to detect early impairment of RV function. Physical stress may unmask RV function abnormalities. This has been described in patients with repaired Tetralogy of Fallot (TOF) who have normal resting RV function (Apostolopoulou et al., 2007, Brili et al., 2008, Ait-Ali et al., 2014, Bhatt et al., 2019b), in pulmonary hypertension (Spruijt et al., 2015). In this chapter we aimed to describe normal RV contractile reserve in healthy adults and to compare RV contractile reserve to patients with defined RV compromise because of previous repair of TOF.

8.3 Methods

8.3.1 Main hypothesis and objectives

1) To describe normal right ventricular augmentation and to establish normal range of normal RV contractile reserve during stress echocardiography in healthy adults (cases).
2) To compare the normal RV contractile reserve to adult patients with repaired TOF, with or without severe pulmonary regurgitation.
3) To establish to what extent contractile reserve relates to exercise capacity.

8.3.2 General characteristics

40 healthy individuals with satisfactory RV windows were randomly selected from previously published study (marathon study) (van Zalen et al., 2021), and retrospectively evaluated. Untrained healthy volunteers were recruited prior starting their first time London marathon and underwent cardiopulmonary exercise test with echocardiography focused on LV assessment. The full protocol of the study was previously published (D'Silva et al., 2020). The most important point about these normal cases is that they were verifiably normal, as in addition to a normal echocardiogram all subjects also had a normal cardiac magnetic resonance scan.

The inclusion criteria were;1) good RV delineation in all stages of exercise; 2) maximum effort achieved (RER>1.1) and; 3) acceptable frame rate (FR>25 Hz) for TDI and retrospective strain analyses. Those healthy participants were compared to 100 adult patients with repaired TOF (rTOF)-60 with severe pulmonary regurgitation (SPR) (RV pathology and loading) and 40 repaired TOF patients with mild or no pulmonary regurgitation (MPR) (RV pathology without loading). We have chosen this group of patients as; (a) they are mainly right heart disease and; (b) with this variable degree of RV pathology and loading in our cases could help in defining the normal RV contractile reserve during stress echo in healthy individuals.

8.3.3 2D Echocardiography

We defined RV systolic function by tricuspid lateral annular systolic velocity (RVS') using Doppler tissue imaging, tricuspid annular plane systolic excursion (TAPSE) using M-mode echocardiography, and fractional area change (FAC) using 2D echocardiography. RV functional contractile reserve was defined by the change in tricuspid annular plane systolic excursion (ΔTAPSE), change in RV fractional area change (ΔFAC), change in Doppler-derived tricuspid lateral annular peak systolic velocity (ΔRVS'). All parameters were evaluated at baseline and at peak stress (when RER >1). The detailed methodology of the 2D echocardiographic measurement of RV systolic function is provided in chapter 3 (section 3.6).

8.3.4 Cardiopulmonary exercise testing (CPET)

Protocols were individually determined with work rate and ranged between 15 to 30w, increased every minute until voluntary exhaustion. The full cardiopulmonary exercise testing (CPET) protocol for the TOF cases was previously described in chapter 3 (section 3.7), while the protocol for healthy individuals was previously published (D'Silva et al., 2020).

8.3.5 Statistical analysis

All data were tested for normality using the Kolmogorov-Smirnov statistical test. Continuous data were presented as mean ±SD. RV functional parameters (RVS', TAPSE, and FAC) at rest and at peak stress were compared between healthy individuals and TOF cases, using Student's t test for independent samples. Student's t test for paired sample was used to compare RV contractile reserve parameters, comparison between gender and magnitude of change (ΔRVS', ΔTAPSE, and ΔRVFAC). ANOVA test was used to compare mean difference between cases and TOF population. Pearson correlation coefficients (r) were used to determine relationships between RV contractile reserve parameters and exercise parameters. All variables that were significant at univariate correlation analysis were entered into multivariable regression analysis for all patients. Pulmonary regurgitation was treated as a binary variable (severe PR/ no or minimal PR) for the purposes of this analysis. The statistical analysis was performed using IBM SPSS statistics version 28. A p value of <0.05 was considered statistically significant.

8.4 Results

8.4.1 Baseline characteristics

As shown in table 8.1, our study included 40 healthy subjects that were randomly selected from a previously published study from a population that were more male (62%), with a mean age of 29±3.3y. These patients were compared against 100 patients with repaired TOF, who were marginally more male (52%). 60 patients had severe pulmonary regurgitation (SPR group), and 40 repaired TOF patients had mild or no pulmonary regurgitation (MPR) (Table 8.1). The full detail on baseline characteristics of the TOF population including surgical history is presented in chapter 5 (section 5.3.1).

Table 8. 1. Baseline characteristics of the entire population.

Baseline characteristics	Healthy individuals Mean ±SD (n=40)	SPR group Mean ±SD (n=60)	MPR group Mean ± SD (n=40)	P value*
Age (yrs)	29.2 ±3.3	35 ±13	33±11	**<0.05**
Sex				
Male	25 (62%)	31 (52%)	19 (48%)	**<0.05**
Female	15 (38%)	29 (48%)	21(52%)	**<0.05**
Height (cm)	175 ±10.3	166±11	169±8	**<0.05**
Weight (kg)	71 ±12.9	71±16	73±14	NS
BMI (Kg/m^2)	23±2	25±5	26±5	**<0.05**
CPET parameters				
Absolute peak VO_2 (ml/min)	2769 ±669	1695±627	1744±521	**<0.05**
Predicted VO_2 (%)	107±17	75±17	76±17	**<0.05**
Peak RER	1.23±.09	1.2±.1	1.2±.1	NS

BMI=body mass index; VO_2=peak absolute maximum oxygen uptake in absolute (ml/min); RER=respiratory exchange ratio. Bold values indicate significant level ($p<0.05$); * Difference between healthy cases and TOF groups; NS=non-significant.

8.4.2 Baseline echocardiographic findings

8.4.2.1 2D Echocardiography-RV size and systolic function

In healthy individuals, normal RV size and systolic function were observed. 98% fell within the published normal ranges for FAC, with 93% for TAPSE and RVS'. The mean values were 45 ±4 %; 21±2 mm and 10±3 cm/s, for FAC, TAPSE and RVS', respectively (Table 8.2). While in the TOF population, larger RV size was observed in the loaded-SPR group, and unloaded-MPR group, with more RV dilation in the loaded-SPR group (mean basal RV diameter 5±.3 vs 4.1±.6, cm, $p<0.05$, Table 8.2). In the loaded-SPR group, 93% of population had moderate to severe RV dilatation, while 48% had mild or moderate RV dilatation in the unloaded group, when compared to the normal range (>4.2, abnormal, (Lang et al., 2015)).

For RV systolic function, RV longitudinal function measured by TAPSE and RVS' was reduced in both groups with more reduced values in the unloaded-MPR group (17±3 vs 14±4, mm, $p<0.05$); (8±2 vs 6±2, cm/s, $p<0.05$) for TAPSE and RVS' in the SPR and MPR group, respectively. For TAPSE, 45% of loaded-SPR group was below the normal range, while 68% in unloaded-MPR group (>18 mm in normal individual, (Lang et al., 2015)). For RVS', 73 % of loaded-SPR group was below the normal range, while 90% in unloaded-MPR group (>10 cm/s in normal individual, (Lang et al., 2015)). They were significantly lower than healthy individuals (Table 8.2). For FAC, normal function was observed in both groups (43±6 vs 41± 5 %, $p<0.05$), with 12% of loaded-SPR group was below the normal range while in 20% in unloaded-MPR group (>35% in normal individual). FAC in the TOF population was also significantly lower than in healthy individuals (Table 8. 2).

8.4.3 RV functional contractile reserve

8.4.3.1 Changes in tricuspid lateral annular systolic velocity (RVS')

In healthy individuals, RVS' increased significantly from 10±3 cm/sec to 16±2 cm/sec, representing an average increase in contractile reserve of 60%. In the repaired TOF (unloaded -MPR) group, the RVS' was lower at baseline 6±2 cm/sec and increased to 9±2, cm/s representing a reduced contractile reserve of 48%. In the repaired TOF (loaded-SPR) group, the RVS' was lower at baseline 8± 2 cm/sec and increased to 11±2, cm/s representing a reduced contractile reserve of 41%. There was a significant difference in contractile reserve between the SPR and MPR groups ($p<0.05$, Table 8.2, Figure 8.1, 8.2).

8.4.3.2 Changes in tricuspid annular plane systolic excursion (TAPSE)

In healthy individuals, TAPSE increased significantly from 21±2 mm to 30±4 mm, representing an average increase in contractile reserve of 48%. In the repaired TOF (unloaded-MPR) group, the TAPSE was lower at baseline 14±4 mm and increased to 20±6 mm representing a reduced contractile reserve of 42%. In the repaired TOF (loaded-SPR) group, the TAPSE was lower at baseline 17±3 mm and increased to 24±5 mm representing a reduced contractile reserve of 39%. There was a significant difference in contractile reserve between the SPR and MPR groups ($p<0.05$, Table 8.2, Figure 8.1, 8.2).

8.4.3.3 Changes in fractional area change (FAC)

In healthy individuals, FAC increased significantly from 45±4% to 57±5%, representing an average increase in contractile reserve of 32%. In the repaired TOF (unloaded-MPR) group, the FAC was lower at baseline 41±5% and increased to 49±9% representing a reduced contractile reserve of 23%. In the repaired TOF (loaded-SPR) group, the FAC was lower at baseline 43±6% and increased to 52±9% representing a reduced contractile reserve of 20%. There was a significant difference in contractile reserve between the SPR and MPR groups ($p<0.05$, Table 8.2, Figure 8.1, 8.2).

Table 8. 2. Baseline and contractile reserve (CR) of FAC, TAPSE and RVS' in the entire population.

Right Ventricle parameters 2D Analyses		Baseline	Peak Intensity	P value Baseline vs peak	Contractile reserve Δ POC (%)	P value Δ Healthy vs all cases	P value Δ SPR vs MPR
RV Size (mm)	**Healthy cases (n=40)**	3.8±.2	3.2±.3	**<0.05**	-	-	-
	TOF SPR (n=60)	5±.3	4.5±.6	**<0.05**	-		
	TOF MPR (n=40)	4.1±.6	3.9±.5	**<0.05**	-		
FAC (%)	**Healthy cases (n=40)**	45 ±4	57±5	**<0.05**	32±10	**<0.001**	**<0.05**
	TOF SPR (n=60)	43±6	52 ±9	**<0.05**	20±15		
	TOF MPR (n=40)	41±5	49±9	**<0.05**	23±16		
TAPSE (mm)	**Healthy cases (n=40)**	21±2	30 ±4	**<0.05**	48±15	**<0.001**	**<0.05**
	TOF SPR (n=60)	17±3	24±5	**<0.05**	39±28		
	TOF MPR (n=40)	14±4	20 ±6	**<0.05**	42±28		
RVS' (cm/s)	**Healthy cases (n=40)**	10±3	16±2	**<0.05**	60±20	**<0.001**	**<0.05**
	TOF SPR (n=60)	8± 2	11±2	**<0.05**	41±28		
	TOF MPR (n=40)	6±2	9±2	**<0.05**	48±20		

FAC=fractional area change; TAPSE=tricuspid annular plane systolic excursion; RVS'=right ventricular systolic velocity; POC=percentage of change; POC=percentage of change; Δ=change between baseline and peak stress. * Bold values indicate significant level (p<0.05, <0.001).

Contractile reserve was defined by the change in tricuspid annular plane systolic excursion (ΔTAPSE), change in RV fractional area change (ΔFAC), change in Doppler- derived tricuspid lateral annular peak systolic velocity (ΔRVS'). The highest RV contractile resrerve was observed in the healthy cases. RVS' was increased by 60±20%, followed by TAPSE by 48±15, %, and the lowest with FAC by 32±10% (Figure 8.2). This range was significantly higher than seen in the TOF population (Table 8.2, Figure 8.2).

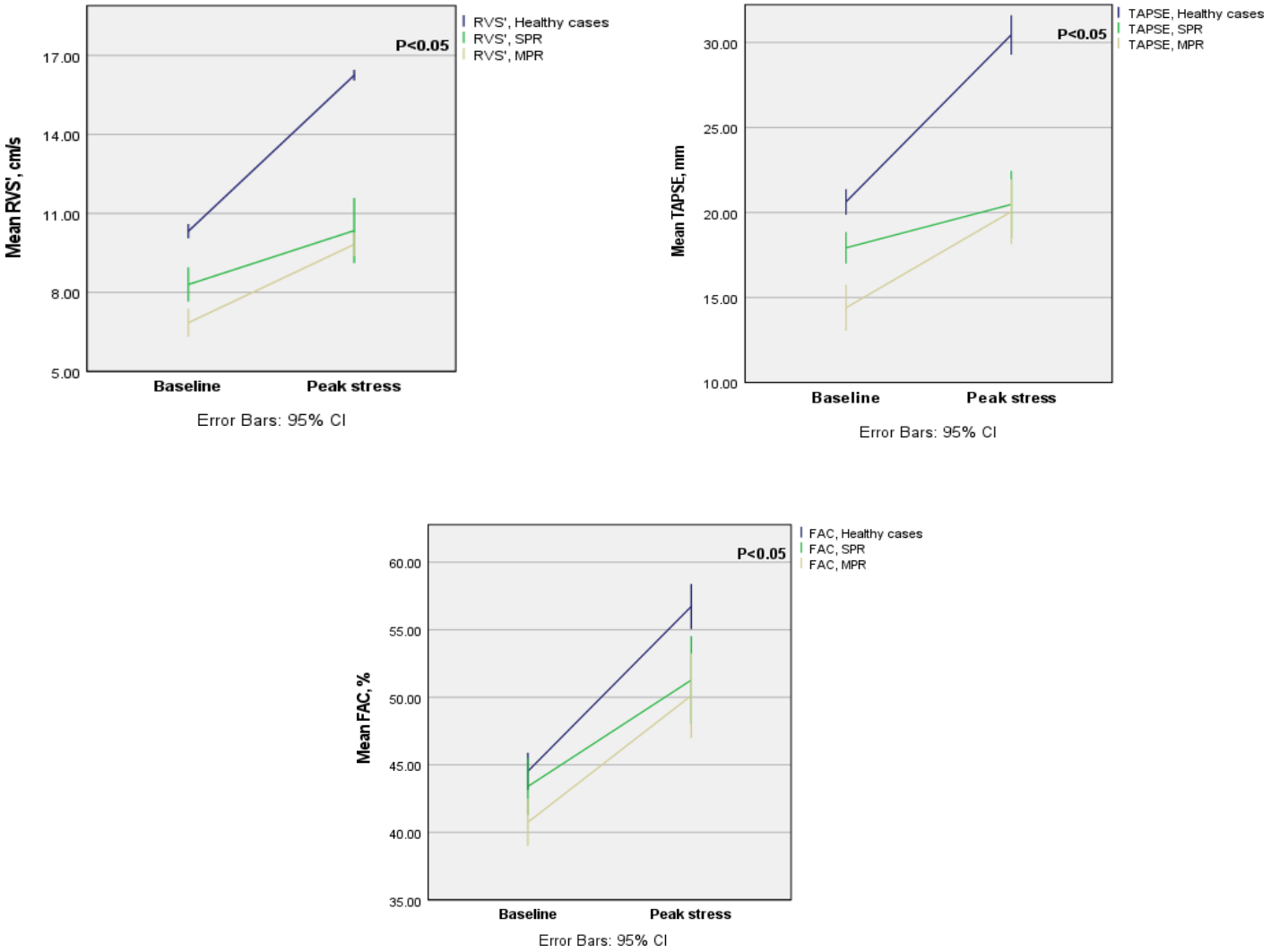


Figure 8. 1. Difference in RVS', TAPSE and in FAC between baseline and peak stress in the entire population.

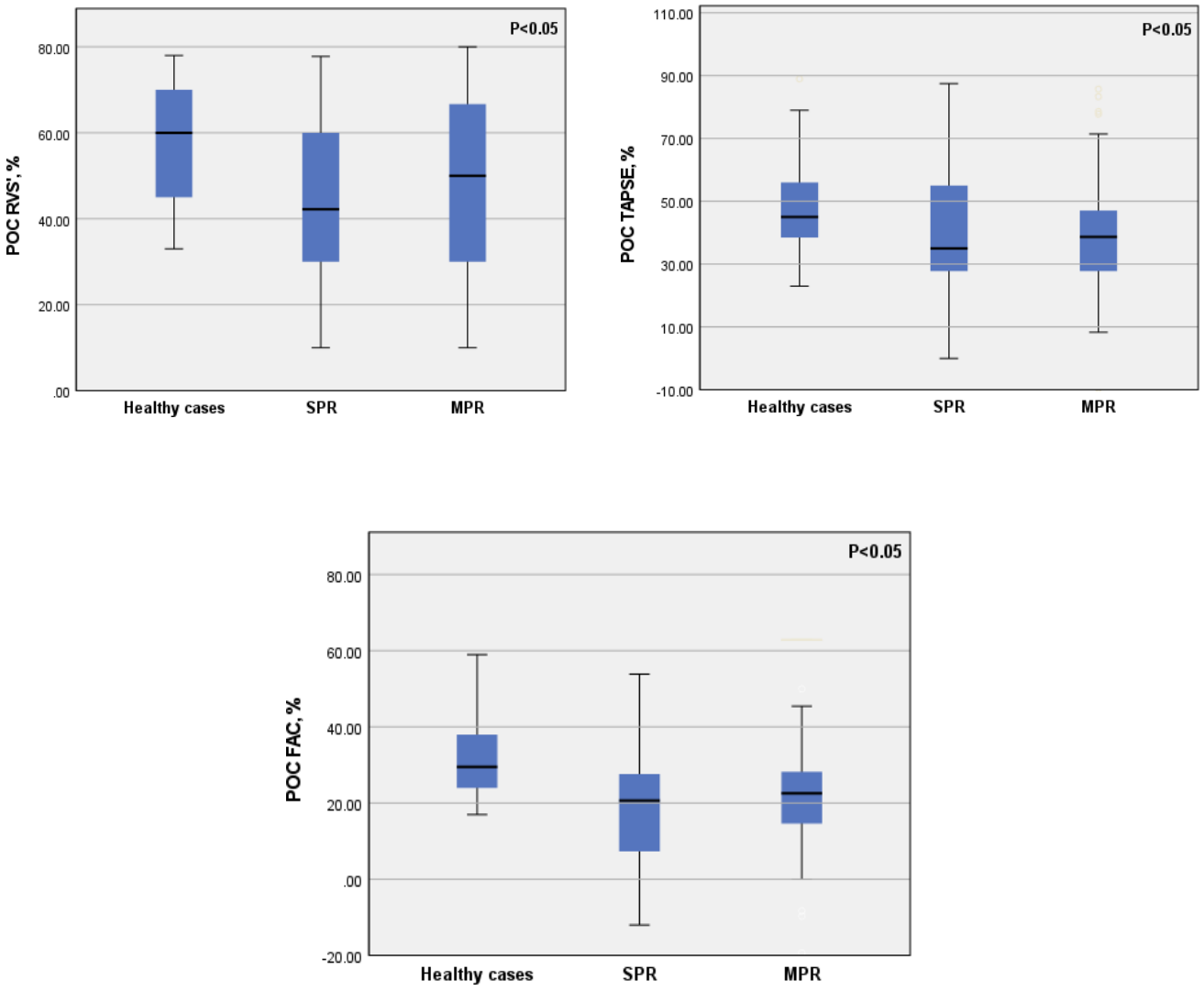


Figure 8. 2. RV contractile reserve parameters difference in healthy, SPR and MPR groups. POC=percentage of change.

In the TOF population we found similar ΔTAPSE and ΔFAC in normal and abnormal RV function groups (36±17 vs 37±34, p>0.05); (22±13 vs 21±14, p>0.05), while lower ΔRVS' was observed in abnormal RV loading group (48±18 vs 41±1, p<0.05) (Table 8.3).

