Early Versus Later Re-valving in Tetralogy of Fallot with Free Pulmonary Regurgitation

Combined cross-sectional and prospective, multi-centre, randomized, parallel-group clinical trial evaluating the efficacy and safety of early re-valving in patients with Tetralogy of Fallot and free pulmonary regurgitation compared to current guideline practice

National Danish research project involving:

Rigshospitalet, Copenhagen
Odense University Hospital
Aarhus University Hospital

Contact person:

Lars Søndergaard
Professor of Cardiology, DMSc
The Heart Centre, Section 2011
Rigshospitalet
Blegdamsvej 9
2100 Copenhagen, Denmark
E-Mail: lars.soendergaard.01@regionh.dk
Phone: +45 35458693 (work), +45 28777161 (private)

Projektidentifikation: H-18016868

Table of Contents

Appendices

Appendix 1: Additional considerations regarding the inclusion criteria and additional information about clinical and paraclinical test procedures

Appendix 2: Abbreviated study protocol

Appendix 3: **Appendices for sub-study 1**

Patient information for adults (Deltagerinformation til voksne)

Patient information for patients aged 15-17 (Deltagerinformation til 15- til 17-årige)

Patient information for children (Deltagerinformation til børn)

Consent form for adults **(**Samtykkeerklæring til voksne)

Consent form for children (Samtykkeerklæring til børn)

Medical power of attorney form (Fuldmagtserklæring)

Appendix 4: **Appendices for sub-study 2**

Patient information for adults (Deltagerinformation til voksne)

Patient information for patients aged 15-17 (Deltagerinformation til 15- til 17-årige)

Patient information for children (Deltagerinformation til børn)

Consent form for adults **(**Samtykkeerklæring til voksne)

Consent form for children (Samtykkeerklæring til børn)

Medical power of attorney form (Fuldmagtserklæring)

Appendix 5: Questionnaires

EQ-5D-3L

EQ-5D-Y

Background

Anatomy and classification

Tetralogy of Fallot (ToF) is a congenital heart disease (CHD) characterized by four anatomic features, consisting of 1) ventricular septal defect (VSD), 2) pulmonary stenosis and 3) dextroposition of the aorta, which arise due to anterior and cephalad deviation of the infundibular septum and 4) right ventricular hypertrophy. The anatomy was first discovered by the Danish anatomist Steno in 1673 and later described more specifically by Fallot in 1888. Four anatomic variants of ToF are considered by The International Society for Nomenclature of Paediatric and Congenital Heart Disease and outlined in The International Paediatric and Congenital Cardiac Code: 1) ToF with pulmonary stenosis, 2) ToF with pulmonary atresia, 3) ToF with common atrioventricular canal and 4) ToF with absent pulmonary valve. Tof with pulmonary stenosis which is the most common type will be addressed in this study.

Etiology and genetics

Although ToF may present af part of a known syndrome, this lesion typically occurs sporadically without other anomalies. In a study of 114 patient with non-syndromic ToF, 4 % had mutations in transcription factor NKX2.5, which appears to have a role in cardiac development. In genome-wide surveys of patients with non-syndromic ToF and their parents, de novo copy number variants were estimated to be present in approximately 10 % of sporadic cases of ToF compared with less than 0.1 % in controls at several chromosomal locations. Several reports have associated ToF with mutations in TBX1 and ZFPM2. MTHFR polymorphisms have also been associated with an increased risk of ToF development.

Further investigation is required to determine the roles of these mutations and polymorphisms in the evolution of ToF.

Approximately 15 % of patients with ToF present with associated syndromes:

1) Down syndrome (trisomy 21)
2) Alagille syndrome (JAG1 mutations). ToF as sole manifestation of JAG1 mutations without other evidence of Alagille syndrome has also been reported.
3) DiGeorge and velocardiofacial syndromes (deletions on chromosome 22q11). There may be susceptibility genes for ToF within the latter region of chromosome 22q11 in children without extracardiac anomalies, and 22q11.2 deletions syndrome is unrecognized in many adult patients with ToF. In one study patients with 22q11 deletion had worse surgical outcomes due to less favorable anatomy of the pulmonary arteries.