RV contractile reserve was greater in males than females for all RV functional measures in healthy cases (34±10 vs 28±10, %, p<0.05); (50±10 vs 44±11, %, p<0.05); (61±12 vs 52±11, %, p<0.05), for ΔFAC, ΔTAPSE, and ΔRVS', respectively (Table 8.3, Figure 8.3). Across all gender groups, there was an influence of gender on CR, and this was not explained by the difference in RV cavity size or body size.

Table 8. 3. Gender and RV contractile reserve parameters in healthy population, and RV contractile reserve parameters in the TOF population.

Right ventricle CR (n=40)	Male (n=25)	Female (n=15)	p value	TOF RV CR (n=100)		p value
				Normal RV function	Abnormal RV function	
ΔFAC (%)	34±10	28±10	**<0.05**	22±13	21±14	NS
ΔTAPSE (%)	50±10	44±11	**<0.05**	36 ±17	37±34	NS
ΔRVS' (%)	61±12	52±11	**<0.05**	48±18	41 ±1	**<0.05**

CR=contractile reserve; ΔFAC=contractile reserve of fractional area change; ΔTAPSE=contractile reserve of tricuspid annular plane systolic excursion; ΔRVS'=contractile reserve of right ventricular systolic velocity. * Bold values indicate significant level (p<0.05); NS=non-significant.

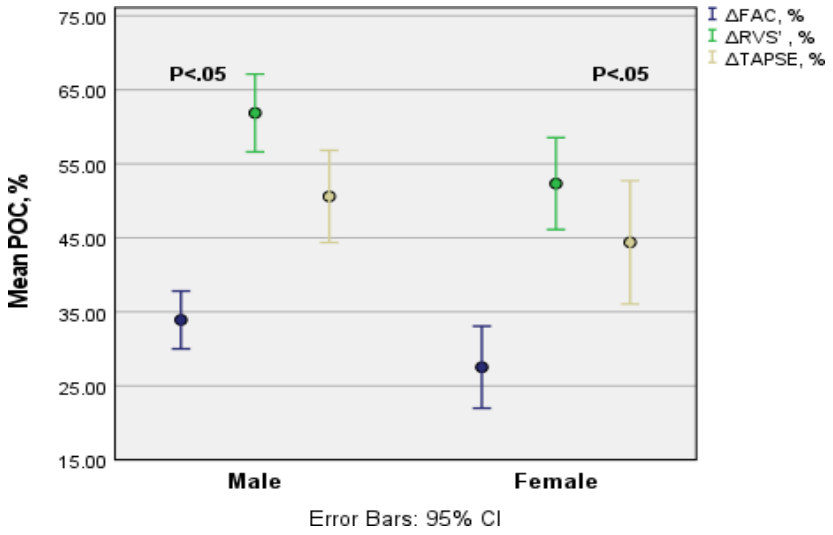


Figure 8. 3. Difference in all RV contractile reserve parameters between male and female in healthy population. POC=percentage of change.

8.4.4 Cardiopulmonary exercise test- association between maximum oxygen uptake (VO_2) and RV functional parameters in the entire population

All subjects successfully completed cardiopulmonary exercise testing using upright bicycle with echocardiography. A respiratory exchange ratio (RER) of >1.01 was achieved in all healthy individuals (mean RER 1.23±1), and 97% in TOF population (mean RER of 1.2±.1) (Table 8.1).

In healthy individuals, 90% of population had an exercise capacity above the normal published range with mean peak absolute VO_2 of 2769± 669.5 ml/min and mean peak percent of predicted VO_2 of 107±17% (Table 8.1) (Takken et al., 2019). In the TOF population, reduced exercise capacity was observed in 80% of the loaded-SPR group and in 70% of the unloaded- MPR group, with no statistically significant difference between two groups in peak absolute VO_2 (1695±627 vs 1744±521, ml/min, $p>0.05$), and in mean peak percent of predicted (75±17 vs 76±17, %, $p>0.05$) in the SPR and MPR group, respectively (Table 8.1).

In healthy adults, among all exercise measures, there was no association observed between peak absolute, predicted VO_2, and all RV parameters including RV FAC, TAPSE and RVS' at rest, at peak and with contractile reserve (Table 8.4). For baseline characteristics, there was no association with age. However, there was a statistically significant association between gender and all RV contractile reserve measures ($r=.34$, $p<0.05$) ;($r=.41$, $p<0.001$), and ($r=.35$, $p<0.05$), for ΔFAC, ΔTAPSE, and ΔRVS', respectively (Table 8.4).

Table 8. 4. Univariate correlation analysis of RV function, and RV contractile reserve parameters in healthy population.

RV parameters	VO_2 (ml/min)		Predicted VO_2 (%)		Contractile reserve Δ			
n=40	Baseline	Peak stress	Baseline	Peak stress	VO_2(ml/min)	Predicted VO_2 (%)	Gender	Age
FAC (%)	.04	.14	.11	.09	.11	.03	**.34***	.01
TAPSE (mm)	.15	.05	.09	.11	.13	.03	**.41****	.08
RVS' (cm/s)	.15	.20	.07	.24	.21	.04	**.35***	.00

FAC=fractional area change; TAPSE=tricuspid annular plane systolic excursion; RVS'=right ventricular systolic velocity; Δ=change between baseline and peak stress. All values in table represent r values. * Bold values indicate significant level ($p<0.05$), ** $p<0.001$.

In the TOF population, for RV function, right ventricular systolic function at baseline and at peak stress measured by FAC correlated with absolute VO_2 (ml/min) (r=.37, r=.39 $p<0.05$). TAPSE correlated with absolute VO_2 at peak stress (r=.27, $p<0.05$), and RVS' (.22, $p<0.05$).

For RV contractile reserve, only change in FAC between baseline and peak stress correlated with absolute VO_2 (ml/min) (r= .36, $p<0.05$). There were no associations between RV function, contractile reserve with baseline characteristics including gender and age in the TOF population (Table 8.5).

Table 8. 5. Univariate correlation analysis of RV function, and RV contractile reserve parameters in the TOF population.

RV parameters	VO_2(ml/min)		Predicted VO_2 (%)		Contractile reserve Δ			
n=100	Baseline	Peak stress	Baseline	Peak stress	VO2(ml/min)	Predicted VO_2 (%)	Gender	Age
FAC (%)	**.37***	**.39***	.11	.09	**.36***	.20	.20	.11
TAPSE (mm)	-0.16	**.27***	.18	.10	.20	.16	.13	.05
RVS' (cm/s)	.04	**.22***	.22	.04	.10	.11	.30	.03

FAC=fractional area change; TAPSE=tricuspid annular plane systolic excursion; RVS'=right ventricular systolic velocity; Δ=change between baseline and peak stress. All values in table represent r values. * Bold values indicate significant level ($p<0.05$).

8.4.5 Right ventricular longitudinal systolic efficiency slopes-The RVS'/ VO_2, TAPSE/ VO_2 and FAC/ VO_2 relationships

We have previously described myocardial efficiency by creating regression line between augmentation of the right ventricle and simultaneous VO_2. We have used the term longitudinal efficiency slope (LES), to describe each RV functional slope. This represents the amount of RVS/TAPSE/ FAC lines augmentation associated with a 1 unit increase in VO_2.

There was a significant difference in the LES RV slope between healthy individuals and the TOF population (Table 8.6). Higher RV LES slope was observed in healthy individuals when compared to the TOF population, in FAC-LES (6.1±5 vs 1.7±2.2 vs 1.4±2.1, p<0.001); in RVS-LES (4.6±3 vs 2.4±2.2 vs 5±3, p<0.001), and in TAPSE-LES (4.2±5.1 vs 1.9 ±2.2 vs 2.4±2, p<0.001, Table 8.6) in healthy, SPR and MPR, respectively.

In the TOF population, higher systolic longitudinal argumentation by RVS' and TAPSE- LES was observed in the unloaded-MPR group, while higher FAC-LES was observed in the loaded-SPR group (Table 8.6).

Table 8. 6. RV Longitudinal efficiency slopes in the entire population.

RV augmentation slopes		Mean Slope	P value Healthy vs entire TOF cohort	P value SPR vs MPR
FAC/VO_2	**Healthy**	6.1±5	**<0.001**	**<0.001**
	SPR	1.7±2.2		
	MPR	1.4±2.1		
TAPSE/VO_2	**Healthy**	4.2±5.1	**<0.001**	**<0.001**
	SPR	1.9 ±2.2		
	MPR	2.4±2		
RVS'/VO_2	**Healthy**	4.6±3	**<0.001**	**<0.001**
	SPR	2.4±2.2		
	MPR	5±3		

FAC/VO_2=longitudinal efficiency slope (LES) for fractional area change; TAPSE/VO_2=LES for tricuspid annular plane systolic excursion; RVS'/VO_2=LES for right ventricular systolic velocity. * Bold values indicate significant level (p<0.001).

8.4.6 Association between maximum oxygen uptake (VO_2) and RV functional longitudinal efficiency slopes (RV LES)

In healthy individuals, there were no significant associations between peak absolute and predicted VO_2 and all the RV LES slopes (Table 8.7). In the TOF population, there were significant correlations observed between absolute VO_2 and RVS'-LES (r= -.40, p<0.001), TAPSE-LES (r=-.32, p<0.001), and with FAC'- LES (r=-.28, p<0.001, Table 8.7).

Table 8. 7. Univariate correlation analysis (r values presented) between maximum oxygen uptake and RV LES slopes in healthy and TOF populations.

RV Slopes*	**VO_2(ml/min)**	**Predicted VO_2 (%)**
Healthy individuals (n=40)		
FAC/VO_2	-.10	-.21
TAPSE/VO_2	-.11	-.33
RVS'/ VO_2	-08	-.10
TOF population (n=100)		
FAC/VO_2	**-.28****	-.15
TAPSE/VO_2	**-.32****	-.09
RVS'/ VO_2	**-.40****	-.24

FAC/VO_2=longitudinal efficiency slope (LES) for fractional area change; TAPSE/VO_2=LES for tricuspid annular plane systolic excursion; RVS'/VO_2=LES for right ventricular systolic velocity. **Bold values indicate significant level (p<0.001). * All values in table represent r values.

A multivariable regression model was constructed with peak absolute VO_2 and the RV LES slopes in the TOF population which showed that the increments of RVS and TAPSE longitudinal velocity during exercise were the most predictive parameters of exercise capacity (r=-.41, r=-.50, p<0.001). The overall model fit was R^2= .36, p<0.001 (Table 8.8).

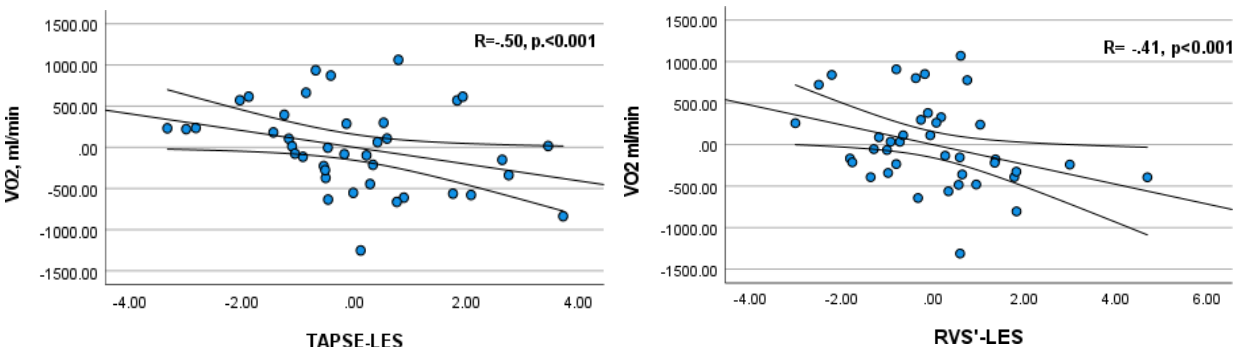


Figure 8. 4. Scatter plots of RV- LES slopes significant exercise predictors in the TOF population (R^2= .36, p<0.001).

Table 8. 8. Multivariable linear regression of RV augmentation slopes (LES) in the TOF population.

RV augmentation slopes TOF population, (n=100) VO_2 (ml/min), (R^2 =.36, p<0.001)	**R**	**Coefficient beta**	**P value**
Pulmonary regurgitation	.27	-.07	NS
FAC/VO_2	-.22	-.11	NS
TAPSE/VO_2	**-.50**	-.37	**<0.001**
RVS'/ VO_2	**-.41**	-.22	**<0.001**

FAC/VO_2=longitudinal efficiency slope (LES) for fractional area change; TAPSE/VO_2=LES for tricuspid annular plane systolic excursion; RVS'/VO_2=LES for right ventricular systolic velocity. * Bold values indicate significant level (p<0.001).

8.5 Discussion

The right ventricle (RV) plays an importance role in the mortality and morbidity in patients with congestive heart failure and pulmonary hypertension (Guazzi et al., 2013). The physiological importance of RV performance during stress and RV contractile reserve (CR) assessment is however poorly described. RV dysfunction has been shown to be an important cause of exercise intolerance predicting adverse outcomes in heart failure (Kusunose et al., 2017, Legris et al., 2022), in pulmonary hypertension, and in congenital heart disease including Tetralogy of Fallot patients (Apostolopoulou et al., 2007, Brili et al., 2008). The objectives of this study were to describe normal augmentation of RV systolic functional parameters in a population clearly defined not to have any form of heart or pulmonary disease and to compare with two populations of patients with predefined right ventricular disease (TOF with and without volume loading from pulmonary regurgitation). We also sought to describe to what extent CR relates to maximum predicted oxygen uptake (VO_2) obtained from coincident cardiopulmonary exercise (CPET) testing in both scenarios.

In this chapter we have described the normal ranges for RV augmentation in a verifiably healthy population which are much higher than in patients with a condition known to compromise RV function, repaired TOF. RV augmentation was also greater in men than in women. We found no association between RV augmentation and functional capacity in healthy individuals, whereas in those with disease there was a significant association, suggesting that the contribution of RV function may be more important in the diseased population than in healthy subjects. Finally, we have verified that, using our newly developed parameter, that RV efficiency slopes, in those with significant disease, can predict functional capacity.

Contractile reserve of the left ventricle has repeatedly been shown to predict symptoms and prognosis (Waddingham et al., 2018, McIntosh et al., 2013), and exercise ability. The ventricular reserve of the right ventricle in response to physical exercise or pharmacological stress still requires better description, lacking both agreed measures and normal ranges. The best RV measures to assess contractile reserve in healthy individuals are not agreed. In our study, we selected three well validated measures describing predominantly long axis (TAPSE and RVS') and one more global index to assess RV contractile reserve (FAC). These measures have a high degree of validity and in practical terms can be measured with relative ease at all levels of exercise stress. We found that in healthy adults, FAC increased by 32±10%, while TAPSE and S' increased by 48±15% and 60±20%, respectively. Given that this population was predefined as normal, we are confident that this represents a normal reference range for these measures. A recent comparable study showed lower TAPSE and RVS' augmentation, with similar FAC contractile reserve. This population was older with mean age of 82y who are at risk of cardiovascular symptoms and arrythmias which could explain the observed difference (Pergola et al., 2021).

Taking our normal population as a comparator, our TOF population had both impaired resting RV functional parameters but also markedly abnormally contractile reserve. This is comparable to few reports in TOF population who have focused on the correlation between exercise predictors rather than evaluation of each parameter response to exercise (Kingsley et al., 2018, Apostolopoulou et al., 2007, Ait-Ali et al., 2014, Bhatt et al., 2019b). RV CR was higher in the unloaded-MPR group when compared to the loaded-SPR group, and this could be explained by the dynamic interactions between PR and the presence of severe volume overload in the SPR group, which prevent further RV CR augmentation provoked by the stress.

The relationship between RV functional parameters measured at peak exercise capacity in patients with heart failure and exercise capacity has been previously described but not to date in healthy subjects (Sljivic et al., 2018, Di Salvo et al., 1995, de Groote et al., 1998, Ben-Gal et al., 2000, Hacker et al., 2003, Witte et al., 2004, Rubiś et al., 2010). The results were inconsistent in finding a relationship between RV measures during exercise and functional capacity in the heart failure population (de Groote et al., 2004, Hacker et al., 2003, Rubiś et al., 2010, Witte et al., 2004), and in patients with dilated cardiomyopathies (Salerno et al., 2011). In our TOF population, we found significant correlations between exercise capacity and all RV longitudinal indices including FAC, RVS' and TAPSE, while in a healthy group, we found no significant relationships between all RV contractile reserve parameters, suggesting that FAC, TAPSE, and RVS' are not major contributors of exercise capacity in a normal population unlike LV contractile reserve (Waddingham et al., 2018, van Zalen et al., 2019, McIntosh et al., 2013). This suggests that the RV contribution to functional capacity is important when there is a pathological RV.

Evaluation of the relationship between RV myocardial augmentation and exercise performance is novel. For LV, this has been proved with LV systolic augmentation that could predict exercise capacity in healthy adults (van Zalen et al., 2021). We have also shown this in the TOF population which was described in chapter 5. Systolic efficiency slope (SES) (with LVS'), and the novel, myocardial efficiency slope (MES) (with LVGLS), are powerful predictors of exercise capacity in this population. Due to our close relationship of RV functional indices and VO_2, we have explored RV longitudinal absolute augmentation and VO_2 throughout exercise and their relationship to peak VO_2 in a healthy and in a TOF population. We have created a new slope, named longitudinal efficiency slope (LES) for RV function, which describes for the first time the incremental relationships between RVS', TAPSE, and FAC to VO_2 during exercise, that could predict VO_2 (the amount of RV LES augmentation required to increase VO_2 by a single unit). We have shown that the increments of RVS' and TAPSE longitudinal velocity during exercise was predictive of exercise capacity in the TOF population while they are not in healthy subjects. This is an important finding which mirrors our RV correlation analysis described above, and this highlights the importance of RV function assessment during exercise echocardiography that could become a surrogate for VO_2 in a population with impaired RV function.

Although it is known that biventricular function might reduce with age, we did not find any association between RV contractile reserve parameters and age in healthy subjects, probably due to different age distributions in our populations and the relatively small number size. We found that RV contractile reserve parameters were affected by gender difference with higher RV contractile reserve in men compared to women, suggesting the importance of taking into account the impact of gender on RV contractile reserve. This was in keeping with one study on RV CR (D'Alto et al., 2017), and in few studies that were performed to assess LV contractile reserve in adults (Wang et al., 2012, Petre et al., 2007). Our study confirms that assessment of RV CR by FAC, RVS' and TAPSE is feasible when RV function is normal or impaired. We proposed an expected range of normal RV exercise-indices of systolic functional changes and contractile reserve for the first time by ΔFAC, ΔTAPSE, and ΔRVS' that seem to be gender related, but not explained by size. We found that RV CR does not contribute to functional capacity in normal individuals, while it is more important when RV function dwindles. We have also described new RV exercise biomarker (LES), longitudinal efficiency slopes for all RV longitudinal functional indices which predict functional capacity when RV function is abnormal. The changes of RV LES slopes during exercise should mirror symptomatic improvement in the TOF population and, therefore we recommend measuring this exercise biomarker in patients with pathological RV.

8.6 Study limitations

The major limitation of this study was the relatively small number of healthy adults and single-centre observational study. We have not assessed 2D RV strain due its technical acquisition difficulties during exercise at very high heart rates which could affect the accuracy of tracing. We did not formally take account of RV/PA coupling, as RV CR may reflect the right ventricular-arterial coupling rather than just measures of RV contractility, and any deterioration in RV CR can reflect RV-arterial impairment (La Gerche et al., 2012). The insufficient response of RV CR to exercise load could result in RV-arterial deterioration during exercise in patients with impaired RV function (Argiento et al., 2010). We have not assessed the prognosis of these finding as there was no follow up data. However, this was an exploratory study to determine the potential utility of RV conventional systolic functional measures to assess RV contractile reserve during stress echocardiography. Future studies should consider assessing markers of RV contractile reserve with more advanced echocardiographic strain imaging linked to its prognosis in different right heart diseases.