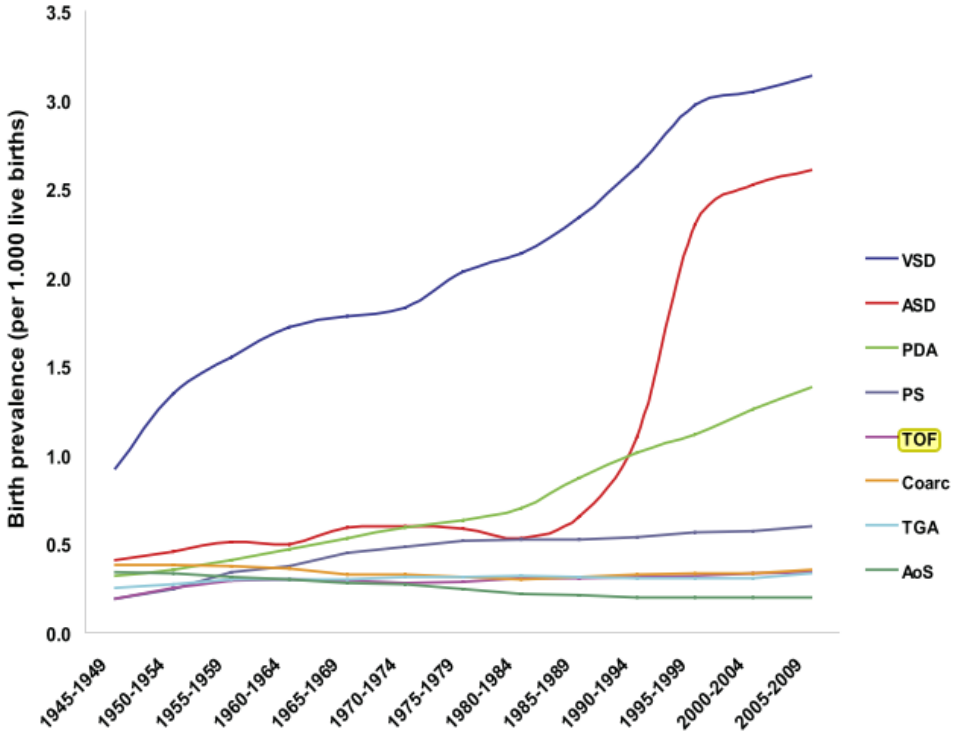
Identification of disease-related genes and associated syndromes provides critical information for diagnosis and a fundamental understanding of cardiac development, as well as important information for counselling of patients with ToF or their parents.

Epidemiology

CHD is the leading cause of infant mortality and the most common congenital malformation, accounting for about one third of all congenital anomalies.[1–3] The best estimate of birth prevalence for CHD based on large systematic reviews is 8-9 per 1.000 live births, corresponding to 1.35 million newborn worldwide with CHD every year[1,4]. Due to the last 70 years of progress in diagnosis, management and care of patients with CHD, the majority of affected children reach adulthood, which has resulted in a substantial rise in prevalence of grown-ups with congenital heart diseases (GUCH) over the last decades, with growth estimates around 3-5

% annually.[5,6] As such CHD represent a major global health burden. There is some evidence that we might see a further increase in the birth prevalence of CHD as rising numbers of women in the western world delay childbearing age and as the offspring of the growing GUCH population is at higher risk of congenital heart anomalies.[7,8] Fetal echocardiography is emerging as an important element in ante- and postnatal treatment of structural heart diseases.[9] Nevertheless, the impact on incidence and prevalence of CHD has yet to be evaluated. In a novel, as yet unpublished Danish study (Rebbekka Lytzen, PhD thesis) the termination rate of pregnancies diagnosed with ToF from 1996 to 2013 was only 7 %. Even though we expect antenatal detection rates for CHD to increase with advances in ultrasound screening, it remains questionable whether termination rates will rise proportionally in case of isolated well-manageable anomalies such as ToF, with an ever-improving prognosis. The worldwide development in birth prevalence of the most common CHD subtypes is displayed in Figure 1. One should note the ongoing increase for almost every CHD subtype.

Figure 1. Time Course of the Eight Most Common Congenital Heart Disease Subtypes From 1945 to 2010.

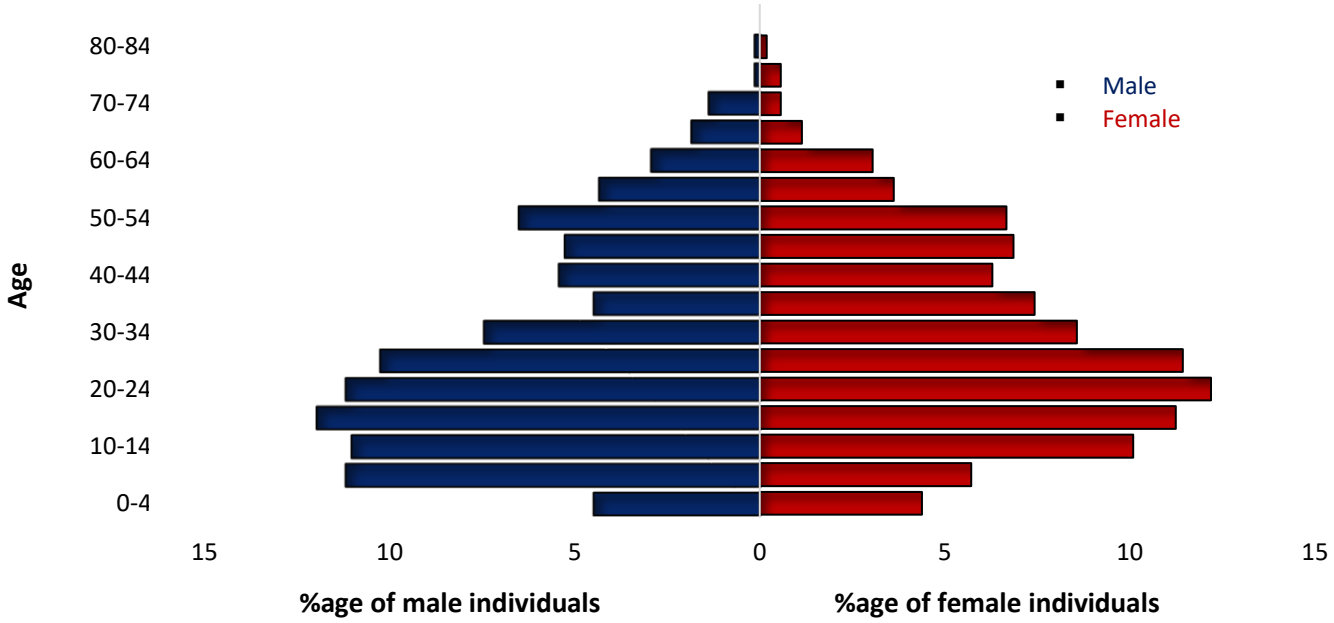


AoS = aortic stenosis; ASD = atrial septal defect; Coarc = coarctation; PDA = patent ductus arteriosus; PS = pulmonary stenosis; TGA = transposition of the great arteries; TOF = Tetralogy of Fallot; VSD = ventricular septal defect.

Adapted from "Birth Prevalence of Congenital Heart Disease Worldwide", Van der Linde et al., Journal of The American College of Cardiology, 2011, Volume 58).[4]

A birth prevalence of 2-5 per 10.000 live births is reported for ToF in international studies.[1,4] According to the Danish National Patient Registry there are currently 1.170 patients with Tetralogy of Fallot in Denmark. 55 % are male and 45 % are female with a median age of 26 years (25th to 75th percentile: 14-42 years) (Figure 2).

Figure 2. Age distribution of patients with Tetralogy of Fallot in Denmark. *The %age of the different male (blue bars) and female (red bars) age groups of the total male and female cohort are displayed on the X-axis. The age groups are displayed on the Y-axis.*



The median number of liveborn with ToF per year in Denmark between 1970 when echocardiography became widely available in clinical practice, to 2017 was 26 (25th to 75th percentile: 23-33 liveborn with ToF) (Figures 3 and 4).

Figure 3. Liveborn with Tetralogy of Fallot in Denmark (1900 - 2017). *The corresponding year is displayed on the X-axis and the total number of liveborn each year on the Y-axis. The years with the lowest and highest number of liveborn with ToF are hightlighted with red.*