8.7 Conclusions

This chapter describes a range of normal values for RV augmentation which are obtainable on a standard stress echocardiogram, these parameters are markedly reduced in patients with TOF and further depressed by the presence of severe PR. RV augmentation and efficiency are an important determinants of exercise function in TOF patients but not in normal subjects.

Chapter 9 Blood biomarkers in patients with repaired Tetralogy of Fallot (rTOF); A systematic review and meta-analysis

This chapter is based on the peer reviewed publication below:

Alborikan, S., Von Klemperer, K., Bhan, A., Walker, F., Pandya, B., Badiani, S., Bhattacharyya, S., Petersen, S.E. and Lloyd, G., 2021. Blood biomarkers in patients with repaired Tetralogy of Fallot (rTOF); A systematic review and meta-analysis. International Journal of Cardiology Congenital Heart Disease, 6, p.100237.

My contribution was performing all comprehensive research of literature, statistical analyses, meta-analyses, quality assessment and writing the whole manuscript.

9.1 Abstract

Background: The clinical use and prognostic value of plasma brain natriuretic peptide (NT-proBNP) and soluble suppression of tumorigenicity-2 (sST2) levels are not known in patients with repaired Tetralogy of Fallot (rTOF).

Objectives: We evaluated blood biomarkers in rTOF patients by combining the available evidence, focussing on prognosis, adverse echocardiographic findings and exercise intolerance.

Methods: This systematic review and meta-analysis were carried out in accordance with the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines. For the primary prognostic outcomes, a meta-analysis was performed. For haemodynamic outcomes, a pooled meta-analysis of correlation coefficients (r) was performed. The study protocol was registered with PROSPERO (CRD42020211897).

Results: We analysed 1479 patients with repaired TOF in 23 studies. Mean age was 22.7±8.3 years. The mean value of NT-proBNP was 174.4.1±56.4 pg/ml while sST2 drawn from two investigations was 26.95 ng/ml. There was no difference in mean NT-proBNP between the older and younger subjects (160.4±37.7 vs 190.6±72.9, pg/ml, respectively; $p>.05$). NT-proBNP levels were higher in studies with transannular patch than others with other RVOT intervention (191.6± 57 vs 151±46, pg/ml, $p<.05$). Elevated NT-proBNP levels were associated with an increased risk of adverse cardiovascular outcomes including death, arrhythmias and acute heart failure with a hazard ratio (HR) of 1.18 (95% CI 1.07-1.31, p=.001). We noted a moderate correlation between NT-proBNP levels and exercise intolerance, RV structural and volumetric changes (r= -.52, r=.41, $p<0.001$).

Conclusions: NT-proBNP levels are elevated in patients with surgically repaired TOF and are associated with an increased risk of cardiovascular adverse outcomes and exercise intolerance.

9.2 Introduction

Despite 90% long term survival in patients with repaired TOF (rTOF), an increasing number of late complications are present such as right and left ventricular dysfunction, arrhythmia and exercise intolerance related to residua which dictate the need for reintervention (Gatzoulis et al., 2000, Geva et al., 2004, Hickey et al., 2009). The degree of pulmonary regurgitation and its relationship to right ventricle (RV) remodelling and symptoms is variable but, in some cases, leads to the development of life-threatening atrial and, ventricular arrhythmias and sudden cardiac death (Geva et al., 2004, Abd El Rahman et al., 2000).

Clinical management is aimed at identifying patients at risk who need intervention. Symptoms are a poor guide to selection as the majority of adult survivors of TOF are asymptomatic (Hickey et al., 2009, Diller et al., 2012). Using blood biomarkers such as plasma brain natriuretic peptide (NT-proBNP) and soluble suppression of tumorigenicity-2 (sST2) is an emerging adjunct to the existing tools to predict disease progression and potentially could help identify appropriate patients for intervention. NT-proBNP is known to be elevated in asymptomatic patients with repaired TOF compared to other types of congenital heart disease (CHD) (Apitz et al., 2009, Mir et al., 2005, Norozi et al., 2005, Tulevski et al., 2001, Khositseth et al., 2007), however, clear data about the usefulness in diagnostics and prognostication in this population is not yet available.

In order to better understand the relationship between NT-proBNP and sST2 in patients with repaired TOF, we undertook this systematic review and meta-analysis to combine the available evidence, focusing on their prognostic value and their association with adverse haemodynamic echocardiographic findings.

9.3 Methods

9.3.1 Systematic review and meta-analysis

The systematic review and meta-analysis were carried out in accordance with the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines. The protocol of this study was previously published and registered in the international Prospective Register of Systematic reviews (PROSPERO) (CRD42020211897). The flow diagram in figure 9.1 demonstrates the literature search based on the PRISMA guidelines.

9.3.2 Inclusion and exclusion criteria

This review was limited to studies published in English. Inclusion criteria were (a) prospectively or retrospectively conducted cohort, cross sectional, single and multicenter studies; (b) Blood biomarkers included in methodology and; (c) adult and adolescent patients with repaired TOF. Studies excluded from the review were those (a) without blood biomarkers data; (b) not published in English and; (c) non rTOF.

9.3.3 Information sources and search strategy

A comprehensive retrospective search of the literature was conducted using databases including MEDLINE, Pubmed, EBM review-Cochrane Database of systematic review, Wiley Online library and EBM reviews, utilising a combination of the following search keywords: “blood biomarkers in Tetralogy of Fallot’”, “NT-proBNP in TOF”, “sST2 in adult survivors of TOF “,’’ Pro-brain natriuretic peptide in CHD’’, “Severe pulmonary regurgitation and blood biomarkers”. There was no limitation on age. The search included all studies between 1988 and 2020.

9.3.4 Study selection and eligibility criteria

The following steps were performed (Figure 9.1). (1) identification of titles through database searching. (2) removal of duplicates. (3) titles and abstracts screening. (4) full text sources for further screening. (5) studies which gave outcomes were selected for quantitative analysis. The primary end points of the study were (a) levels of NT-proBNP in patients with repaired TOF; (b) prognostic value of NT-proBNP to composed major cardiovascular outcomes (MACE), defined as the occurrence of acute heart failure, arrhythmias or death from any cause. The secondary end points were (a) association of elevated NT-proBNP levels with haemodynamic echocardiographic changes and to exercise capacity into pooled meta-analysis.

9.3.5 Data extraction

Data collected by one reviewer (Sahar Alborikan, S.A) who determined the eligibility of the studies, according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines, and verified by a second expert reviewer (Guy Lloyd, G.L). To prevent bias, the screening was performed independently. The following data were extracted: first author's name, country, year of publication, number of patients included, study design, age, plasma NT-proBNP levels, sST2 when available, correlation coefficients (r) and the conclusion together with main findings of the study. For prognostic analysis, hazard ratios, outcomes measure (mortality or major adverse cardiovascular outcomes), and follow up duration were collected.

9.3.6 Statistical analysis

Studies were divided into (i) those that reported in an adult population and (ii) those reported in a population younger than 18 years old. For the continuous outcome variables, data were presented as mean differences (MD)±standard deviation. Statistical analyses for the primary results were conducted using SPSS statistics version 26 (IBM corp, London, United Kingdom). For the prognostic studies, a comprehensive meta-analysis was performed using Review Manager (version 5.4, Copenhagen: The Nordic Cochrane Centre, The Cochrane

Collaboration, 2012). The generic inverse variance method was used to combine log hazard ratios (log HR) and standard errors of the log HR (SElogHR). A fixed-effect model was used to pool the log hazard ratios and 95% confidence interval for all-cause mortality in each study.

For the secondary outcomes, a pooled meta-analysis of correlation coefficients (r) (random effects) using MedCalc (MedCalc software, Belgium, Version 19.6.4) was performed. We pooled the values of correlation coefficients and number recruited in each study. The appraisal of the heterogeneity among studies was conducted via the Q statistics and I^2 statistics, with a value of 0%–24.9% considered insignificant, 25%–49.9% mild, 50%–74.9% moderate, and ≥75% considered severe (Higgins et al., 2003). All p values were two tailed, and the statistical significance was set at <0.05.

9.3.7 Quality assessment

The methodological quality of the individual studies was evaluated using the Newcastle-Ottawa Scale (NOS) (Wells et al., 2000). Each study was judged on eight items, categorised into three groups: cohort selection, the comparability of the groups and the outcome. Stars were awarded for each item, with a maximum score of 9. It assigns a maximum of 4 stars for selection, 2 stars for comparability, and 3 stars for exposure/outcome assessment. Studies with <5 stars were considered low quality, 5–7 stars moderate quality, and >7 stars high quality (Table 9.1). The quality assessment showed an acceptable overall quality, risk of bias and applicability concerns.

9.3.8 Publication bias

We assessed publication bias by visual inspection of the funnel plot of all included studies. The absence of any asymmetric distribution suggested there was no publication bias. In the subgroup analysis of the comprehensive correlation meta- analysis, Begg's test and Egger's test were used to assess the possibility of publication bias in our analysis and any p value <0.05 was considered a significant publication bias.

9.4 Results

9.4.1 Literature search outcomes

The literature search identified a total of 300 potentially eligible studies. After the exclusion of irrelevant studies, 224 were screened thoroughly through abstract and/or full text. 99 articles were excluded due to duplication. Of the remaining 125 articles, 50 articles were excluded, as they did not meet the inclusion criteria. Full text article assessment was performed for the remaining 75 articles and 52 articles were further excluded due to unavailability of the full text, leading to a total of 23 articles included in this review. Figure 9.1 shows the PRISMA flow chart for the systematic selection of studies during the literature search.

9.4.2 Characteristics of selected studies

Of the 23 articles, 13 (57%) reported adult survivors of rTOF (Geenen et al., 2019, Laqqan et al., 2018, Heng et al., 2014, Westhoff-Bleck et al., 2016, Menting et al., 2015, Eindhoven et al., 2014, Zatocil et al., 2007, Oosterhof et al., 2006, Norozi et al., 2006, Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006, Luijnenburg et al., 2013), while 10 studies (43%) were reported on a younger group (Kitagawa et al., 2015, Pietrzak and Werner, 2015, Koch et al., 2010, Valverde et al., 2015, Tatani et al., 2010, Apitz et al., 2009, Khositseth et al., 2007, Dodge-Khatami et al., 2006, Cheung et al., 2007, van den Berg et al., 2009). 4 studies (17%) were cross-sectional studies (Luijnenburg et al., 2013, Eindhoven et al., 2014, Oosterhof et al., 2006, Festa et al., 2007), and 19 studies (83%) were cohort studies (Geenen et al., 2019, Laqqan et al., 2018, Heng et al., 2014, Westhoff-Bleck et al., 2016, Menting et al., 2015, Zatocil et al., 2007, Norozi et al., 2006, Norozi et al., 2005, Trojnarska et al., 2006, Kitagawa et al., 2015, Pietrzak and Werner, 2015, Koch et al., 2010, Valverde et al., 2015, Tatani et al., 2010, Apitz et al., 2009, Khositseth et al., 2007, Dodge-Khatami et al., 2006, Cheung et al., 2007, van den Berg et al., 2009).

Of those, 18 studies (95%) were conducted prospectively (Geenen et al., 2019, Laqqan et al., 2018, Heng et al., 2014, Westhoff-Bleck et al., 2016, Menting et al., 2015, Norozi et al., 2006, Zatocil et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006, Kitagawa et al., 2015, Pietrzak and Werner, 2015, Valverde et al., 2015, Tatani et al., 2010, Apitz et al., 2009, Khositseth et al., 2007, Dodge-Khatami et al., 2006, Cheung et al., 2007, van den Berg et al., 2009) and one study retrospectively (Koch et al., 2010). All of the included studies reported NT-proBNP and only 2 studies included sST2 in their methodology (Geenen et al., 2019, Laqqan et al., 2018). 10 articles (43%) reported the association of NT-proBNP with echocardiographic haemodynamic and structural changes (Eindhoven et al., 2014, Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006, Pietrzak and Werner, 2015, Koch et al., 2010, Valverde et al., 2015, Tatani et al., 2010, Khositseth et al., 2007, Cheung et al., 2007). 4 articles (17%) reported the association of NT-proBNP and exercise capacity (Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006, Cheung et al., 2007). 3 articles (13%) reported the prognostic value of NT-proBNP for adverse outcomes (Laqqan et al., 2018, Heng et al., 2014, Westhoff-Bleck et al., 2016) and 2 articles investigated the levels of NT-proBNP following pulmonary valve replacement (PVR) (Kitagawa et al., 2015, Dodge-Khatami et al., 2006). Sample size ranged from 16 to 177, and the publication year ranged from 2005 to 2019. Mean age of included participants ranged from 12 to 35 years. All included studies were single centre with different designs. Baseline characteristics of the included studies are shown in table 9.1.

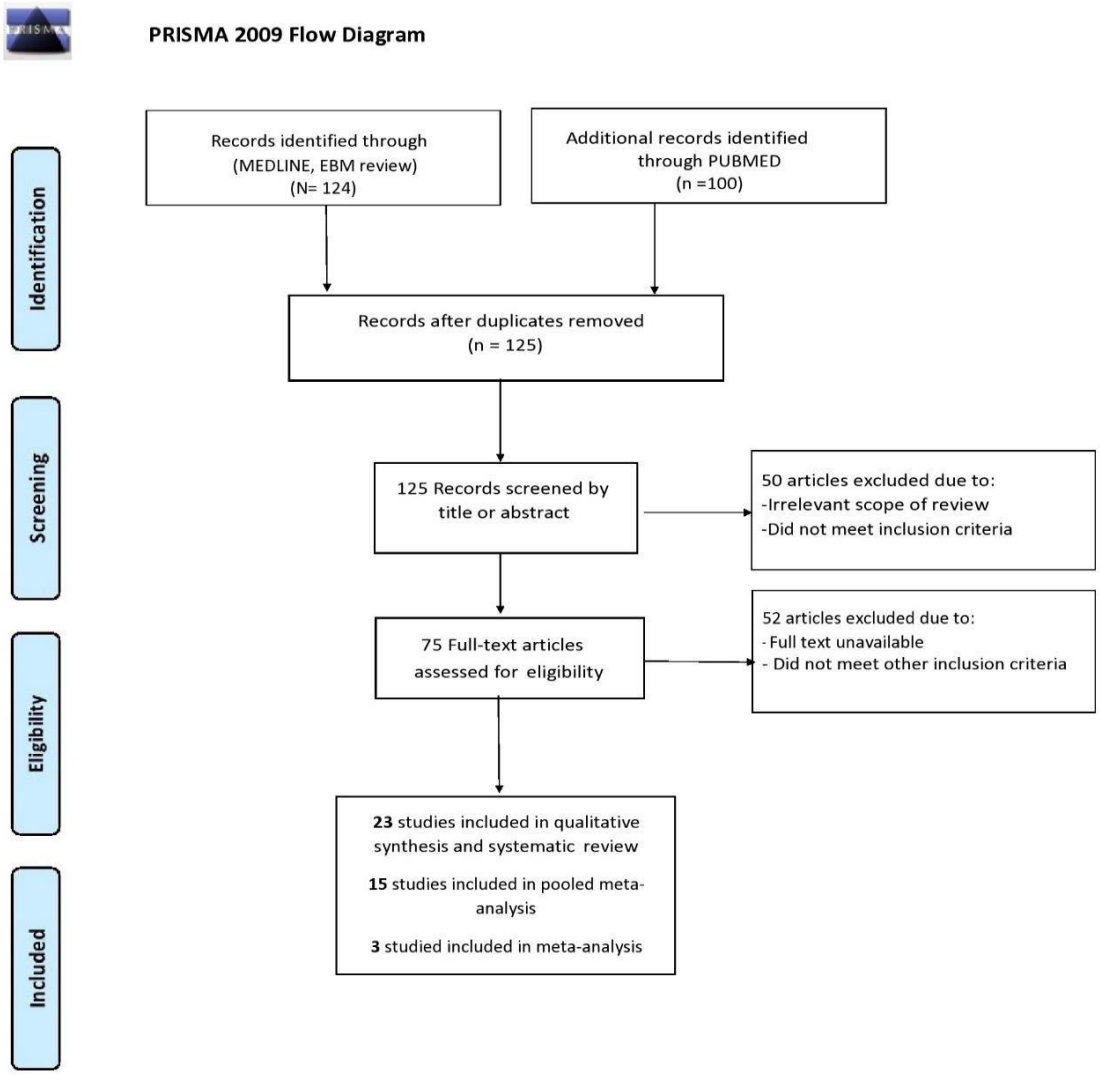


Figure 9. 1. The PRISMA flow chart displaying the selection of studies and reasons for exclusion.

Table 9.1. Study characteristics of the included studies, results and the quality assessment.

Author	Country	Study type	N*	Age, years	NT-proBNP pg/ml	sST2, ng/ml	Clinical Outcomes	Quality assessment
Geenen et al., 2019	Netherlands	Prospective cohort	176	33(25-41)	141.45	24	-ST2 was associated with adverse cardiovascular events. -No association between ST2 and NT-proBNP levels	Moderate quality
Laqqan et al., 2018	Germany	Prospective cohort	61	28.2±12	164	29.9	-ST2 levels is significantly elevated in patient with CHD -Elevated NT-proBNP and ST2 levels are strong predictors of all-cause mortality in complex CHD	Moderate quality
Westhoff-bleck et al., 2016	Germany	Prospective cohort	81	26.3±7.4	168±148	n/a	-NT-proBNP levels and pulmonary regurgitation were strong predictors of adverse outcomes in asymptomatic patients -NT-proBNP levels were associated with LV parameters and not with RV	Moderate quality
Kitagawa et al., 2015	USA	Prospective cohort	33	14.5±2.8	71.4±46.1	n/a	-Elevated NT-proBNP levels were reduced after PVR	Poor quality
Pietrzak et al., 2015	Poland	Prospective cohort	52	13.7±3.42	286±269.2	n/a	-Elevated NT-proBNP levels were associated with right ventricular function and exercise intolerance. -Elevated NT-proBNP levels were associated with exercise intolerance	Moderate quality
Valverde et al., 2015	Spain	Prospective Cohort	40	14.3±6.7	175±109	n/a	-NT-proBNP levels were associated with RV dilatation and PR -NT-proBNP >145 pg/ml could predict the presence of RV volume overload and dilatation	Poor quality
Menting et al., 2015	Netherlands	Prospective cohort	94	32.8±9.5	124±221	n/a	-Elevated NT-proBNP level, -No relationship between NT-proBNP and exercise capacity	High quality
Heng et al., 2014	United Kingdom	Prospective cohort	90	32.7±11.3	147 ±254	n/a	-Elevated levels of NT-proBNP in asymptomatic TOF patients. - NT-proBNP >132 pg/ml was a predictive value of mortality	Moderate quality
Eindhoven et al., 2014	Netherlands	Cross sectional	177	34.6±11.8	151±317	n/a	-Elevated NT-proBNP levels were associated with LV dysfunction, RVD and significant PR -NT-proBNP levels were not associated with exercise capacity	Moderate quality
Luijnenburg et al., 2013	Netherlands	Cross sectional	51	21±8	132±94	n/a	-Elevated NT-proBNP levels were associated with RV dysfunction and diastolic dysfunction	Poor quality
Koch et al., 2010	Germany	Retrospective cohort	130	16.1±7.1	200 ±110	n/a	-Elevated NT-proBNP levels. -Elevated NT-proBNP levels were correlated with right ventricular volume, severe PR and exercise capacity	High quality
Tatani et al., 2010	Brazil	Prospective cohort	49	14.7	211±219	n/a	-Elevated NT-proBNP levels were associated with RVD, diastolic dysfunction and severity of PR	Moderate quality
Apitz et al., 2009	Germany	Prospective cohort	16	14.2	179±396	n/a	-Elevated NT-proBNP levels	Poor quality