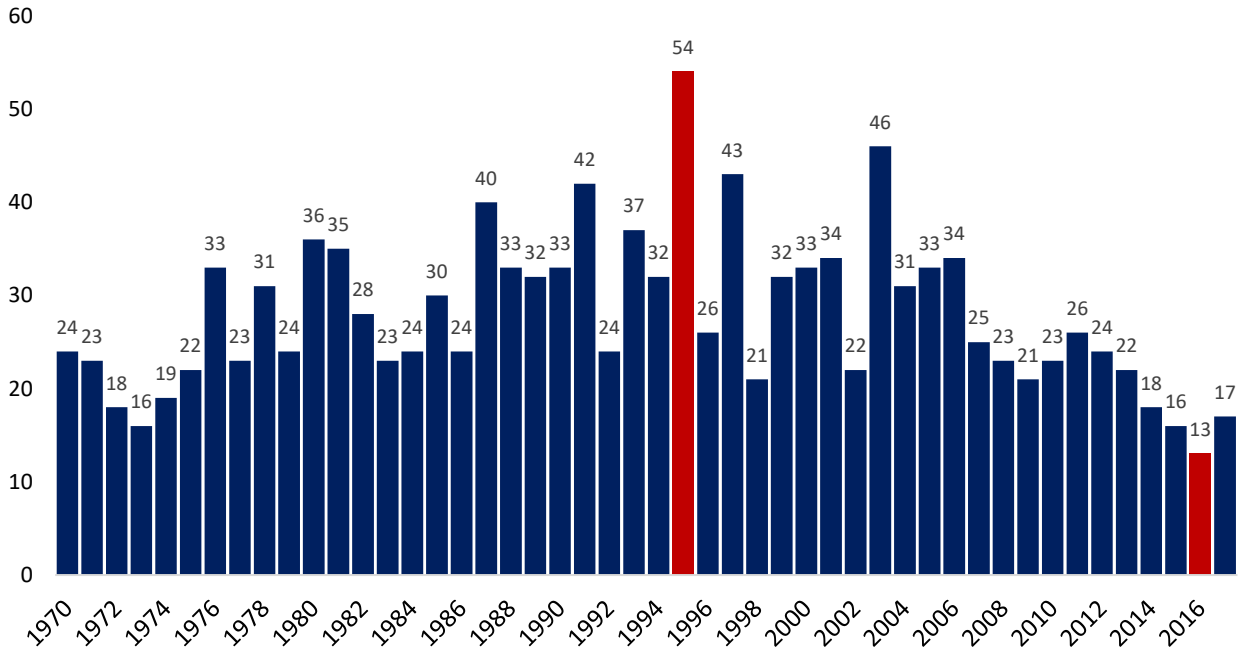
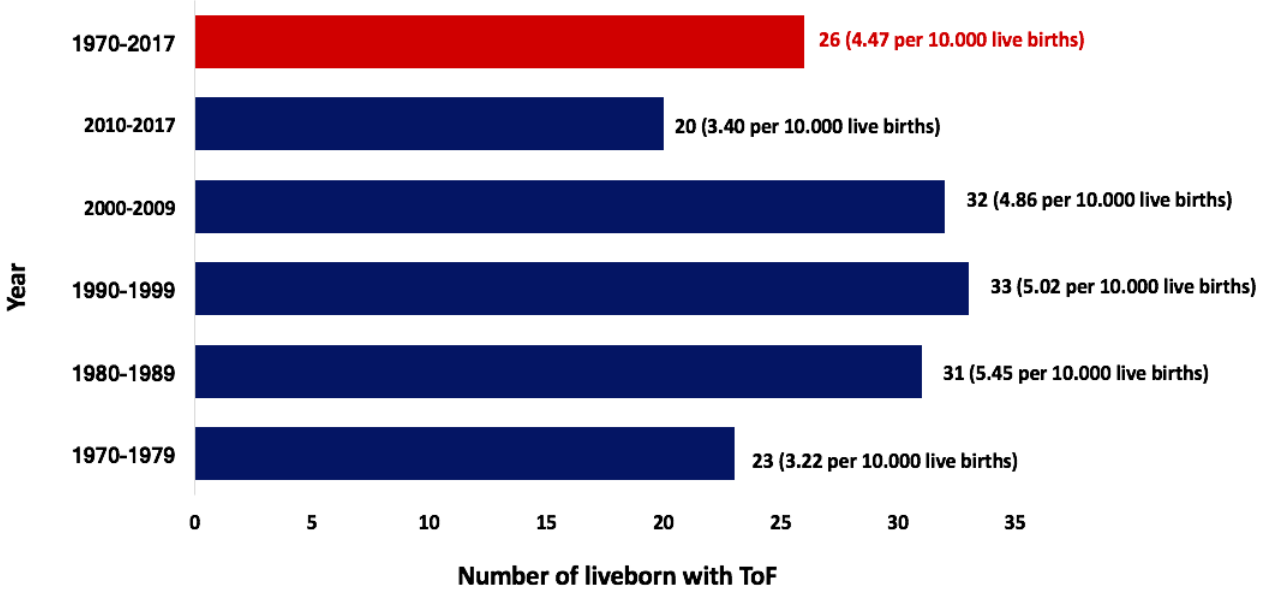


Figure 4. Liveborn with Tetralogy of Fallot in Denmark per 10.000 Live Births (1970-2017). *The median number of liveborn with Tetralogy of Fallot per year aggregated for different periods and median number of liveborn with Tetralogy of Fallot per 10.000 live births is displayed on the X-axis. The different time periods are displayed on the Y-axis.*



Management

Successful surgical therapy using a palliative subclavian-to-pulmonary-artery shunt was first reported by Blalock and Taussig in 1945. Almost ten years later the first intra-cardiac corrective repairs with resection of the pulmonary stenosis and closure of the VSD were performed by Lillehei in 1954, using another patient as oxygenator during surgery, and by Kirklin in 1955 using a pump oxygenator. In 1959 Warden and Lillehei introduced the use of a patch for enlargement of the right ventricular outflow tract (RVOT) and subsequently the first reconstruction with an aortic homograft was performed by Ross and colleagues in 1966. RVOT reconstructions with valved conduits were first performed in Denmark in the 1970's. In the current era, most patients undergo intra-cardiac repair during the first year of life. This is often done by VSD-closure witch a patch, resection of the subvalvular pulmonary stenosis and commissurotomy of the pulmonary valve. However, in up to 50 % of the patients the pulmonary annulus is too small and a transannular patch (TAP) is inserted to achieve relief of outflow tract obstruction, although resulting in severe/free pulmonary regurgitation (PR).[10] In some of these patients valve-sparing repair is applied instead of using a TAP, although this approach is associated with the risk of residual pulmonary stenosis when used in case of a small RVOT.[11] Furthermore, although early outcomes after valve-sparing surgery might seem favourable, studies suggest that over time most patients experience PR in the same range as those repaired with a TAP. Extra-cardiac palliative shunts are no longer commonly applied, but may be needed in infants with severe outflow tract obstruction who are not suited for intra-cardiac repair (i.e. due to prematurity or small pulmonary arteries) or in those with anatomic variants (i.e. ToF with pulmonary atresia). Later in life, most patients require RVOT reconstruction with a valved conduit due to symptoms arising from longstanding PR. The most commonly used conduits are pulmonary and aortic homografts or bovine jugular vein grafts (Contegra® Pulmonary Valved Conduit, Medtronic Inc., Minneapolis, Minnesota). Due to time-dependent conduit deterioration, patients require re-intervention at some point. In the majority of cases, this re-valving can be performed percutaneously with a transcatheter valve (Melody® Transcatheter Pulmonary Valve, Medtronic Inc., Minneapolis, Minnesota).

Follow-up

The use of of paraclinical testing in Tetralogy of Fallot is directed towards assisting with surgical planning during the neonatal period and later in life, individualized supportive medical treatment, as well as prediction of long-term morbidity and mortality.

Strategies for investigation of anatomy and physiology in patients with congenital heart defects are changing rapidly, with a shift from invasive studies to non-invasive protocols involving imaging studies such as echocardiography, cardiovascular magnetic resonance imaging (CMR), computed tomography, evaulation of arrhythmias using electrocardiograms, Holter-monitoring, event recorders and eventually electrophysiology testing as well as cardiopulmonary exercise testing (CPET) which has gained particular importance in the longitudinal follow-up and timing of intervention and re-intervention. Due to the ongoing improvement of these non-invasive methods, the use of invasive studies, such as cardiac catherization can often be reserved for resolution of specific anatomical and physiological qestions, or for intervention.

In adult patients with heart failure but without congenital heart defects, the role of cardiac biomarkers has increased exponentially over the last decades, including markers of myocardial injury and stress (e.g. cardiac troponins, creatine kinase, B-type natriuretic peptide and the N-terminal segment of pro-B-type natriuretic peptide, ST2, serum heart-type fatty-acid binding protein, adrenomedullin, growth differentiation factor 15), as well as markers of cardiac remodelling and fibrosis (e.g. connective tissue growth factor, type 1 procollagen C-terminal propeptide, carboxy-terminal telopeptide of collagen type I, tissue-inhibitor of metalloproteinases-1). The role of these biomarkers is emphasized in the current guidelines, as they can reflect important biological processes, but also because these biomarkers open up the possibility of possible therapeutic targets.