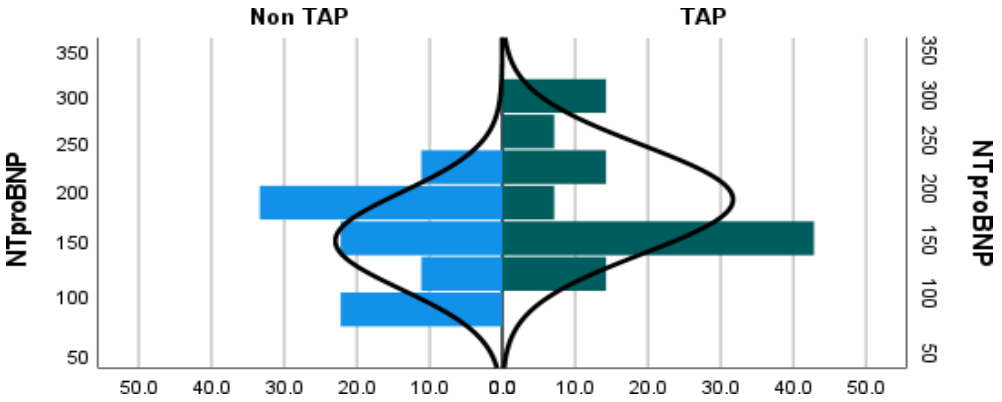
Zatocil et al., 2007	Czechs	Prospective Cohort	21	35	168±92	n/a	-Elevated NT-proBNP levels in asymptomatic adult survivors of TOF	Moderate quality
Khositseth et al., 2007	Thailand	Prospective cohort	21	12.1±2.5	295.75±389.11	n/a	-Elevated NT-proBNP levels were associated with RVD, RVEDV and dysfunction -NT-proBNP>115 pg/ml could be used as a marker in the detection of RV dilatation and dysfunction	Moderate quality
Oesterhof et al., 2006	Netherla nds	Cross sectional	42	30 (17-57)	108±133	n/a	-Elevated NT-proBNP levels were associated with RV volume overload and severity of PR	Moderate quality
Khatami et al., 2006	Switzerl and	Prospective cohort	23	13.2	231±228	n/a	-Elevated NT-proBNP levels in asymptomatic patients with chronic PR. -NT-proBNP levels were significantly reduced after 6 months post PVR	Poor quality
Norozi et al., 2006	Germany	Prospective cohort	59	30±8	150±141	n/a	-NT-proBNP levels could be replaced (Vo_2, %) max to re-stratify CHD patients with impaired cardiac function	Moderate quality
Exercise investigations								
Berg et al., 2009	Netherla nds	Prospective Cohort	51	15(7-26)	94.3	n/a	-Normal NT-pro BP levels with preserved exercise capacity	Poor quality
Festa et al., 2007	Italy	Cross-sectional study	70	21±1	218±283	n/a	-Elevated NT-proBNP levels were associated with RVD, function and RVEDV and with exercise intolerance	Moderate quality
Cheung et al., 2007	China	Prospective cohort	32	14.7±3.1	154	n/a	-Elevated NT-proBNP levels were associated with RVD, RVEDV, PR and with exercise intolerance	Moderate quality
Trojnarska et al., 2006	Poland	Prospective cohort	60	27.6±8.2	250±200	n/a	-Elevated NT-proBNP levels were associated with exercise intolerance	Moderate quality
Norozi et al., 2005	Germany	Prospective cohort	50	27.8±1.7	164±23	n/a	-Elevated NT-proBNP levels were associated with RVD, RVS' and with exercise intolerance	Poor quality

VO_2 = maximum predicted oxygen uptake; PVR= pulmonary valve replacement; PR= Pulmonary regurgitation; RVD= Right ventricular dimensions; RVEDV= right ventricular end diastolic volume; RVS'= right ventricular peak systolic velocity; N/A= not available.

9.4.3 Plasma brain natriuretic peptide level (NT-proBNP) in asymptomatic adult and adolescent patients with repaired TOF

Data was collected on 1479 patients with repaired TOF in 23 studies. Mean age in the total population was 22.7±8.3 years. Mean age of the older group was 29±4.5 years and the mean age of the younger group was 14±1.1 years. The mean value of NT-proBNP was 174.4.1±56.4 pg/ml, but ranged from 71.4 to 295 pg/ml. There was no significant difference in mean NT-proBNP between older and younger subjects (160.4±37.7 vs 190.6±72.9, pg/ml, respectively; $p>0.05$). Mean value of sST2 drawn from two investigations was 26.95 ng/ml (Table 9.2).

14 studies (61%) reported NT-proBNP in a population where a trans-annular patch (TAP) was used as a surgical approach (Laqqan et al., 2018, Luijnenburg et al., 2013, Westhoff-Bleck et al., 2016, Menting et al., 2015, Eindhoven et al., 2014, Zatocil et al., 2007, Norozi et al., 2006, Festa et al., 2007, Trojnarska et al., 2006, Pietrzak and Werner, 2015, Tatani et al., 2010, Khositseth et al., 2007, Dodge-Khatami et al., 2006, Cheung et al., 2007). 9 studies (39%) were reported in a population with other RVOT intervention, such as pulmonary valvotomy or infundibulectomy and/or no reports (Geenen et al., 2019, Heng et al., 2014, Oosterhof et al., 2006, Norozi et al., 2005, Kitagawa et al., 2015, Koch et al., 2010, Valverde et al., 2015, Apitz et al., 2009, van den Berg et al., 2009). Mean NT-proBNP levels were higher in TAP studies than others (191.6± 57 vs 151±46, pg/ml, $p<0.05$) (Figure 9.2).



TAP = studies with transannular patch; non-TAP = studies with other type of RVOT intervention such as pulmonary valvotomy or infundibulectomy and/or no reports.

Figure 9. 2. Difference in mean NT-proBNP between TAP and non-TAP studies.

Table 9. 2. Baseline characteristic of population included.

Baseline characteristics	**N=1479 patients**	**Older (13 studies)**	**Younger (10 studies)**	**P value**
Age± SD, years	22.7±8.3	29±4.5	14±1.1	**<0.05**
NT-proBNP, pg/ml	174.4.1±56.4	160.4±37.7	190.6 ±72.9	>0.05
sST2, ng/ml	26.95*	n/a	n/a	n/a
Surgical type	**TAP (14)**	**Non-TAP (9)**	**-**	**P value**
NT-proBNP, pg/ml	191.6± 57	151±46		**<.05**

TAP= Transannular patch; N/A = Not available. *A value of sST2 drawn from two investigations.

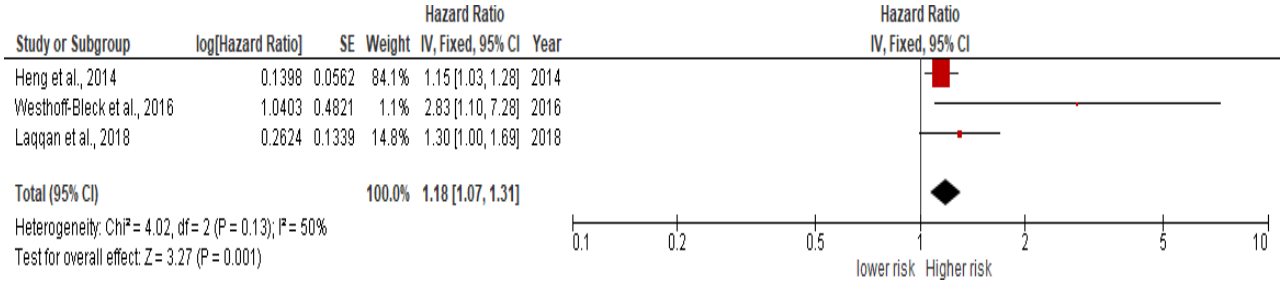
9.4.4 Meta-analysis on the effect of NT-proBNP on cardiovascular outcomes

The prognostic primary outcomes of all-cause mortality, heart failure and sustained ventricular arrhythmias occurred in 113 patients, as reported in table 9.3. Three studies (13%) in asymptomatic adult populations investigated the relationship between NT-proBNP and cardiovascular outcomes. The mean NT-proBNP drawn from these investigations was 243± 113 pg/ml. Heng et al demonstrated that NT-proBNP levels were significantly related to all-cause mortality with the longest mean observation time of 10 years among the studies (HR 1.15, 95% CI 1.03-1.28, p<.01). In univariate Cox analysis, the best cut off value of NT-proBNP level to predict all-cause mortality was 147 pg/ml (AUC .68, p=.04) (Heng et al., 2014).

In the second study, elevated NT-proBNP levels were the strongest predictor of other adverse outcomes such as sustained ventricular arrhythmias and heart failure, with a mean observation time of 6.9 years (HR 2.83, 95% CI 1.1-7.28, p<.029). In multivariate Cox analysis, the best cut off value of NT-proBNP level to predict adverse clinical events was 232 pg/ml (AUC .873, p=.004) (sensitivity of 76.9%, specificity of 85.3%) (Westhoff-Bleck et al., 2016).

In the last study, Laqqan et al reported that elevated NT-proBNP levels were significant predictor of all-cause mortality with a mean observation time of two years (HR 1.3, 95% CI 1.0-1.69, p<0.001) (Table 9.3). The authors constructed ROC curves demonstrating the best cut off value of NT-proBNP level to predict heart failure, which was 349.5 pg/ml (AUC .875, p=.001) (sensitivity of 71.4%, specificity of 88.9%) (Table 9.3) (Laqqan et al., 2018).

After an average 6.3 years of follow up, high NT-proBNP levels were associated with an increased risk of cardiovascular outcomes including death, arrhythmias and acute heart failure with a hazard ratio (HR) of 1.18 (95% CI 1.07-1.31, p=.001) (Figure 9.3).



Data are displayed as HR (95% CI). Heterogeneity as reported by I^2 (I^2= 50%, p=.13).

Figure 9. 3. Forest plot of the hazard ratios of high NT-proBNP values with cardiovascular outcomes in patients with repaired TOF.

Table 9. 3. Studies included in meta-analysis.

Author	Cohort size	Age (years)	NT-proBNP pg/ml	Follow up (years)	Cardiovascular events
Heng et al., 2014	90	32+5	147±254	10 (.7-12.4)	**83** **7 deaths:** **2** sudden cardiac death **2** perioperative right ventricular failure **1** severe Aortic stenosis **1** respiratory sepsis **1** unknown
Weshoff-Bleck et al., 2016	81	26.3+7.4	232±175	6.9+2.6	**13** sustained supraventricular arrhythmias or heart failure
Laqqan et al., 2018	61	28.2+12	349.5	2.11+2	**17** **Major adverse cardiac events**, defined as the occurrence of acute heart failure or death from any cause

9.4.5 Meta-analysis of the association between NT-proBNP and haemodynamic echocardiographic changes

The relationship between NT-proBNP and RV structural, functional and volumetric changes was investigated in 10 (43%) articles. Of these 10 articles, 4 (40%) were reported in the older group, while 6 (60%) were reported in the paediatric group. Most of these articles demonstrated that elevated NT-proBNP levels were associated with either RV dilatation, higher RV end diastolic volumes, RV systolic dysfunction and with severity of pulmonary regurgitation or diastolic dysfunction (Eindhoven et al., 2014, Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006, Pietrzak and Werner, 2015, Koch et al., 2010, Valverde et al., 2015, Tatani et al., 2010, Khositseth et al., 2007, Cheung et al., 2007). Only three studies (13%) evaluated the association between raised levels of NT-proBNP and left ventricular parameters. Higher NT-proBNP levels were associated with LV dysfunction (Westhoff-Bleck et al., 2016, Eindhoven et al., 2014), with LV indexed volume (Westhoff-Bleck et al., 2016), and with LV indexed dimensions (Cheung et al., 2007). Two studies proposed a cut-off NT-proBNP values of 145 pg/ml that could predict the presence of RV dilatation (ROC .95, sensitivity 71%, specificity 100%) (Valverde et al., 2015), and a value of 115 pg/ml (ROC .87, sensitivity 71%, specificity 78%) that could predict the presence of RV dilatation and/or dysfunction (Khositseth et al., 2007).

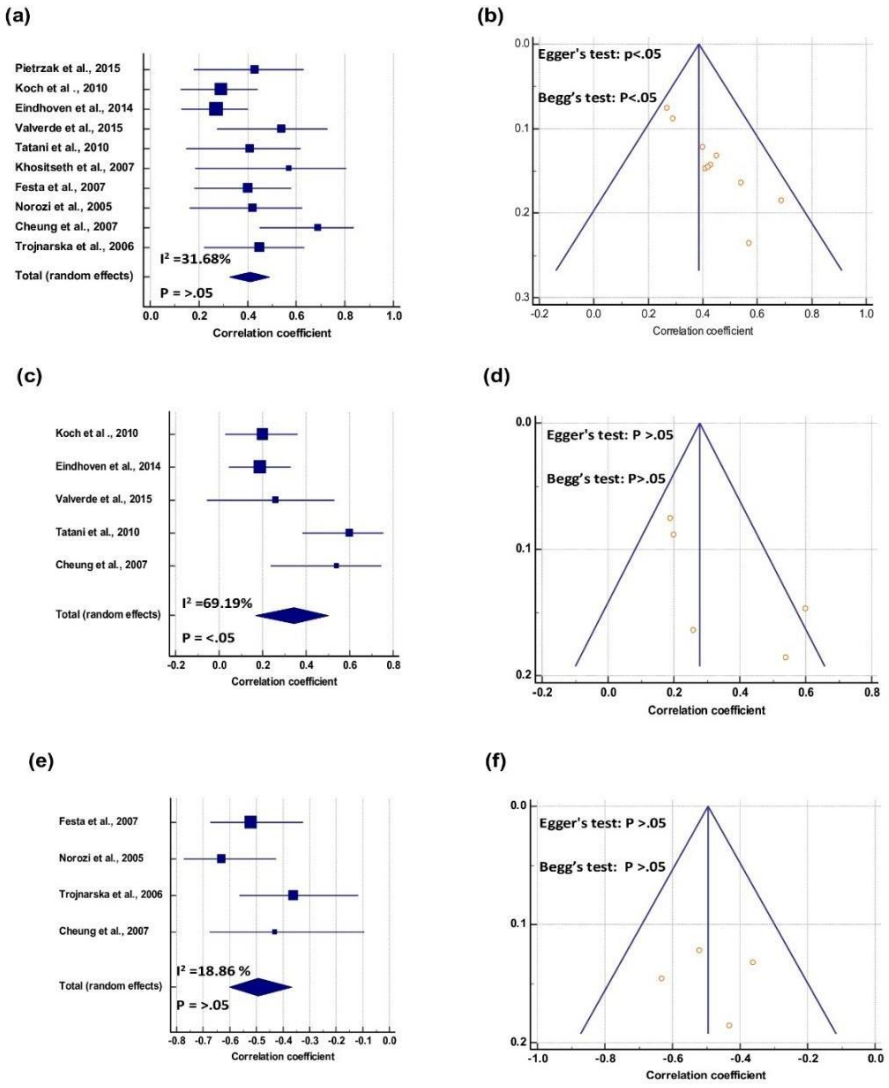
The meta- analysis showed a statistically significant association of elevated NT-proBNP levels to RV size and right ventricular end diastolic volume (RVEDV). The weighted average (random effects) correlation coefficient among 10 studies was .41 (95% CI .32-.48, p<.001) (Table 9.4). The study with the highest correlation was by Cheung et al., 2007 (r=.69, 95% CI .44-.83, p<.001), however, this study was one of the lowest weighted studies included in the analysis, with 31 participants (Cheung et al., 2007). Figure 9.4a shows the forest plot of pooled correlation coefficients. Visual evaluation of the forest plot suggests that there is a mild degree of heterogeneity which confirmed by I^2 (values of 31.7%, p=.15). We observed publication bias by Begg's test (p<.05) or Egger's test (p<.05) (Figure 9.4b).

There was a strong association between NT-proBNP and the severity of pulmonary regurgitation. The pooled analysis showed statistically significant association of elevated NT-proBNP levels to severity of pulmonary regurgitation in 5 studies (50%) (Eindhoven et al., 2014, Koch et al., 2010, Valverde et al., 2015, Tatani et al., 2010, Cheung et al., 2007). The weighted average (random effects) correlation coefficient was .34 (95% CI .16-.50, p<.001). Figure 9.4c shows the forest plot of pooled correlation coefficients. Visual assessment of the forest plot suggests a modest degree of heterogeneity, which confirmed by I^2 (values of 69.2 %, p<.05). We observed no publication bias by Begg's test (p>.05) or Egger's test (p>.05) (Figure 9.4d).

9.4.6 Meta-analysis of the association between NT-proBNP and reduced exercise capacity in patients with repaired TOF

The relationship between NT-proBNP and exercise intolerance was investigated in 4 articles (17%) (Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006, Cheung et al., 2007). Of these 4 articles, 3 (75%) were reported in adults (Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006), while 1 article was reported in paediatrics (Cheung et al., 2007). All 4 studies supported the relationship between increased NT-proBNP levels and exercise intolerance in asymptomatic patients with repaired TOF (Table 9.4).

The pooled analysis showed a statistically significant association of elevated NT-proBNP levels and reduced exercise capacity in this population. The weighted average (random effects) correlation coefficient among 4 studies was -.54 (95% CI -.60- -.36, p<.001) (Table 9.4). Figure 9.4e shows the forest plot of pooled correlation coefficients. Visual evaluation of the forest plot suggests that there is insignificant degree of heterogeneity which confirmed by I^2 (values of 18.9 %, p>.05). We observed no publication bias by Begg's test (p>.05) or Egger's test (p>.05) (Figure 9.4f).



(a) Forest plot of the 10 studies evaluated in meta-analysis for the assessment of the association between NT-proBNP levels and RV size and RVEDV. **(b)** Funnel plot of all the 10 studies, where there is publication bias. **(c)** Forest plot of the 5 studies evaluated in meta-analysis for the assessment of the association between NT-proBNP levels and PR. **(d)** Funnel plot of all the 5 studies, where there is no publication bias. **(e)** Forest plot of the 4 studies evaluated in meta-analysis for the assessment of the association between NT-proBNP levels and VO_2 max. **(f)** Funnel plot of all the 4 studies, where there is no publication bias.

Figure 9. 4. A pooled meta-analysis in 10 studies.

Table 9. 4. Studies included into pooled meta-analysis of the correlation coefficients.

Study	N number	Correlation coefficients, r (95% CI)	P.value	Association conclusions
Pietrzak et al., 2015	52	r= -.43 (0.178-0.629) *	p<.001	-With RVD and RVS'
Koch et al., 2010	130	r= 0.29 (0.124-0.440) * r= 0.20 (0.0288-0.360) **	p<.001	-With RVD and severity of PR
Valverde et al., 2015	40	r= 0.54 (0.275-0.729) * r= 0.26 (-0.0561-0.529) **	p<.001	-With RVD and severity of PR
Westhoff-bleck et al., 2016	81	r= -.412**** r= 0.019 *****	p<.05	-With LV dysfunction and not with RV structural changes -With LVEDVI
Eindhoven et al., 2014	177	*r* = 0.27 (0.128-0.402) * *r* = 0.19 (0.0437-0.328) ** *r* = - 0.367****	p<.001	-With RVD and severity of PR -With LV dysfunction
Tatani et al., 2010	49	r = 0.41 (0.146-0.620) * r = 0.60 (0.384-0.754) **	p<.001	-With RVD, diastolic dysfunction and severity of PR
Khositseth et al., 2007	21	r = 0.57 (0.183-0.804) *	p<.001	-With RVD, RVEDV and dysfunction
Festa et al., 2007	70	r = 0.40 (0.182-0.580) * r = - 0.52 (-0.673- -0.325) ***	p<.001	-With RVD, systolic function and RVEDV -With exercise capacity
Norozi et al., 2005	50	r = .42 (0.160-0.625) * r = -.63 (-0.773- -0.426) ***	p<.001	-With RVD, RVS'. -With exercise capacity
Cheung et al., 2007	32	r = 0.69 (0.449-0.837) * r = 0.54 (0.236-0.748) ** r = -0.43 (-0.677- -0.0956) *** r = .47, p.006******	p<.001	-With RVD, RVEDV and severity of PR -With exercise capacity -With LV Indexed dimensions
Trojnarska et al., 2006	60	r = .45 (0.221-0.632) * r = - 0.36(-0.563- -0.117) ***	p<.001	-With RVD -With exercise capacity
Pooled correlation coefficients	**-**	**-NT-proBNP & RV structural changes=.41 (95% CI .32-.48) * -NT-proBNP & severity of PR =.34 (95% CI .16-.50) ** -NT-proBNP & Vo_2 max= -.54 (95% CI -.60- -.36) *****	**p<.001**	**-Moderate correlation between NT-proBNP and RV structural and haemodynamic changes -Higher moderate correlation with reduced exercise capacity**

RVD=Right ventricular dimensions; RVS'=right ventricular peak systolic velocity PR= pulmonary regurgitation; LVEDVI= Left ventricular end diastolic volume index; RVEDV=right ventricular end diastolic volume; N/A= not available; *Correlation coefficient between NT-proBNP and RVD and RVEDV; ** Correlation coefficient between NT-proBNP and severity of pulmonary regurgitation; *** Correlation coefficient between NT-proBNP and Vo_2 max; **** Correlation coefficient between NT-proBNP and LV dysfunction; ***** Correlation coefficient between NT-proBNP and LV end diastolic volume index; ****** Correlation coefficient between NT-proBNP and LV indexed dimensions.