There is however, a substantial lack of data regarding the validity of biochemical biomarkers in patients with congenital heart defects, which is demonstrated by an almost complete abscence of recommendations for the utilization of biomarkers in the most recent guidelines for the management of grown- up congenital heart disease from the European Society of Cardiology.[12]

Aims and hypotheses

Even though management and care of CHD has improved, they are still associated with increased long-term morbidity, usually requiring life-long follow up and death rates twice to seven times higher compared to the general population.[13–15] Free PR as a consequence of RVOT repair with a TAP is the most significant haemodynamic lesion in patients suffering from sudden cardiac death late after initial repair of ToF.[16] Furthermore, PR is correlated to right ventricular dilatation and dysfunction, exercise intolerance and supra-ventricular as well as ventricular arrhythmias. The importance of maintaining a competent pulmonary valve has been emphasized, and preservation or reconstruction of pulmonary competence at an appropriate point in time has resulted in haemodynamic improvement of patients with PR after repair of ToF. However, optimal timing of re-valving in patients with ToF and free PR remains to be established in order to avoid irreversible impairment of right ventricular function. Although delayed intervention may result in ventricular dysfunction and fibrosis, valve replacement is associated with risks, including procedure related complications, altered ventricular geometry, infective endocarditis as well as prosthetic valve deterioration and the need for re-intervention.

American, European and Canadian guidelines for re-valving in asymptomatic patients with free PR after repair of ToF recommend re-valving when the right ventricle end-diastolic volume index (RVEDVi) has nearly doubled (> 160 ml/m2), the exercise capacity declines or when arrhythmias arise (Table 1).

Table 1. Summary of published guidelines for surgical pulmonary valve replacement in the asymptomatic patient.

Criteria	AHA [13]	ESC [11]	CCS [12]
RVEDVi	≥Moderate	>160 mL/m^2	>170 mL/m^2
RVESVi	Not specified	Not specified	Not specified
RV function	≥Moderate RV dysfunction	Progressive RV dysfunction	≥Moderate RV dysfunction
RVOT obstruction[a]	PIG ≥50 mm Hg or RV/LV pressure ratio ≥0.7	PIG ≥80 mm Hg (4.3 m/s)	RV systolic pressure ≥2/3 systemic pressure
PR[a]	Severe	Severe	Free
TR	≥Moderate	≥Moderate	"Important"
QRS duration	Not specified	>180 msec	Not specified
Arrhythmia	Symptomatic or sustained AT or VT	Sustained AT or VT	AT or VT
Exercise cardiopulmonary function	Not specified	Objective decrease	Not specified
Other considerations	Significant residual VSD or AR	Not specified	Significant residual VSD

It may be argued that these guidelines are too conservative, as more than half of the patients reaching an RVEDVi > 160 mL/m2 do not reverse-remodel to normal right ventricular size or recover from reduced right ventricular function after re-valving.[17,18] Furthermore, pulmonary valve replacement does not have major impact on exercise impairment or the risk of ventricular tachycardia, at least when performed in adults with ToF and long-standing free PR.[19]

The current guidelines are based solely on retrospective studies, rather than prospective controlled trials. The aims of this study are to evaluate the long-term impact of free PR on right ventricular dimensions, function, fibrosis, exercise capacity and arrhythmias, as well as to compare outcomes and safety of an early re-valving strategy versus current guideline practice in patients with ToF initially repaired with a TAP.

Based on the abovementioned, our main hypotheses are, that in patients after repair of ToF using a TAP:

1. The duration of PR is correlated with progressive right ventricular dysfunction, dilatation and fibrosis, as well as impaired exercise capacity and increased burden of arrhythmias.
2. The earlier re-valving is performed, the greater is the chance of beneficial reverse remodelling of the right ventricle, preservation of exercise capacity and absence of arrhythmia.
3. Early re-valving is safe with regards to procedural outcome and infective endocarditis (Appendix 1).

Study design

The study will be divided in two sub-studies:

Sub-study I: Cross-sectional evaluation of the impact of long-standing PR after initial repair of ToF with pulmonary stenosis.

Sub-study II: Randomized clinical trial comparing an early and later re-valving strategy in patients with ToF and free PR after repair with a TAP.

A detailed description of the sub-studies is given in the following sections.

Sub-study I: Cross-sectional evaluation of the impact of long-standing PR after initial repair of ToF with pulmonary stenosis.

Aims

1. To compare right ventricular dimensions and function, functional class, exercise capacity, presence of sustained arrhythmia, biochemical biomarkers of myocardial injury, myocardial stress, remodelling and fibrosis as well as other measures at different ages in patients with ToF repaired using a TAP compared to patients with ToF who have undergone valve-sparing repair in the Danish cohort of patients with ToF.
1. To evaluate whether the patient age (early: 10-18 years of age, intermediate 19-27 years of age, late: > 27 years of age) at re-valving in patients with ToF repaired with a TAP affects right ventricular remodelling, functional class, exercise capacity, burden of arrhythmia, biochemical biomarkers of myocardial injury, myocardial stress, remodelling and fibrosis, as well as assessment of the correlation between biomarkers of fibrosis to fibrosis assessed by CMR.
2. and other measures compared with patients repaired with a valve-sparing approach.
3. To evaluate the safety of re-valving in patients with ToF repaired with a TAP with regards to post-operative mortality, stroke, acute kidney impairment, endocarditis etc.
4. To evaluate the durability of the bioprosthetic pulmonary valve using time to re-intervention as end-point. Furthermore, to assess whether the patients' age (early: 10-18 years of age, intermediate 19-27 years of age, late: > 27 years of age) at the time of re-valving affects the durability of the bioprosthesis.

Inclusion criteria

1. ToF with pulmonary stenosis repaired with or without TAP during the first 2 years of life.

Exclusion criteria

1. ToF with pulmonary atresia, ToF with common atrioventricular canal, ToF with absent pulmonary valve syndrome, major aortopulmonary collateral arteries and other significant associated anomalies.
2. Palliation with a shunt (Blalock-Taussig or central) at any time.
3. Co-morbidity preventing exercise testing (e.g. genetics, neuro-cognitive dysfunction, physical disability).
4. Contraindication for CMR (e.g. permanent pacemaker, intra-cardiac defibrillator, intracranial ferro-magnetic device).
5. Pregnancy at time of inclusion.
6. Age < 12 or unable to comply with instructions given during CMR or exercise testing.

Study population

Of the 1.170 living patients with ToF in Denmark 947 patients (81 %) are 12 years of age or older and as such potential candidates for the cross-sectional study assuming all other conditions are satisfied. 503 (53 %) are male and 444 (47 %) are female. Median age is 29 years (range 12-82 years, IQR 21-46 years). 50 % of the patients are living in the Capital Region of Denmark and Zealand, 18 % in the Southern Denmark Region, 23 % in the Central Region of Denmark and the remaining 9 % are living in the Noth Denmark Region.

Methods

The following data will be collected:

1. Patient demographics.
2. Medical history including surgical notes.
3. Basic assessment in order to rule out significant neuro-cognitive dysfunction, factors limiting the ability to perform exercise testing and contra-indications for CMR.
4. NYHA/functional-class (Appendix 1) and occurrence of arrhythmias assessed during interview.
5. Health-related quality of life assessed using a generic measure (EQ-5D-3L). The youth version of the EQ-5D-3L (EQ-5D-Y) is used for patients aged 12-15 and allows for later mapping onto the corresponding adult scale. The generic questionnaires are available in Danish.

The following will be performed and obtained:

1. ECG
2. Blood samples (Appendix 1)
3. 24-hour Holter-monitoring
4. Transthoracic echocardiography (Appendix 1)
5. Cardiac CMR (Appendix 1)
6. Cardio-pulmonary exercise testing (Appendix 1)

Statistical considerations

Efficacy

Several small and uncontrolled CMR studies have evaluated the effect of re-valving on right and left ventricular dimensions and function in patients with ToF initially repaired with a TAP (Table 2). Little is known regarding long-term outcome for these patients compared to patients who have undergone valve-sparing repair. Therefore, this study is of explorative nature with the purpose to acquire more knowledge about the magnitude of these important issues. We expect about half of the patients in the Danish ToF cohort who are 12 years of age or older to be included in the study (approximately 500 patients).

Table 2. Effects of pulmonary valve replacement on ventricular mechanics, QRS duration, peak oxygen consumption and functional class.