9.5 Discussion

This systematic review and meta-analysis demonstrate that NT-proBNP levels are generally elevated in asymptomatic patients with repaired TOF irrespective of age. It showed that NT-proBNP is associated with a range of echocardiographic changes including structural RV changes, RV volume, severity of pulmonary regurgitation and most importantly the deterioration in exercise capacity, clinical condition and mortality. We found that patients with a TAP surgical approach had higher values of NT-proBNP compared to other approaches, suggesting an adverse haemodynamic response.

Plasma brain natriuretic peptide concentration is known to be high in patients with repaired TOF with reference values previously reported as age and gender related (Apitz et al., 2009, Mir et al., 2005, Norozi et al., 2005, Tulevski et al., 2001, Khositseth et al., 2007, Koch et al., 2010, Baggen et al., 2017, Schwachtgen et al., 2005). In contrast in this meta-analysis, we found no difference in NT-proBNP levels between adults and adolescents (160.4 ±37.7 vs 190.6 ±72.9 pg/ml, $p>.05$), suggesting that other anatomical and functional factors, are more important in these patients. Unlike other pathologies such as LV systolic dysfunction, NT-proBNP levels appear to be linked more to RV volumes and surgical selection techniques than underlying patient characteristics. This mirrors the finding that reduced exercise tolerance has no relation to age, but to the surgical selection (Alborikan et al., 2020).

NT-proBNP concentrations correlated with RV structural and volumetric changes in the majority of included investigations in this review (Eindhoven et al., 2014, Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006, Pietrzak and Werner, 2015, Koch et al., 2010, Valverde et al., 2015, Tatani et al., 2010, Khositseth et al., 2007, Cheung et al., 2007). Our pooled meta-analysis results revealed a moderate association between NT-proBNP levels and RV structural and volumetric changes ($r=.41$, 95% CI .32-.48, $p<.001$) (Table 9.4). High concentrations of plasma natriuretic peptide are associated with reduced exercise capacity in adult congenital heart disease (ACHD), but this has not to date been specifically demonstrated in patients with repaired TOF (Schoonbeek et al., 2016). In the studies included in this analysis that evaluated exercise capacity using cardiopulmonary exercise testing (CPET), the majority of patients with high levels of NT-proBNP were asymptomatic, but with reduced exercise

capacity (Festa et al., 2007, Norozi et al., 2005, Trojnarska et al., 2006). There were two studies that showed contrary findings (Menting et al., 2015, Eindhoven et al., 2014). Looking at these study populations, they were heavily drawn from populations with low NT-proBNP levels and nearly normal functional reserve (124 and 151, pg/ml), and (max VO_2 of 80 and 77, %), suggesting that they were biased towards the most clinically stable patients. In our pooled meta-analysis, despite relatively fewer studies investigating the link between NT-proBNP and exercise capacity, we found a higher modest correlation with exercise if it was compared with the structural and volumetric RV changes (r= -.54 vs r=.41, p<.001).

In terms of intervention studies and NT-proBNP, only two investigations in the included analysis observed a notable reduction in NT-proBNP levels after 6 months following PVR (Kitagawa et al., 2015, Dodge-Khatami et al., 2006). These findings may have an important clinical implication that could contribute to the complex debate about the decision-making of timing PVR by having an additive value in detecting any deterioration in cardiac function and functional capacity (Oosterhof et al., 2006, Buechel et al., 2005, Therrien et al., 2005). Rare documentation of sST2 data which drawn from only two investigations in this review was evident and this limits our sST2 assessment in patients with repaired TOF (Geenen et al., 2019, Laqqan et al., 2018). A raised NT-proBNP could potentially detect early RV dysfunction and identify patients who are in need of surgical intervention. This would require identifying specific cut off value of NT-proBNP which predict adverse outcomes.

The prognostic value of NT-proBNP in adult congenital heart disease has not been widely described, and particularly in patients with repaired TOF. Our meta-analysis evaluating the prognostic data from three investigations in adults showed that elevated NT-proBNP levels (mean of 243± 113 pg/ml) in asymptomatic patients were significantly associated with the increased risk of all-cause mortality and major cardiac events, such as acute heart failure, sustained ventricular arrhythmias and death (HR of 1.18 (95% CI 1.07-1.31, p=.001). NT-proBNP levels were reported by Laqqan et al as an excellent predictor of adverse outcomes in a low-risk population, with an AUC of .875 corresponding to NT-proBNP>349 pg/ml (Laqqan et al., 2018). This was much greater than the other two studies included with observed prognostic relevance (147 and 232 pg/ml) (Heng et al., 2014, Westhoff-Bleck et al., 2016). This suggests that the magnitude of the mortality deficit is closely linked to the BNP value, with higher values indicating significant risk.

This meta-analysis in 1479 patients with repaired TOF is the first meta-analysis in this group with the proposed end points. Currently, evaluation of NT-proBNP levels is not routinely assessed for systematic categorisation used in risk assessment in these patients. Although all included studies were observational investigations with different methodology, definitive conclusions could be drawn. Firstly, our results demonstrate that despite wide variability, elevated NT-proBNP levels seems to be more related to surgical selection than advancing age. This mirrors our recent publication that showed subsequent exercise intolerance in this population is more a function of surgical technique than a function of age (Alborikan et al., 2020). Secondly, these studies suggests that NT-proBNP is a reliable non-invasive tool as which could improve risk stratification in these patients if it is integrated into routine clinical care. Finally, our sub-group analysis results of a wide range of NT-proBNP values in relation to haemodynamic changes and exercise capacity suggests that the use of blood biomarkers could be more sensitive to other existing pathology, which could help in risk assessment in a low-risk population. Future prospective studies will need to investigate the use of index and sequential NT-proBNP measurements to predict adverse outcomes and to facilitate clinical decision making in this population.

9.6 Study limitations

This systematic review and meta-analysis were limited by inconsistent characteristics of the study population, the variability of NT-proBNP with some degree of heterogeneity and the rare documentation of sST2. All studies were observational cohorts with limited follow up data but with an overall good quality. Larger prospective and longitudinal studies are warranted to draw an accurate prognostic value of NT-proBNP in relation to subsequent haemodynamic changes in these patients.

9.7 Conclusions

Our findings form the first summary and meta-analysis on the prognostic effect of NT-proBNP in patients with repaired TOF. We have demonstrated that elevated levels NT-proBNP were associated with an increased risk of cardiovascular adverse outcomes that were not age or gender related, but seems to be more dependent on surgical selection, the stress on the right ventricle and on the subsequent exercise intolerance. NT-proBNP shows promise in improving risk assessment and guiding timing for intervention in patients with repaired TOF.

Chapter 10 Blood biomarkers in adult patients with repaired TOF

10.1 Abstract

Background: The clinical use of plasma brain natriuretic peptide (NT-proBNP) and soluble suppression of tumorigenicity-2 (sST2), and their association to exercise capacity are not known in patients with repaired Tetralogy of Fallot (rTOF).

Methods: Peripheral venous blood samples were drawn for 99 patients with repaired TOF, 59 patients with severe pulmonary regurgitation (PR) (SPR group) and 40 patients with no or mild PR as a control group. NT-proBNP was measured using enzyme-linked immunosorbent assays (Roche Diagnostics, Indianapolis, IN). Soluble ST2 levels were assessed on Aspect-plus ST2 test using a low and high level of external quality control (Critical diagnostics, Cambridge, United Kingdom).

Results: The mean value of NT-proBNP was 160±137 pg/ml, and sST2 was 29±13 ng/ml in the entire population. 58% of all population had an elevated NT-proBNP, while sST2 was abnormal in 40%. Mean NT-proBNP was significantly higher in the SPR group (169±150 vs 145±118, pg/ml, $p<0.001$), while similar sST2 levels were observed in both groups (29±14 vs 30±12, ng/ml, $p>0.05$). NT-proBNP and sST2 levels were higher in patients with transannular patch when compared to other RVOT intervention (174±145 vs 107±100, pg/ml, $p<0.001$); (31±13 vs 29±15, ng/ml, $p<0.05$), for NT-proBNP and sST2, respectively. NT-proBNP was associated with RV contractile reserve (CR), while sST2 was associated with both LV and RV CR. Both biomarkers were significant predictors of exercise capacity, but it was stronger with NT-proBNP ($r=-.60$, $p<0.001$).

Conclusions: Serum levels of sST2 and NT-proBNP are elevated in patients with repaired TOF, and higher values are more dependent on surgical selection than the severity of PR. NT-proBNP reflects changes in the RV while sST2 is more sensitive to both changes in the LV and RV. NT-proBNP is the most predictive blood biomarker of functional capacity in patients with repaired TOF.

10.2 Introduction

Plasma brain natriuretic peptide (NT-proBNP) is cardiac neurohormone secreted by ventricular myocytes in response to pressure, volume overload and increased wall stress, and it is the most recommended laboratory biomarker for supporting heart failure diagnosis (Ponikowski et al., 2016, Gopal et al., 2011, Yoo et al., 2004), however, several reports have shown its inconsistent results due to its affect with age, gender and renal function (Dupuy et al., 2016, Yoo et al., 2004). Soluble suppression of tumorigenicity-2 (sST2) is emerging as a new prognostic biomarker in heart failure, and it overcomes these limitations by having fewer biological variabilities and stronger ability to predict mortality risk in heart failure (Daniels and Bayes-Genis, 2014, Gaggin et al., 2014). It also reflects myocardial stress that stimulated by myocardial myocytes, and it is associated with increased mortality (Aldous et al., 2012, Ky et al., 2012). NT-proBNP is well established diagnostic and prognostic tool in congenital heart disease (CHD), including patients with repaired TOF (Mir et al., 2005, Norozi et al., 2005, Khositseth et al., 2007). Unlike NT-proBNP, studies specifically investigating sST2 in adult survivors of TOF are lacking (Geenen et al., 2019, Laqqan et al., 2018).

Little is known about the usefulness, potential diagnostic, and prognostic value of these biomarkers in patients with repaired TOF. Early surgical repair has improved survival in these patients with technique refinement adding to the prognostic advantage, however, chronic pulmonary regurgitation with subsequent RV dilatation and dysfunction are still thought to be the major causes of late morbidity and mortality in these patients (Davlouros et al., 2002, Gatzoulis et al., 2000, Geva et al., 2004). Repeated measures of combined NT-proBNP and sST2 may offer direct physiological marker of the presence of adverse cardiac remodelling in patients with repaired TOF.

Specific cut-off values of these biomarker indicating RV pressure and volume overload or to predict the need of reintervention in this population are unknown, and therefore we undertook this additional study to (1) evaluate blood biomarkers in asymptomatic adult survivors of TOF repair; (2) to describe for the first time the association between these biomarkers and adverse haemodynamic echocardiographic findings at rest and during exercise, and; (3) to what degree they are related to impaired functional capacity in this population.

10.3 Methods

10.3.1 Main hypothesis and objectives

We aimed to describe levels of blood biomarkers including NT-pro BNP and the novel sST2 in asymptomatic adult patients with repaired TOF comparing those with and without severe PR as well as the effect of surgical technique. We also set out to establish whether these results are associated with adverse cardiac remodelling and functional capacity during stress echocardiography.

10.3.2 General characteristics

This sub-study was constructed with the same cohort that was described in chapter 5. Details of the repaired TOF patients, cardiopulmonary exercise stress echocardiography protocols are as described before (section 3.6, 3.7, and section 5.3.1). Additional details below:

10.3.3 Blood biomarkers assays

10.3.3.1 Plasma brain natriuretic peptide (NT-proBNP)

Peripheral venous blood samples were drawn for 99 patients before cardiopulmonary exercise testing visit (CPET), into EDTA-containing tubes and then centrifuged immediately and stored at −70°C for subsequent analysis. Amino-terminal pro-brain natriuretic peptide (Nitro-pro BNP) was measured using enzyme-linked immunosorbent assays (Roche Diagnostics, Indianapolis, IN). One patient refused to give a blood sample. All NT-proBNP analyses were performed at Royal London Hospital core laboratory blinded to clinical data.

10.3.3.2. Soluble suppression of tumorigenicity-2 (sST2)

Soluble ST2 levels were assessed on Aspect-plus ST2 test using a low and high level of external quality control (Critical diagnostics, Cambridge, United Kingdom). The measurement of sST2 ASPECT-Plus ST2 test was manually performed for 99 patients following the manufacturer's instruction. In brief, sST2 test cassette was warmed to room temperature for 15 minutes, the foil pouch was removed and 35 µl of plasma sample was pipetted into the sample well. 2 drops (i.e., ~110 µL) of test buffer were added into the test buffer well after 60s of loading the plasma sample. Finally, the ASPECT-PLUS sST2 test cassette was inserted into the ASPECT reader and the quantitative sST2 results were analysed approximately in 20 min and calculated in ng/ml. sST2 plasma concentrations were measured with a lower limit of detection of 25 ng/ml, and upper limit of detection of 200 ng/ml.

10.3.4 Statistical analysis

All data were tested for normality using the Kolmogorov-Smirnov statistical test. Continuous data were presented as mean ±SD. Blood biomarkers values were compared between groups using Student's paired t test for independent samples. Pearson correlation coefficients (r) were used to determine relationships between blood biomarkers parameters and exercise parameters. All variables that were significant at univariable correlation analysis were entered into multivariable regression analysis in all patients. Pulmonary regurgitation was treated as a binary variable (severe PR / no or minimal PR) for the purposes of this analysis. Details of the regression model were provided as corresponding R, R^2, standardised beta (regression coefficient). The statistical analysis was performed using IBM SPSS statistics version 28. A p value of <0.05 was considered statistically significant.

10.4 Results

10.4.1 Baseline characteristics, TOF population (99 patients)

The results from this chapter are drawn from the same population described in chapter 5. 59 adult patients with repaired TOF, with severe pulmonary regurgitation (SPR group), and 40 patients with repaired TOF with mild, or without pulmonary regurgitation (control group) were included. SPR group was older than controls (35±13 vs 33±11, years, $p<0.05$) and were marginally more male. Reduced peak VO_2 was observed in both groups with no significant difference (1695±627 vs 1744±521, ml/min, $p>0.05$) between those with and without severe PR. Detailed baseline characteristic, surgical history and exercise performance are outlined in chapter 5 (section 5.3.1 and 5.3.6). There were no significant baseline differences between the two groups apart from age and gender (Table 10.1).

Table 10. 1. Baseline characteristics of the entire population.

Baseline Characteristics	**SPR Group Mean ± SD**	**Control group Mean ± SD**	**P value**
Age (yrs)	35 ±13	33±11	**<0.05**
Sex **Male** **Female**	 31 (52%) 29 (48%)	 19 (48%) 21(52%)	**<0.05**
Height (cm)	166±11	169±8	NS
Weight (kg)	71±16	73±14	NS
BMI (Kg/m2)	25±5	26±5	NS
ORS duration (ms)	153±20	150±22	NS
Peak oxygen uptake VO_2 (ml/min)	1695±627	1744±521	NS

BMI=body mass index; QRS=depolarization of ventricles; VO_2=peak absolute maximum oxygen uptake in absolute (ml/min);
* Bold values indicate significant level ($p<0.05$); NS=non-significant.

10.4.2 Plasma brain natriuretic peptide level (NT-proBNP) in asymptomatic adult patients with repaired TOF

The mean value of NT-proBNP in all population was 160±137 pg/ml but ranged from 35 to 703 pg/ml. 58% of the entire population had an elevated NT-proBNP, when compared to normal range (normal <100 pg/ml, (Binder et al., 2005)) (Figure 10.1a). Higher NT-proBNP levels were observed in patients with TAP surgical approach when compared to other RVOT approaches (174±145 vs 107±100, pg/ml, p<0.001, Figure 10.2). In comparison between groups, 58% of SPR group had an elevated level of NT-proBNP, while 53%, in the control, p <0.001. Mean NT-proBNP was significantly higher in the SPR group (169±150 vs 145±118, pg/ml, p<0.001, Figure 10.1b). Regarding the interaction with surgical technique, NT-proBNP was higher in patients with TAP compared with non-TAP in the SPR group (187±157 vs 69±29, pg/ml, p<0.001), but also the group without PR (150±118 vs 134±12, pg/ml, p<0.001, Table 10.2).

a) **b)**

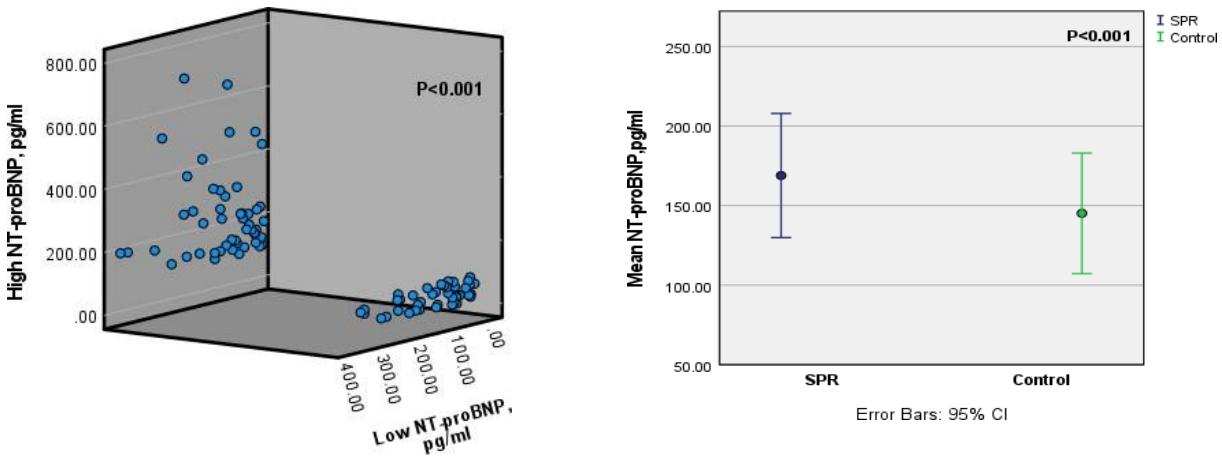


Figure 10. 1. Difference in mean NT-proBNP levels in (a) entire population, n=99 (high and low NT-proBNP), and (b) between groups (SPR and control), p<0.001.

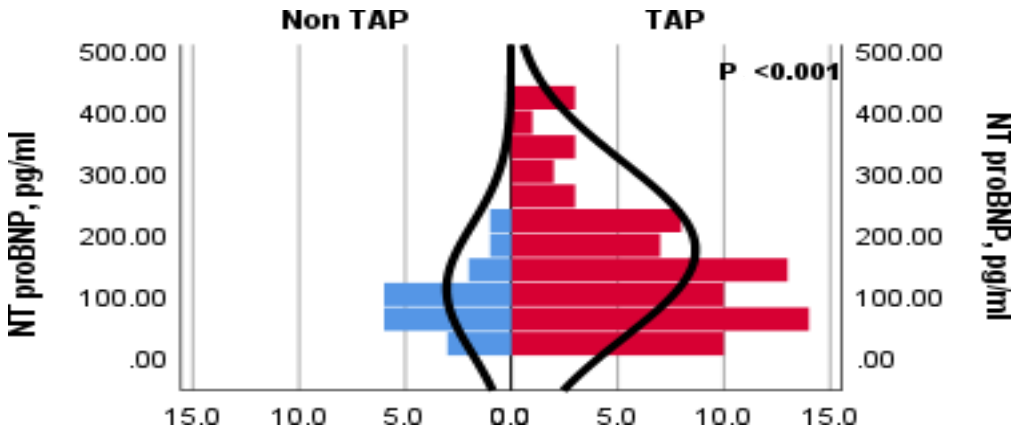


Figure 10. 2. NT-proBNP levels comparison between TAP and non-TAP in the entire population (n=99).

10.4.3 Soluble suppression of tumorigenicity-2 (sST2) in asymptomatic adult patients with repaired TOF

The mean value of sST2 in all population was 29±13 ng/ml but ranged from 15 to 80 ng/ml. 40% of the entire population had an elevated sST2, when compared to the normal range (normal <30 ng/ml, (Wu et al., 2013)) (Figure 10.3a). There was no significant difference in the mean sST2 in both groups (29±14 vs 30±12, ng/ml, p>0.05, Figure 10.3b). Higher sST2 levels were observed in all patients with TAP surgical approach compared to other RVOT approaches regardless of the presence or absence of severe pulmonary regurgitation (31±13 vs 29±15, ng/ml, p<0.05, Figure 10.4). In comparison between groups, 45% of control group had higher levels of sST2, while 37 % in the SPR, p<0.001. Likewise, similar sST2 levels were observed in subgroup analysis with the type of surgery in both groups (TAP vs non-TAP, Table 10.2).

a) **b)**

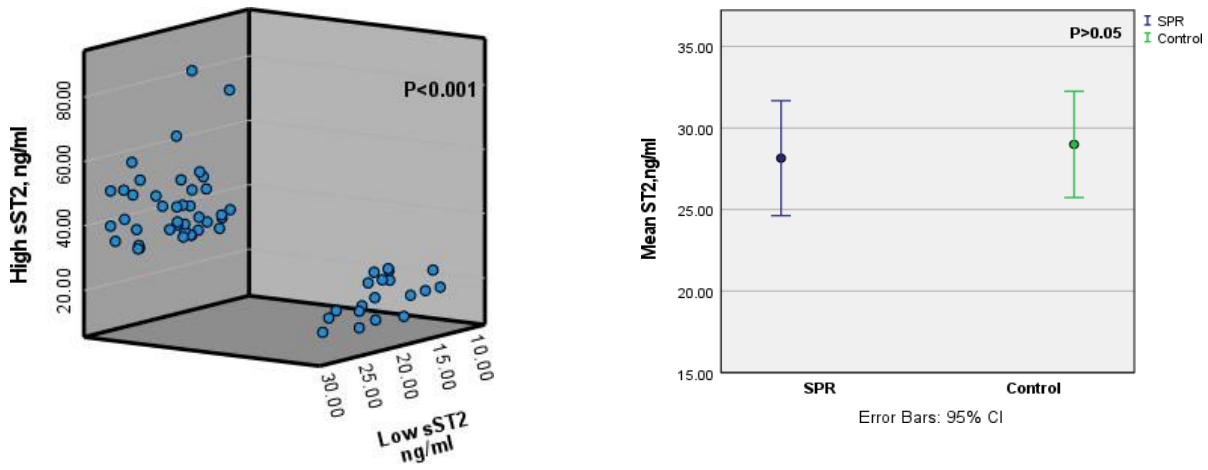


Figure 10. 3. Difference in mean sST2 levels in (a) entire population, n=99 (High and low sST2), and (b) between groups (SPR and control), p<0.001.

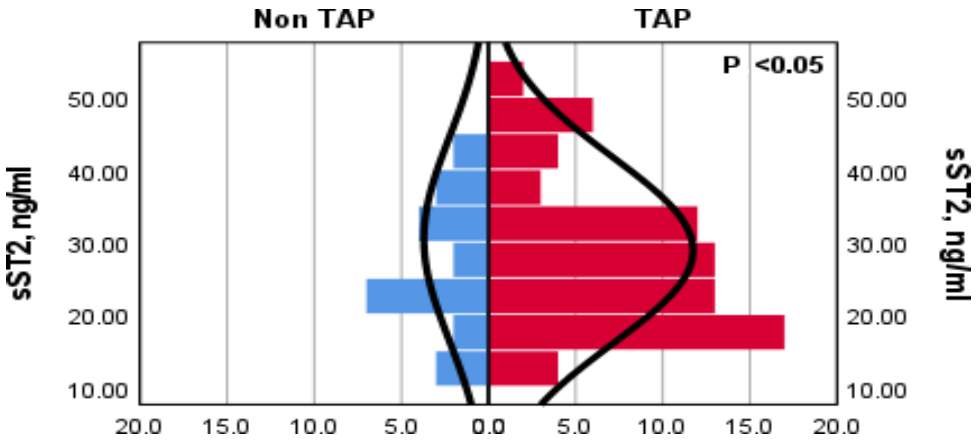


Figure 10. 4. sST2 levels comparison between TAP and non-TAP in the entire population (n=99).

Table 10. 2. Baseline blood biomarkers in the entire population and subgroups analysis.

Blood biomarkers	**NT-proBNP pg/ml (n=99)**	**sST2, ng/ml (n=99)**	**P value**	**Blood biomarkers subgroup**	**SPR Group Mean ± S. D (n=59)**	**Control Group Mean ± S (n=40)**	**P value**
All population (n=99)	160±137	29±13	-	**NT-proBNP**	169±150	145±118	**<0.001**
				sST2, ng/ml	29±14	30±12	NS
Surgical type	**TAP**	**Non-TAP**		**Surgical type**			
NT-proBNP (n=99)	174±145	107±100	**<0.001**	**NT-proBNP, TAP**	187±157	150±118	**<0.001**
sST2 (n=99)	31±13	29±15	**<0.05**	**NT-proBNP, non-TAP**	69±29	134±124	**<0.001**
-		-	-	**sST2, TAP**	28±13	30±12	NS
-	-	-	-	**sST2, non-TAP**	29±15	30±15	NS

NT-proBNP=plasma brain natriuretic peptide; sST2=soluble suppression of tumourigenicity-2; TAP=transannular patch; non-TAP=other type of surgery (e.g., pulmonary valvotomy) * Bold values indicate significant level (p<0.001, <0.05); NS=non-significant.

10.4.4 Association between blood biomarkers and baseline echocardiographic findings

Both biomarkers were, as expected significantly correlated (r=.67, p<0.001) (overall model fit of R^2=-.60, p<0.001), although the clear relationship was not strong for them to be considered absolute co linear variables (Table 10.3).

At baseline, NT-proBNP was associated with RV size, both at basal and at mid dimensions (r=.21, r=.31, p<0.001), inversely associated with RV longitudinal function by TAPSE (r=-.22, p<0.001), and with RV global longitudinal strain (RVGLS) (r=-.17, p<0.001). For the LV, neither LV volume, function or strain were correlated with NT-proBNP levels (Table 10.3).

Conversely sST2 while not correlated with LV or RV volumes, nor conventional functional parameters, there was a significant inverse association with both RVGLS (-.39, p<0.001), and LV global longitudinal strain (LVGLS) (r=-.49, p<0.001, Table 10.3).

Table 10. 3. Univariate correlation analysis (r values presented) between blood biomarkers and baseline echocardiographic parameters.

Baseline echocardiographic Parameters (n=99)	**NT-proBNP (pg/ml)**	**sST2 (ng/ml)**
Right ventricle		
RV mid (mm)	**.21***	.02
RV base (mm)	**.31***	.11
RVEDVI (ml/m^2)	.18	.19
RVS' (cm/s)	-.09	-.05
TAPSE (mm)	**-.22***	-.11
FAC (%)	-.10	-.05
RVGLS (%)	**-.17***	**-.39***
RVGFWS (%)	-.20	-.13
Pulmonary regurgitation		
PG (mmHg)	-.01	-.04
PHT (ms)	-.12	-.06
Left ventricle		
LVEDVI (ml/m^2)	.03	.14
LVS' (cm/s)	-.19	-.09
LVGLS (%)	-.30	**-.49***
Blood biomarkers	**sST2 (ng/ml)**	-
NT-proBNP	**.67***	-

RV mid=right ventricular mid-size; RV base=right ventricular basal-size; RVEDVI=indexed right ventricular end diastolic volume; RVS'=right ventricular systolic velocity; TAPSE=tricuspid annular plane systolic excursion; FAC=fractional area change; RVGLS=right ventricular global longitudinal strain; RVGFWS=right ventricular global free wall stain; PG= pulmonary regurgitation pressure gradient; PHT=pulmonary regurgitation pressure half time; LVEDVI=indexed left ventricular end diastolic volume; LVS'=left ventricular systolic velocity; LVGLS=left ventricular global longitudinal strain.* Bold values indicate significant level (p<.0.001). All values in table represent r values.

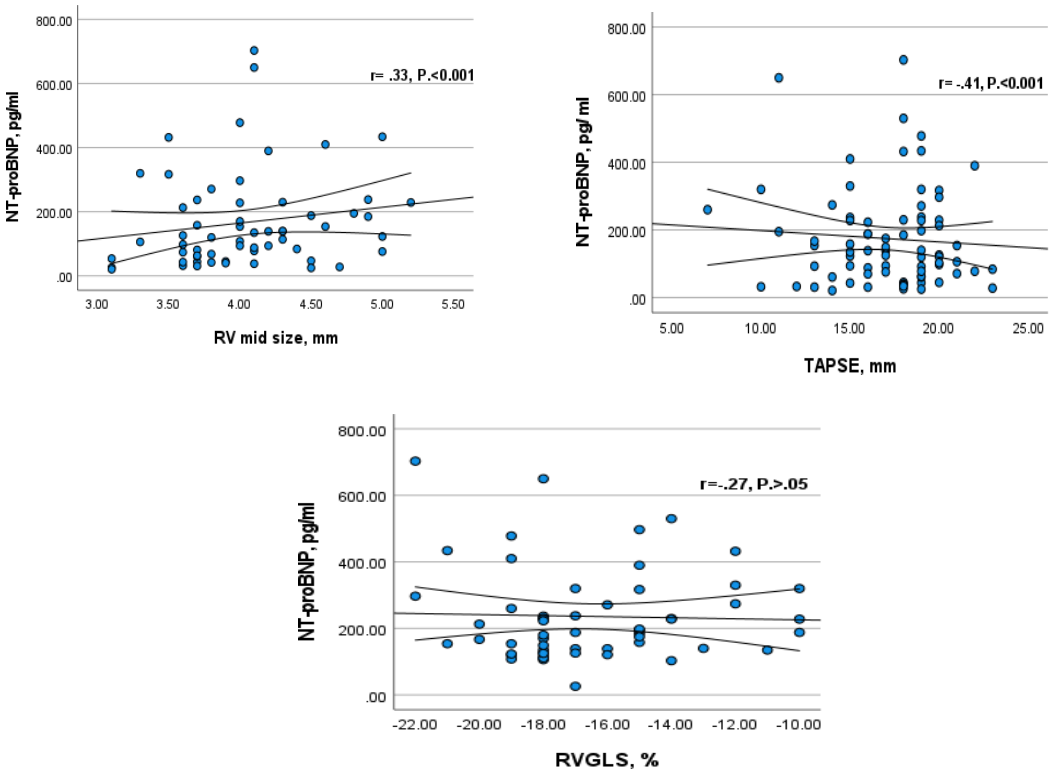
All significant univariate correlations were entered into a generalised multivariable linear regression model to allow the effect of PR in this population which showed that PR has no effect on biomarkers levels. Blood biomarkers were independently associated with RV size at mid-level, and RV longitudinal function by TPASE and LVGLS (Figure 10.5). The best resting echo predictors of NT-proBNP were TAPSE (r= -.41, p<0.001), and RV size at mid-level (r=.33, p<0.001). LVGLS was the best predictor of sST2 (r=-.36, p<0.001) (Table 10.4). The overall model fit was R^2=.40, p<0.001.

Table 10. 4. Multivariable linear regression of potential resting echo predictors of blood biomarkers as dependent variable.

Multivariate linear regression Outcome variable: blood biomarkers R^2=.40, p.<0.001	**B-coefficient**	**R**	**P value**
PR	3.2	.20	NS
RV mid (mm)	.38	.33*	**<0.001**
RV base (mm)	.10	.12*	NS
TAPSE (mm)	-.29	-.41*	**<0.001**
RVGLS (%)	-.17	-.27*, -.22***	NS
LVGLS (%)	-.39	-.36**	**<0.001**

RV mid=right ventricular mid-size; RV base=right ventricular basal-size; TAPSE=tricuspid annular plane systolic excursion; RVGLS=right ventricular global longitudinal strain; LVGLS=left ventricular global longitudinal strain. *Correlation with NT-proBNP; ** Correlation with sSt2 *** Correlation with NT-proBNP and sSt2; Bold values indicate significant level (p<.0.001); NS=non-significant.

a) NT-proBNP resting echocardiographic predictors



b) sST2 resting echocardiographic predictors

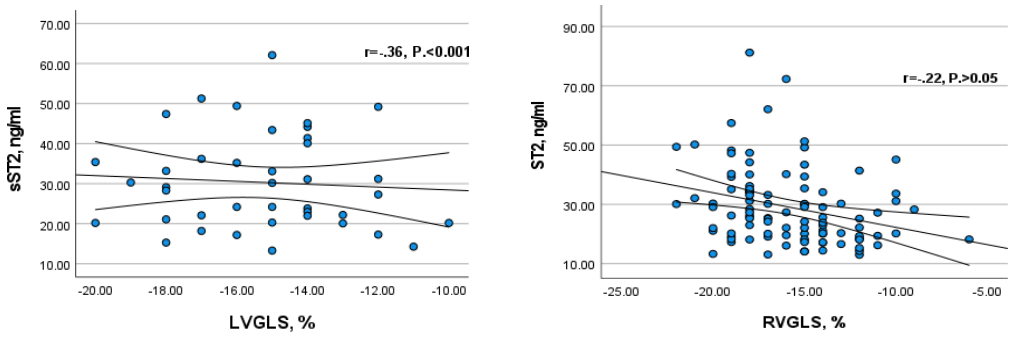


Figure 10. 5. Scatter plots of potential resting echocardiographic parameters predictors of increased levels of blood biomarkers in the entire population (n=99) (overall model fit R^2=.40, p<0.001).

10.4.5 Association between blood biomarkers and echocardiographic biventricular contractile reserve parameters during exercise

For NT-proBNP, only RV contractile reserve parameters were significantly correlated with increased levels of NT-proBNP. There were significant correlations observed with the measures of RV longitudinal function including RV global free wall strain (ΔRVGFWS) (r=-.69, p<0.05), RV global strain ΔRVGLS (-.44, p<0.05), longitudinal function by ΔRVS' (r=-.33, p<0.05), and by ΔTAPSE (r=-.22, p<0.05). There was also an association with change in RV volume (Table 10.5). There was no association observed with all LV contractile reserve parameters and NT-proBNP (Table 10.5).

Unlike NT-proBNP, sST2 was associated with both RV and LV contractile reserve parameters. For RV contractile reserve, the highest correlation was found with the change in RV volume ΔRVEDVI (r=-.73, p<0.05), followed by the change in RV strain, ΔRVGFWS (r=-.38, p<0.05), and in ΔRVGLS (-.23, p<0.05). For LV contractile reserve, modest correlation was observed with change in LV longitudinal strain ΔLVGLS (r= -.49, p<.05), followed by LV longitudinal function ΔLVS (r=-.48, p<0.05), and change in LV volume ΔLVEDVI (r=-.38, p<0.05, Table 10.5).

Table 10. 5. Univariate correlation analysis between blood biomarkers and biventricular contractile reserve parameters in the entire population.

Contractile reserve (n=99) **	**NT proBNP (pg/ml)**	**sST2, (ng/ml)**
Right Ventricle		
Δ RVS' (%)	**-.33***	-.11
Δ FAC (%)	-.10	-.20
Δ TAPSE (%)	**-.22***	-.10
Δ RVEDVI (%)	**-.34***	**-.73***
Δ RVGLS	**-.44***	**-.23***
Δ RVGFWS	**-.69***	**-.38***
Left Ventricle		
Δ LVS'	.03	**-.48***
Δ LVEDVI	.11	**-.38***
Δ LVGLS (%)	-.06	**-.49***

ΔRVS'=contractile reserve of right ventricular systolic velocity; ΔFAC=contractile reserve of fractional area change; ΔTAPSE=contractile reserve of tricuspid annular plane systolic excursion; ΔRVEDVI=contractile reserve of indexed right ventricular end diastolic volume; ΔRVGLS=contractile reserve of right ventricular global longitudinal strain; ΔRVGFWS= contractile reserve of right ventricular global free wall stain; ΔLVS'=contractile reserve of left ventricular systolic velocity; ΔLVEDVI=contractile reserve of indexed left ventricular end diastolic volume; ΔLVGLS=contractile reserve of left ventricular global longitudinal strain.* Bold values indicate significant level (p<.0.05). ** All values in table represent r values.

10.4.6 Association between blood biomarkers and maximum oxygen uptake (VO_2) in patients with repaired TOF

Univariate correlation showed that there were inversed correlations between NT-proBNP with peak absolute (ml/min) and peak weighted (ml/min/kg) VO_2. The relationship with sST2 was less strong although there was some correlation between sST2 and peak ansoluteVO_2 (ml/min) (r=-.39, p<0.001, Table 10.6).

A multivariable regression model was constructed to allow the effect of the type of surgical approach (TAP, and non-TAP), on blood biomarkers and peak oxygen uptake VO_2 (ml/min). Adding type of the surgery makes the association stronger between both blood biomarkers independently and peak oxygen uptake. The overall model fit was R^2= -.60, p<0.001 (Table 10.7, Figure 10.6).

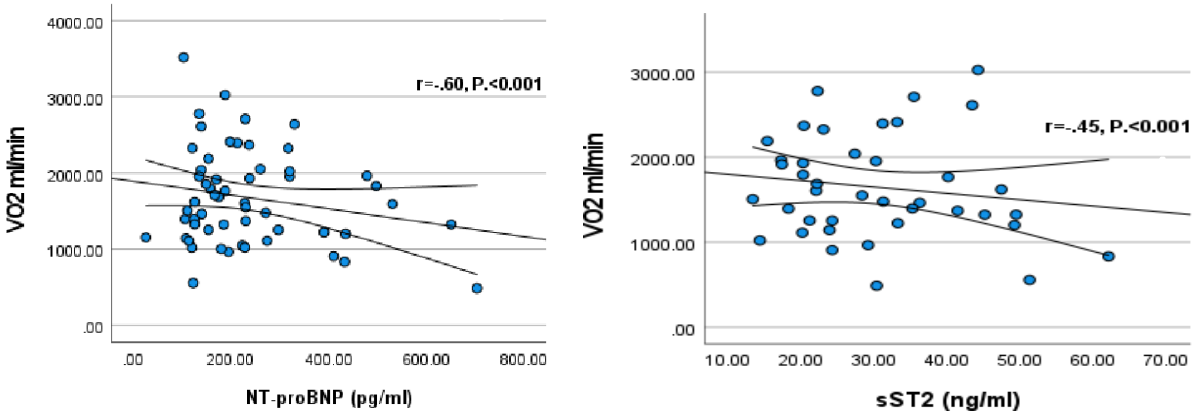


Figure 10. 6. Scatter plots of blood biomarkers as significant predictors of exercise capacity (overall model fit R^2= -.60, p<0.001).

Table 10. 6. Univariate correlation analysis (r values presented) between exercise capacity and blood biomarkers in the entire population.

Blood biomarkers **	**VO_2 (ml/min)** **n=99**	**VO_2 (ml/kg/min)** **n=99**
NT-proBNP (pg/ml)	**-.50***	**-.38***
sST2 (ng/ml)	**-.39***	-.20

NT-proBNP=plasma brain natriuretic peptide; sST2=soluble suppression of tumourigenicity-2. * Bold values indicate significant level (p<.0.001). ** All values in table represent r values.

Table 10. 7. Multivariable linear regression of blood biomarkers predictors of exercise capacity in the entire population.

Multivariable linear regression VO2(ml/min) R^2= -.60, p.<0.001 TAP (0/1)	**B-coefficient**	**R**	**P value**	**R^2**
Surgical approach (TAP/non-TAP)	-.20	.40	**<0.001**	-.30
NT-proBNP (pg/ml)	-9.3	**-.60**	**<0.001**	-.45
sST2 (ng/ml)	-7.5	**-.45**	**<0.001**	-.33

NT-proBNP=plasma brain natriuretic peptide; sST2=soluble suppression of tumourigenicity-2**.** * Bold values indicate significant level (p.<.0.001).

10.4.7 Predictive modelling of increased plasma concentration of blood biomarkers in patients with repaired TOF

Multivariate regression model for the resting and contractile reserve echo parameters was constructed to identify which echocardiographic parameter is associated with increased level of blood biomarkers. For this part of the analysis SPR and controls were considered together and the presence of severe PR where required (0/1) was entered into the models as a factor.

Multivariate regression models for resting and exercise echo potential predictors of increased levels of blood biomarkers showed that resting RV longitudinal function by TAPSE (r=-.47 p<0.001), change in RV volume during the stress (ΔRVEDVI) (r=-.60, p<0.001), and change in RV global free wall strain (ΔRVGFWS) (r=-.52, p<0.001), were the most important determinants of increased levels of NT-proBNP. The overall model fit was R^2= .60, p<0.001 (Model1, Table 10.8, Figure 10.7).

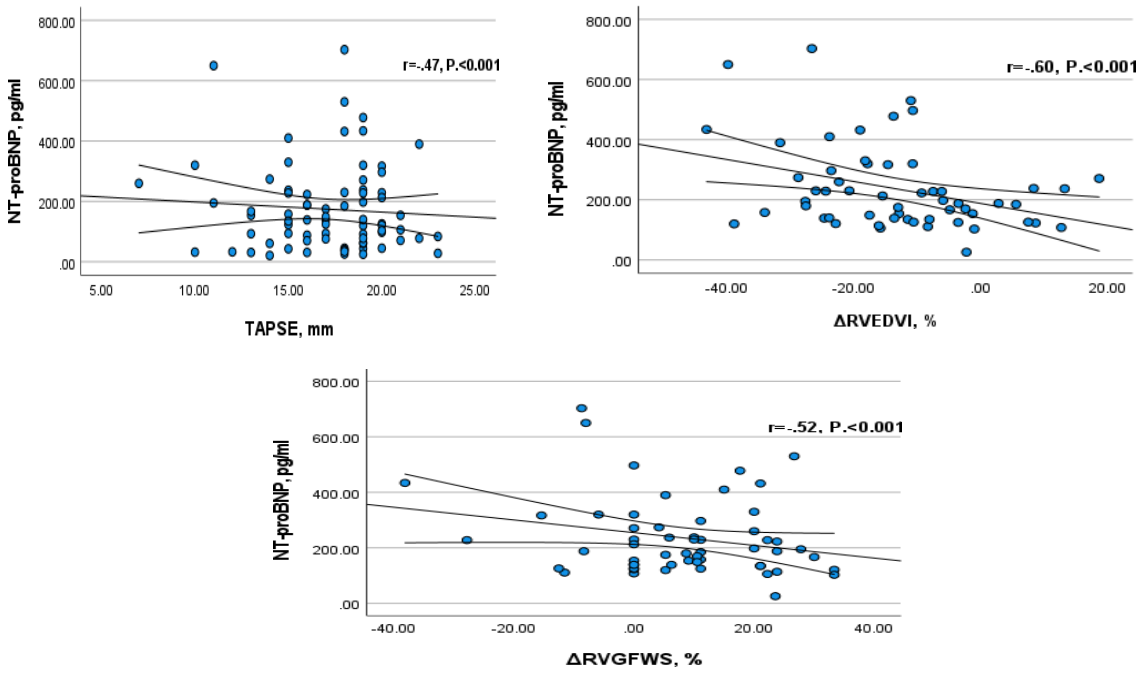
For sST2, LVGLS at rest (r=-.27, p<0.001), LVGLS contractile reserve during stress (r=-.55 p<0.001) and LV volume change (r=-.40 p<0.001), were the most important predictors of increased levels of sST2. The overall model fit was R^2 =.40, p<0.001 (Model 2, Table 10.8, Figure 10.7).

Table 10. 8. Multivariable linear regression of potential echo predictors of increased levels of biomarkers in the entire population.

Multivariable prediction model 1 (NT-proBNP, pg/ml) R^2**= .60. P<0.001, PR (0/1)**	**B-coefficient**	**R**	**P value**	**Multivariable prediction model 2 (sST2, ng/ml)** R^2**= .40. P<0.001, PR (0/1)**	**B-coefficient**	**R**	**P value**
Pulmonary regurgitation	NS	NS	NS	**Pulmonary regurgitation**	NS	NS	NS
Resting Echo Parameters				**Resting Echo Parameters**			
RV mid (mm)	NS	NS	NS	RVGLS (%)	NS	NS	NS
TAPSE (mm)	-.37	-.47	**<0.001**	**LVGLS (%)**	-.31	-.27	**<0.001**
RVGLS (%)	NS	NS	NS	-	-	-	-
Exercise echo parameters				**Exercise Echo parameters**			
Δ TAPSE (%)	NS	NS	NS	ΔRVEDVI (%)	NS	NS	NS
Δ RVS' (%)	NS	NS	NS	Δ RVGLS (%)	NS	NS	NS
ΔRVEDVI (%)	**-.58**	**-.60**	**<0.001**	Δ RVGFWS (%)	NS	NS	NS
ΔRVGLS (%)	NS	NS	NS	ΔLVS' (%)	NS	NS	NS
ΔRVGFWS (%)	-.30	-.52	**<0.001**	**ΔLVEDVI (%)**	-.12	-.40	**<0.001**
-	-	-	-	**ΔLVGLS (%)**	-.43	-.55	**<0.001**

RV mid=right ventricular mid-size; TAPSE=tricuspid annular plane systolic excursion; RVGLS=right ventricular global longitudinal strain; ΔTAPSE=contractile reserve of tricuspid annular plane systolic excursion; ΔRVS'=contractile reserve of right ventricular systolic velocity; ΔRVEDVI=contractile reserve of indexed right ventricular end diastolic volume; ΔRVGLS= contractile reserve of right ventricular global longitudinal strain; ΔRVGFWS=contractile reserve of right ventricular global free wall stain; LVGLS=left ventricular global longitudinal strain; ΔLVS'=contractile reserve of left ventricular systolic velocity; ΔLVEDVI=contractile reserve of indexed left ventricular end diastolic volume; ΔLVGLS=contractile reserve of left ventricular global longitudinal strain.* Bold values indicate significant level (p.<0.001);); NS=non-significant.

a) NT-proBNP resting and exercise echocardiographic predictors



b) sST2 resting and exercise echocardiographic predictors

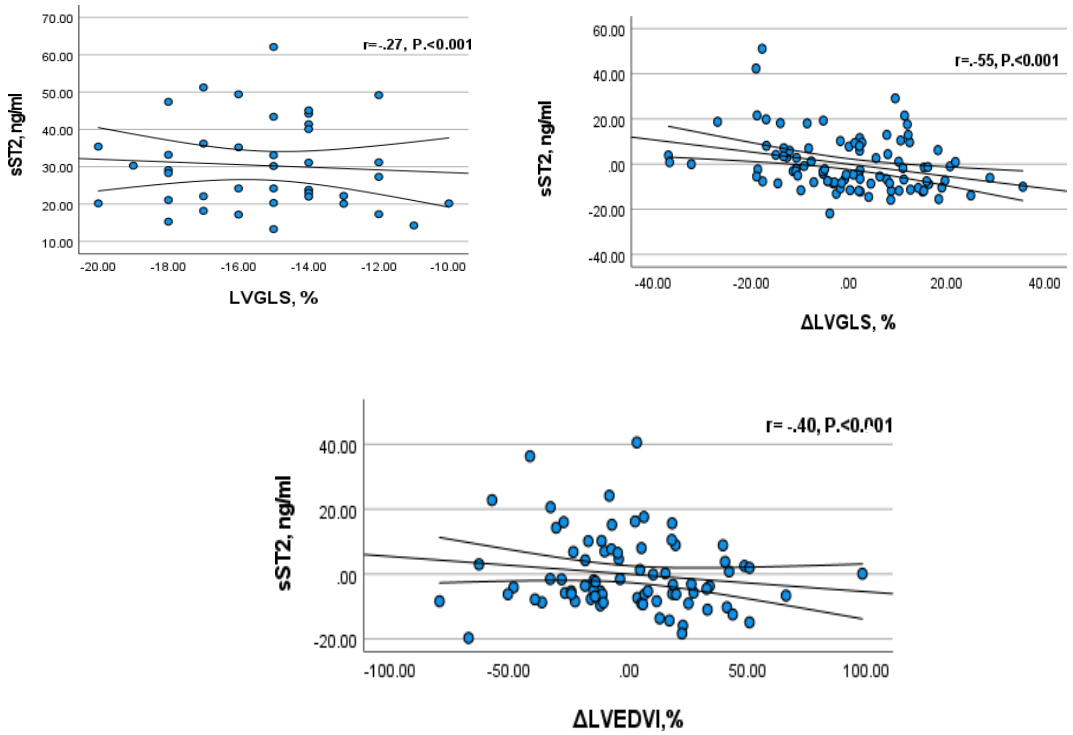


Figure 10. 7. Scatter plots of significant independent resting and exercise echocardiographic predictors of increased levels of blood biomarkers (overall model fit R^2= .60, model 1 (NT-proBNP), R^2=.40, model 2 (sST2), p<0.001).

10.5 Discussion

In this study we have examined the relationships between two key biomarkers of myocardial distress (NT-proBNP and sST2) and biventricular structure and function in asymptomatic patients with repaired TOF and how they may reflect different aspects of cardiac performance. We have demonstrated that NT-proBNP is abnormal in 58% of patients while sST2 was abnormal in 40%. The presence of PR resulted in an elevated NT-proBNP but not an elevated sST2, while the use of a transannular patch as the surgical technique was associated with higher biomarker signal in both groups irrespective of the presence of PR. NT-proBNP was associated with RV size and function, while sST2 was influenced by both RV and LV size and function, LV dysfunction being an important component of the pathophysiology of TOF. Reflecting this differential association NT-proBNP was associated with RV contractile reserve, while sST2 was associated with both LV and RV contractile reserve, suggesting that sST2 may be more sensitive to signals from the LV in this group of patients. Both biomarkers showed an association with VO_2 max, confirming that the observed findings are clinically tangible tracking directly to functional capacity. Overall, the most predictive biomarker was NT-proBNP.

NT-proBNP and sST2 are the key blood biomarkers of myocardial distress which are both released by ventricular myocytes in response to volume, pressure overload and increased wall stress (Dupuy et al., 2016). They are both reflective of myocardial remodelling and stretching, however the major difference is that sST2 offer fewer biological variabilities and being not influenced by confounders such as age and renal function which make it superior to NT-proBNP in risk stratification in heart failure (Dupuy et al., 2016, Daniels and Bayes-Genis, 2014, Gaggin et al., 2014). Combining both biomarkers is recommended to guide treatment strategies for better prediction of increased mortality in high-risk population including adult patients with congenital heart disease (ACHD) (Geenen et al., 2019). Despite improved survival in adult patients with repaired TOF in the recent years, developing new biomarkers that are sensitive enough to detect ongoing haemodynamic adverse changes that could help in patient categorisation, improving treatment strategies and monitoring the impact of treatment is of major importance. This is the first study that evaluated blood biomarkers in adult patients with repaired TOF including NT-proBNP and the novel sST2 comparing their effect on biventricular function, volume, and exercise intolerance.

NT-proBNP is known to be high in this population (Apitz et al., 2009, Norozi et al., 2005, Khositseth et al., 2007), but the underlying causes of its elevation are not known yet. We found that NT-proBNP levels were elevated in asymptomatic patient with repaired TOF, with higher degree found in patients with severe pulmonary regurgitation compared to patients who do not have volume overload. When looking at these patients according to the surgical history regardless of the presence or absence of severe pulmonary regurgitation, we found even higher degree of NT-proBNP in patients where trans-annular patch (TAP) was used as a surgical approach than others with other RVOT intervention such as pulmonary valvotomy or infundibulectomy. This has been highlighted in our recent meta-analysis on blood biomarkers that was collected on 1427 patients with repaired TOF which showed that elevated levels of NT-proBNP in TOF population with mean of 174 ±56 pg/ml were related to chosen surgical techniques and ongoing adverse haemodynamic response in this population (Alborikan et al., 2021).

Assessment of sST2 assays is a novel approach in cardiovascular medicine, and to date, there is no data that has been specifically evaluated the levels of sST2 in patients with repaired TOF (Laqqan et al., 2018, Geenen et al., 2019). Our result forms the first evaluation of sST2 in asymptomatic patients who are clinically stable which showed that mean sST2 was 29 ±1 ng/ml for the population with 40% of subjects abnormal, with no significant difference between patient with severe pulmonary regurgitation and patients with no loading condition. Interestingly, when looking at sST2 levels in the entire TOF population, we have seen higher values of sST2 in patients with TAP surgical approach than patients with other surgical approaches (31±13 vs 29±15, ng/ml, $p<0.001$), suggesting an adverse haemodynamic response. When compared to the only two published reports in ACHD patients, our sST2 levels were higher (29 vs 26, ng/ml), although their population is more clinically deteriorated than ours. This suggests that even in asymptomatic patients with repaired TOF who have elevated levels of NT-proBNP are most likely to have increased levels of sST2.

High concentrations of plasma natriuretic peptide are known to be associated with reduced exercise capacity in ACHD (Schoonbeek et al., 2016), with few reports in patients with repaired TOF (Norozi et al., 2006, Festa et al., 2007, Trojnarska et al., 2006). Looking at these studies who have evaluated functional capacity by cardiopulmonary exercise testing (CPET) in adult patients with repaired TOF, the majority of patients who had reduced exercise capacity had elevated levels of NT-proBNP (Trojnarska et al., 2006, Festa et al., 2007, Norozi et al., 2006). Our findings showed that reduced exercise capacity in patient with repaired TOF is significantly associated with increased levels of NT-proBNP. This has been shown also in our recent meta-analysis which demonstrated a modest correlation between high levels of NT-proBNP and impaired exercise capacity in this population (Alborikan et al., 2021). Our results also showed such association with sST2, but however less than NT-proBNP with reduced exercise capacity, and this is a novel finding that could encourage the use of sST2 in addition to NT-proBNP for the blood biomarkers evaluation in asymptomatic patients with repaired TOF.

There is ongoing debate about the echocardiographic haemodynamic causes behind the raised levels of NT-proBNP and sST2 in patients with repaired TOF. It might be because the excessive load imposed by severe pulmonary regurgitation, or RV structure and RV volume. In our study, we found that there was no association between indices of pulmonary regurgitation and blood biomarkers. Our results also showed that resting and contractile reserve of RV size and function parameters were associated with abnormal NT-proBNP levels while both RV and LV volume and function parameters were associated with sST2 increased levels. This suggests that NT-proBNP in this population is more explained by the change in RV volume and function, while sST2 is more reflective of LV subtle changes. In our NT-proBNP analysis, we found that raised levels of NT-proBNP were associated with resting RV size and function parameters by TAPSE and RVGLS, and the association was more powerful during exercise. This is a novel finding, however, the importance of RV volume and size to explain raised levels of NT-proBNP is shown in few investigations (Norozi et al., 2005, Pietrzak and Werner, 2015, Valverde et al., 2015, Apitz et al., 2009).

In the sST2 analysis, we found that raised levels of sST2 were associated with resting LV longitudinal function by LVGLS. During exercise, interestingly, sST2 was more sensitive to detect changes in RV and LV volume and function. The importance of this novel finding is unclear but given the known importance of LV dysfunction in this population we can speculate that this might be a prognostically important signal. In our prediction analysis, we found that NT-proBNP is the most predictive parameter of impaired exercise capacity in this population. We found that resting RV function and the change in RV volume and free wall strain during stress were the most predictive echo parameters of increased levels of NT-proBNP, while changes in LV strain and volume were the most predictive echo parameters of increased levels of sST2. This reflects what we have concluded in the correlation section above, which suggests that sST2 in patients with repaired TOF is more sensitive to the change in LV function more than NT-proBNP.

This study forms the first description of blood biomarkers including NT-proBNP and sST2 levels in asymptomatic patients with repaired TOF. Our study introduced major findings in this population, first, we have demonstrated that NT-proBNP and sST2 are elevated in patients with repaired TOF who are clinically stable. Second, their raised levels are more related to surgical selection rather than to the severity of pulmonary regurgitation. Third, elevated levels of blood biomarkers in this population are major contributors of reduced exercise tolerance. Fourth, changes in RV volume, longitudinal function and RV strain are the major predictors of further deterioration in NT-proBNP, while LV dysfunction is stronger predictor of high sST2 levels. Our results confirm that raised levels of blood biomarkers could explain reduced exercise capacity, changes in biventricular volume and longitudinal function during exercise echocardiography in this population. Currently, their evaluation in this patient group is not routinely assessed for systematic categorisation used in risk assessment, however we found that they are more sensitive to existing pathology that could help in risk assessment, and we therefore recommend the use of these novel blood biomarkers into risk assessment in a low-risk TOF population.

10.6 Study limitations

This study is limited by the surgical history of our patients that might be not representative of the whole TOF population, but our novel findings represent the first analysis of the blood biomarkers in asymptomatic patients with repaired TOF investigating their increased levels with the ongoing adverse haemodynamic changes.

10.7 Conclusions

NT-proBNP and sST2 levels are elevated in asymptomatic patients with repaired TOF. NT-proBNP reflects changes in the RV while sST2 reflects changes in the LV and RV. NT-proBNP is a stronger predictive biomarker of exercise capacity and echocardiographic haemodynamic changes than sST2 in asymptomatic patients with repaired TOF.

Chapter 11 General discussion and future research

11.1 Background

With increasing longevity of the long-term complications of management of repaired TOF patients, these patients provide a unique challenge, in particularly for the management of severe pulmonary regurgitation (PR). Guidelines are based on observational data and the precise balance between volumes and function as predictors of exercise ability or indication for reintervention. I undertook two formal meta-analyses. The first demonstrated that there was a marked reduction in functional capacity in these patients which was more dependent on surgical approach and developing techniques than advancing age. The second, on blood biomarkers showed that serum levels of plasma brain natriuretic peptide (NT-proBNP) were elevated in these patients with higher risk of adverse cardiovascular outcomes and exercise intolerance.

In this thesis, I sought to harness the unique capabilities of cardiopulmonary exercise testing (CPET) combined with echocardiography to describe the impact of severe PR in adult survivors of TOF on cardiac structure, volume and haemodynamic functional performance with the mechanistic insights of echocardiography in a prospective cohort, as well as harnessing data from normal individuals to better understand RV augmentation. I critically described biventricular contractile reserve (CR), and provided the expected biventricular CR in patients with repaired TOF which linked to exercise capacity for the first time. Myocardial mechanics of the RV and LV are complex so I sought to understand these in more detail by developing new functional mechanical exercise markers which can describe the extent of functional limitations further. There is an increasing use of biomarkers in clinical practice, but how these relate to structural and functional parameters is less well described. Finally, many of the techniques I have used sometimes for the first time in this context are complex, so I have put a special emphasis on describing the reproducibility and coefficient of variables for these techniques.

11.2 Technical development

With the evolution of the IRLM-TOF study, I developed a new stress echocardiographic protocol as a research-based test in the cardiac department at St Bartholomew's Hospital, which now has been adopted in the clinical practice of the assessment of patients with repaired TOF and encouraged incorporating the stress echocardiography into Grown Up Congenital Heart Disease "GUCH" routine clinical examination at Barts Heart Centre. The results of IRLM-TOF attained from CPET combined with echocardiography were highly reproducible. Test-retest reproducibility for CPET showed an excellent reproducibly with minimal degree of variability. For stress echocardiography reproducibility, semiautomated 2D strain outperformed manual conventional echocardiographic measures during stress echocardiography with an excellent inter and intra-observer variabilities. The novel 4D biventricular functional and volumetric parameters during exercise were reliable, but with higher variability. A reproducibility study showed that contractile reserve of biventricular function, volume and strain parameters by 2D and 4D echocardiography can be performed with an acceptable reproducibility, whilst more caution should be applied to 4D volume analysis under strenuous conditions.

11.3 Key findings

11.3.1 Myocardial functional and mechanical augmentation during exercise; how do they predict exercise capacity?

In this main study, I found there was no difference in exercise performance between patients who have severe PR and those who do not have PR. The presence of severe PR does not influence impaired exercise capacity in this population. Despite the presence of right ventricular dilatation and severe volume overload in the severe PR group, exercise performance was similar to patients with no loading conditions. This is a very striking finding which undermines the use of PR severity, and RV volume as indicators of symptomatic treatment in this population, and this call into question the basis for the current indication for surgery or percutaneous intervention. Marked exercise capacity in adult survivors of TOF is more dependent on biventricular functional ability to augment longitudinal systolic function with stronger ability of LV (by LV systolic longitudinal function (LVS') and global longitudinal strain function (GLS)) to predicts exercise capacity. Opposite balanced functional and

volumetric biventricular contractile reserve response was observed in both groups in our cohort which explains the key finding of similar exercise intolerance, and ultimate failure of compensatory mechanisms in this population. This study highlighted the critical role of LV and RV remodelling in asymptomatic patients with repaired TOF for better explanation of functional limitations that could be a more appropriate target for risk stratification in this population.

11.3.2 Normal right ventricle contractile reserve in healthy individuals

In this sub study I have proposed the normal expected range of RV contractile reserve in verifiably healthy population that was gender related but not explained by size. The range of RV CR was much greater in healthy subjects than in patients with a deteriorated RV function, such as repaired TOF. We found a very important finding that RV longitudinal augmentation parameters were important determinants of exercise limitations in a population with abnormal RV, but not in normal subjects. I have developed a new exercise marker, RV longitudinal efficiency slopes (LES) for all RV longitudinal augmentation during stress echocardiography which explains the amount of rise of RV systolic function required to increase 1 unit of VO_2. These new developing slopes (RV longitudinal efficiency slopes) were shown to be powerful predictors of exercise capacity in patients with abnormal RV structure and function, but not in healthy subjects.

11.3.3 Complex myocardial mechanics assessment by novel LV myocardial work and mechanical dispersion in patients with repaired TOF

I sought to undertake this sub-study to investigate the novel LV myocardial work and biventricular mechanical dispersion in adult survivors of TOF. Data showed that all LV MW indices were abnormal in these patients at rest and during exercise that were associated with objective exercise measures irrespective of severity of pulmonary regurgitation. This emphasises the ongoing LV adverse remodelling and fibrosis in this population that require more attention. Biventricular mechanical dispersion was prolonged at rest with more pronounced dispersion during exercise observed and related to exercise intolerance. This study has uniquely proposed the novel myocardial work and dispersion which have a powerful ability to determine cardiopulmonary capability in adult survivors of TOF.

11.3.4 Contribution of LV longitudinal and strain functional augmentation to exercise intolerance

In this sub study, I found that the trajectory of LV systolic function by LVS' and GLS', and the augmentation per unit VO_2 were powerful exercise predictors in adult survivors of TOF. I have created new exercise mechanical slopes, one for LVS', named systolic efficiency slope (SES), and other for GLS which named myocardial efficiency slope (MES). MES describes for the first-time myocardial strain augmentation during exercise which reflects the incremental relationship between VO_2 and GLS. The SES which represents the LV incremental relationship between systolic augmentation (S') and VO_2 was more predictive of functional limitations than MES, but these unique exercise markers introduced for the first time imply stronger evidence into the key finding of left ventricular mechanisms and contribution to the known exercise intolerance in this population.

11.3.5 Blood biomarkers in adult patients with repaired TOF

I undertook this novel study to evaluate for the first time the combination of the most recommended laboratory biomarkers for supporting heart failure diagnosis which are the NT-proBNP, and soluble suppression of tumorigenicity-2 (sST2) in adult patients with repaired TOF. NT-proBNP levels were abnormal in 58% of population, while sST2 was abnormal in 40%, and the use of a transannular patch as the surgical technique was associated with higher biomarkers signal in all patients irrespective of the presence of PR. Elevated NT-proBNP serum level in patients with repaired TOF reflects changes in the RV while sST2 was more sensitive to changes in the LV and RV. We found that increased serum levels of NT-proBNP and sST2 were major contributors of reduced exercise tolerance, but NT-proBNP was a superior predictor in this population. These results form key biological findings in this population which confirmed that NT-proBNP and sST2 are more sensitive to existing pathology that can detect ongoing haemodynamic adverse changes for a better guide of treatment strategies.

11.4 Implication of findings: Clinical insights and future direction

The present thesis has critically demonstrated exercise markers as determinants of exercise capacity by combining stress echocardiography and CPET in adult survivors of TOF. Combining these techniques provided greater insights into biventricular subtle changes in asymptomatic patients with repaired TOF. Main study findings addressed several significant gaps in the literature by the description of myocardial deformation during exercise, introducing novel exercise determinants of functional limitations, and providing for the first time the expected range of bi-ventricular contractile reserve which all have strong ability to predict cardiopulmonary capability. Severe PR does not influence exercise capacity in this population which challenges the current management strategies that mainly rely on the onset of symptoms, severe PR or reaching specific cut-off RV volume. The extent of functional limitations in this study was explained by unique exercise predictors which was heavily dependent on biventricular longitudinal functional augmentation by (LVS'), (ΔLVGLS), and (ΔFAC). The data from this study with the highly reliable techniques suggests the test has a great potential into routine clinical care of adult survivors of TOF which can influence their management in terms of decisions surrounding intervention to correct severe PR.

Study 2 investigated a novel technique, LV myocardial work which was significantly abnormal and linked to reduced exercise tolerance, and this suggests that these patients are at higher risk of ongoing fibrosis which deserves further investigation. Additionally, we have clearly shown the existing abnormal biventricular mechanical dispersion that was exaggerated during exercise, suggesting the inhomogeneous LV and RV myocardial contraction in response to exercise. These are very exciting findings which have major clinical implication as an increased mechanical dispersion in addition to lower MW performance during exercise may identify which part of myocardium is at risk of arrhythmias and sudden cardiac death at earlier stage of disease progression in these patients. Study 3 expanded the methodology further for exercise prediction model by developing new exercise slope parameters that explain the shape and extent of myocardial augmentation of the left ventricle in this population during incremental exercise, named SES and MES of the LV systolic longitudinal function which can predict exercise limitations more superiorly than contractile reserve parameters. These slopes have major clinical importance as they do not require a prespecified maximal exercise or heart rate to be achieved which is important in our population who are known to often struggle to reach

their maximal effort. This suggests that these new markers may form the basis for clinical diagnostic tools which are suitable methods for assessing disease progression in adult survivors of TOF.

Study 4 included 40 healthy individuals, which proposed normal RV augmentation response that was gender but not age-related during exercise echocardiography compared to TOF population with compromise RV function. RV contractile reserve does not contribute to exercise capacity in healthy subjects, while the importance of contribution increases when RV function is distorted. Data from this study confirmed that assessment of RV functional augmentation during stress conditions is feasible and the magnitude of RV function during exercise should mirror symptomatic improvement in patients with pathological RV. Study 5 has powerfully demonstrated the plasma brain natriuretic peptide (NT-proBNP), and the novel soluble suppression of tumorigenicity-2 (sST2) which were marginally abnormal in a large percent of the population. It also provided a pivotal relationship between increased levels of serum blood biomarkers and reduced exercise intolerance in these patients, with stronger ability of NT-proBNP in exercise prediction. To date, their evaluation in this patient group is not routinely assessed for systematic categorisation used in risk assessment, but our data imply a new strategy into the clinical assessment of adult survivors of TOF by involving these novel blood biomarkers which are sensitive enough to detect functional deterioration into risk assessment in a low-risk TOF population.

The above studies highlighted the strong capability of biventricular longitudinal mechanistic performance, and functional augmentation in addition to blood biomarkers to predict exercise capacity which entirely introduced new way of looking at the prediction of exercise performance in adult survivors of TOF. The novel findings of this thesis call into the question some of the basis of current guidelines, particularly when seeking to quantify irreversible myocardial remodelling and predict outcome. This multi parametric approach including functional characteristics of contractile reserve, newly developing exercise markers, and blood biomarkers for better explanatory models to identify adverse LV, and RV remodelling can easily be incorporated into daily clinical practice in this population and by studying how these parameters perform in longitudinal studies of outcome, better treatment algorithms will emerge.

The complex relationship between haemodynamic echocardiographic parameters and CPET in patients with repaired TOF in addition to the healthy myocardium has been critically evaluated. Future work will extend these results to various cardiac patients developing the key findings achieved in this thesis. Studies extending these findings are already underway at St Bartholomew's Hospital including asymptomatic patients with severe aortic stenosis to enhance the clinical understanding of ventricular involvement into symptomatic progression in these patients, and in patients with primary mitral regurgitation to determine the optimal time cut-off for intervention. As demonstrated, there are a variety of ways to predict exercise intolerance of taking forward the key findings attained from the present thesis which will be valuable not only for research purposes, but in the long run for diagnosis, treatment strategies and ultimately for the benefit to the patient.

11.5 Conclusions

In this thesis I have demonstrated that the relationship between exercise ability and myocardial function is complex, but not predominantly effected by PR and is more a function of contractile reserve potential of the right and left ventricles. This thesis has shown deeper diagnostic insights into functional augmentation, mechanistic exercise markers, and blood biomarkers which powerfully explain the known functional limitations in a large TOF cohort. All novel exercise markers in addition to blood biomarkers were explained for the first time in this population that is not available by any other means. The results of this thesis challenging the current management strategies of repaired TOF patients which question the main focus of the current risk assessment in terms of decisions surrounding intervention.

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Appendices

Appendix A: HRA Ethical Approval

NHS
Health Research Authority

Ms Sahar Alborikan
Barts Heart Centre
St Bartholomew's Hospital
West Smithfield
London
EC1A 7BE

Email: hra.approval@nhs.net

16 February 2018

Dear Ms Alborikan

Letter of HRA Approval

Study title:	**Insights from Right and Left Ventricular Mechanics During Exercise Stress in Relation to Functional Capacity and Recovery Following Surgery in Patients with Tetralogy of Fallot and Pulmonary Regurgitation.**
IRAS project ID:	**232328**
REC reference:	**18/LO/0092**
Sponsor	**Barts Health NHS Trust**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

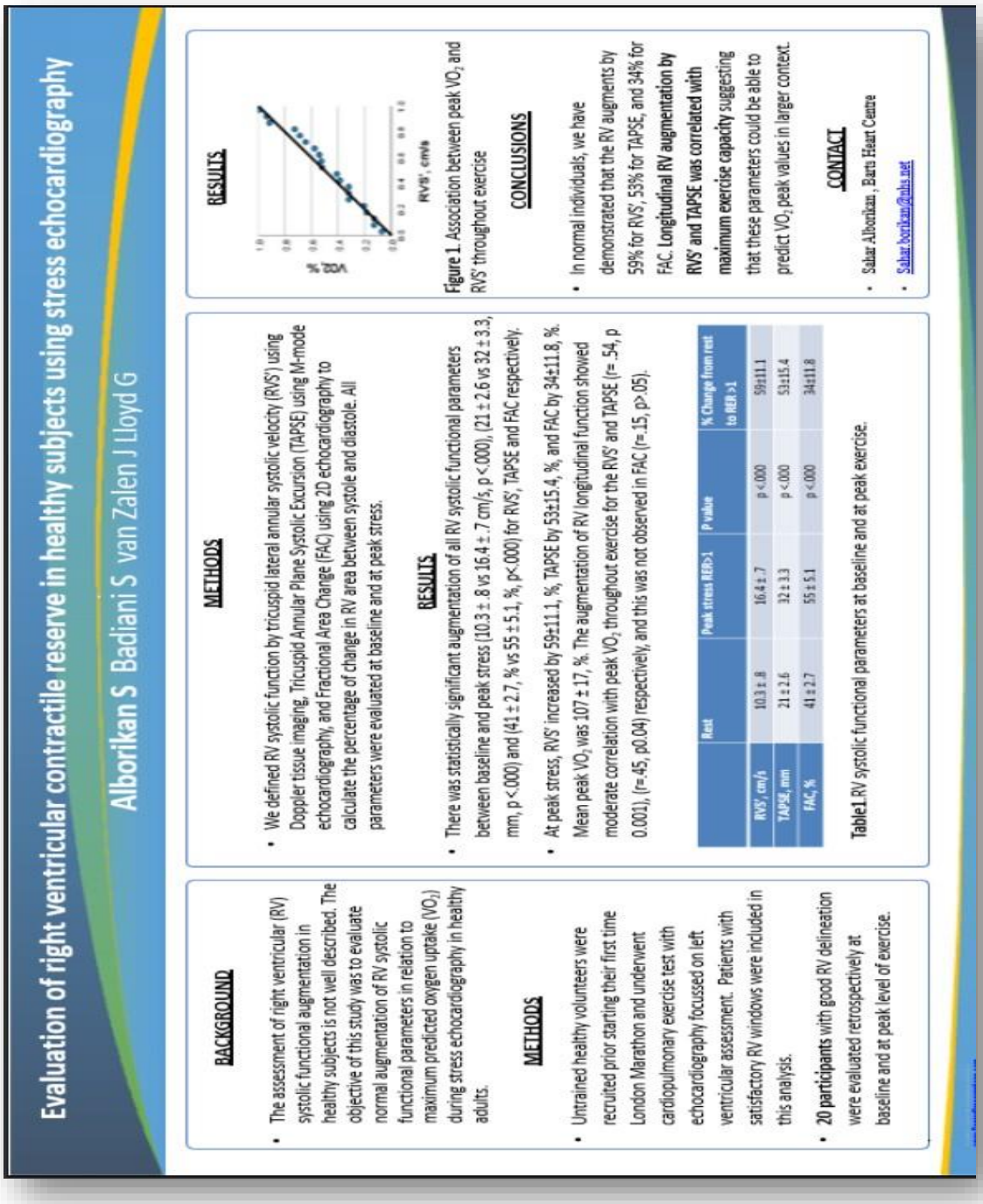
The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

Appendix B: Conference poster publications



Evaluation of right ventricular contractile reserve in healthy subjects using stress echocardiography

Alborikan S Badiani S van Zalen J Lloyd G

BACKGROUND

- The assessment of right ventricular (RV) systolic functional augmentation in healthy subjects is not well described. The objective of this study was to evaluate normal augmentation of RV systolic functional parameters in relation to maximum predicted oxygen uptake (VO_2) during stress echocardiography in healthy adults.

METHODS

- Untrained healthy volunteers were recruited prior starting their first time London Marathon and underwent cardiopulmonary exercise test with echocardiography focussed on left ventricular assessment. Patients with satisfactory RV windows were included in this analysis.
- **20 participants** with good RV delineation were evaluated retrospectively at baseline and at peak level of exercise.

METHODS

- We defined RV systolic function by tricuspid lateral annular systolic velocity (RVS') using Doppler tissue imaging. Tricuspid Annular Plane Systolic Excursion (TAPSE) using M-mode echocardiography, and Fractional Area Change (FAC) using 2D echocardiography to calculate the percentage of change in RV area between systole and diastole. All parameters were evaluated at baseline and at peak stress.

RESULTS

- There was statistically significant augmentation of all RV systolic functional parameters between baseline and peak stress (10.3 ± .8 vs 16.4 ± .7 cm/s, p <.000), (21 ± 2.6 vs 32 ± 3.3, mm, p <.000) and (41 ± 2.7, % vs 55 ± 5.1, %, p<.000) for RVS', TAPSE and FAC respectively.
- At peak stress, RVS' increased by 59±11.1, %, TAPSE by 53±15.4, %, and FAC by 34±11.8, %. Mean peak VO_2 was 107 ± 17, %. The augmentation of RV longitudinal function showed moderate correlation with peak VO_2 throughout exercise for the RVS' and TAPSE (r= .54, p 0.001), (r=.45, p0.04) respectively, and this was not observed in FAC (r=.15, p> 05).

	Rest	Peak stress RER>1	P value	% Change from rest to RER >1
RVS', cm/s	10.3 ± .8	16.4 ± .7	p <.000	59±11.1
TAPSE, mm	21 ± 2.6	32 ± 3.3	p <.000	53±15.4
FAC, %	41 ± 2.7	55 ± 5.1	p <.000	34±11.8

Table1 RV systolic functional parameters at baseline and at peak exercise.

RESULTS

Figure 1. Association between peak VO_2 and RVS' throughout exercise

CONCLUSIONS

- In normal individuals, we have demonstrated that the RV augments by 59% for RVS', 53% for TAPSE, and 34% for FAC. **Longitudinal RV augmentation by RVS' and TAPSE was correlated with maximum exercise capacity** suggesting that these parameters could be able to predict VO_2 peak values in larger context.

CONTACT

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BACKGROUND AND OBJECTIVES

- Cardiopulmonary exercise testing (CPET) provides a comprehensive objective assessment in patients with repaired Tetralogy of Fallot (rTOF). However, the evidence underpinning this practice is scanty as are the mechanisms which drive exercise ability.
- To describe the current evidence linking CPET data and prognosis in addition to review the most important determinants of exercise.

METHODS

- The preferred reporting items (PRISMA) guidelines were followed.
- A systematic search of CPET studies, with/without echo-cardiography was carried out on PubMed MEDLINE, EBM review-Cochrane Database, Wiley Online library and EBM reviews.

RESULTS

- Of 400 studies identified, 21 met the inclusion criteria. 17 articles (81%) were reported in young adults, and 4 articles (19%) in younger group. The sample size ranged from 15 to 875, and the publication year ranged from 2002 to 2019. Mean age was 25 ± 7 years.
- Overall mean predicted VO2peak was 68 ± 2.8% (95% CI. 62.3–74%). There was no difference in mean predicted VO2 between older and younger groups (68 ± 2.7 vs 69 ± 2.9,%, p > 0.05). Peak predicted VO2 was found to be higher in contemporary studies than historic investigations (76 ± 6 vs 59 ± 13,%, p.001).
- Submaximal measures were rarely reported. Determinants of exercise capacity were reported in 9 studies (43%). Prognostic findings qualitatively suggested that mild exercise intolerance with preserved ventilatory-equivalent for carbon dioxide is associated with better outcomes and lower mortality rate.

RESULTS

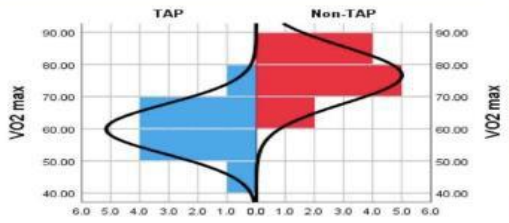

Figure 1. Distribution of exercise capacity in TAP and non-TAP studies, **TAP**= studies with transannular patch; **non-TAP**= studies with other type of surgery)e.g. pulmonary valvotomy) or not known.

CONCLUSION and SUMMARY

- The literature showed a high degree of heterogeneity which limited comparability. Marked reduction in functional capacity in patients with repaired TOF seems to be more dependent on surgical selection and developing technique than advancing age.

Queen Mary University of London

Barts Health NHS Trust