	PR (%)		RVEDVi (ml/m²)		RVESVi (ml/m²)		RV EF (%)		LVEDVi (ml/m²)		LV EF (%)		QRS duration (ms)		Peak O_2 consumption (ml/kg/min)		NYHA class	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Vliegen et al. [97] N = 26 Age: 29 ± 9 years	46 ± 10	4 ± 8	167 ± 40	114 ± 35	99 ± 36	66 ± 35	42 ± 10	42 ± 11	86 ± 29	87 ± 17							2.0 ± 0.6	1.3 ± 0.5
Therrien et al. [15] N = 17 Age: 32 years			163 ± 34	107 ± 26	109 ± 27	69 ± 22	32 ± 7	34 ± 10									2.0 ± 1.0	1.4 ± 0.5
van Straten et al. [148] N = 16 Age: 29 years	48 ± 10	3 ± 5	164 ± 43	113 ± 26	94 ± 33	61 ± 18	44 ± 8	47 ± 12										
Doughan et al. [84] N = 21 Age: 34 ± 9 years													153 ± 34	142 ± 29				
Buechel et al. [16] N = 20 Age: 14 ± 3 years	49 ± 14	9 ± 8	190 ± 33	109 ± 26	102 ± 27	58 ± 16	47 ± 7	45 ± 9	77 ± 10	84 ± 12	53 ± 6	56 ± 7	150 ± 18	148 ± 17				
Henkens et al. [98] N = 27 Age: 31 ± 8 years	48 ± 11		166	100	98	58	42 ± 10	43 ± 10	89 ± 31	87 ± 18	56 ± 12	55 ± 9					2.0 ± 0.6	1.3 ± 0.3
Oosterhof et al. [93] N = 71 Age: 29 years	44 ± 13	5 ± 9	171 ± 44	119 ± 34	102 ± 38	70 ± 29	42 ± 10	43 ± 10	85 ± 22	94 ± 20	52 ± 9	53 ± 8	155 ± 29	144 ± 29			53% grade ≥II	11% grade ≥II
Gengsakul et al. [99] N = 82 Age: 28 ± 13 years													164 ± 21	168 ± 21			54% grade ≥II	13% grade ≥II
Frigiola et al. [94] N = 71 Age 22 ± 11 years	41 ± 9	5 ± 7	142 ± 43	91 ± 18	73 ± 33	43 ± 14	51 ± 10	54 ± 7	66 ± 12	73 ± 13	61 ± 8	64 ± 7			25 ± 10	25 ± 9	2.0	1.0
Geva et al. [95] N = 64 Age: 21 years	49 ± 11	5 ± 9	201 ± 37	123 ± 25	107 ± 29	68 ± 24	47 ± 8	45 ± 9	89 ± 15	94 ± 17	58 ± 8	57 ± 7	154 (82-200)	150 (80-202)	26.5 (8-47)	27 (10-48)	47% grade ≥II	8% grade ≥II

Adapted from "Repaired Tetralogy of Fallot: The Roles of Cardiovascular Magnetic Resonance in Evaluating Pathophysiology and for Pulmonary Valve Replacement Decision Support", Geva, Journal of Cardiovascular Magnetic Resonance, 2011, Volume 13).[20]

Data analysis

Using propensity score matching for sex and age at follow-up, patients initially repaired with a TAP will be compared to patients treated with valve-sparing surgery with regards to:

1. NYHA functional class
2. Right and left ventricular dimension (RVEDVi, RVESVi, LVEDVi, LVESVi) and function (PR, RVEF, LVEF) measured by cardiac CMR
3. Localized (late myocardial enhancement) and diffuse (increased septal and left ventricular free wall extracellular volume) fibrosis measured by cardiac CMR
4. Peak VO2 and exercise time
5. Presence or absence of arrhythmia
6. NT-proBNP, TnT and CK-MB levels
7. Quality of life
8. Number of children for female patients

Furthermore, for patients initially repaired with a TAP and later re-valved, the safety of re-valving will be evaluated with regards to:

1. Procedural mortality (within 30 days after surgery)
2. Disabling stroke
3. Infective endocarditis
4. Longevity of the right ventricular to pulmonary artery conduit (time to re-intervention)

Sub-study II: Randomized clinical trial comparing an early and later re-valving strategy in patients with ToF and free PR after repair with a TAP.

Aims

The primary endpoints for the randomized trial are efficacy and safety of early re-valving, defined as:

2. Primary efficacy endpoint:
   RVEDVi is 35 mL/m2 less in the early re-valving group, compared to the later re-valving group three years after randomization

2. Primary safety endpoint:
   All-cause mortality within three years after randomization is less than 0.6 % in the early re-valving group

Secondary endpoints in both groups are assessed once every year, for at least ten years after randomization, with the exception of procedure related endpoints (1-3), which are only registered 30 days after surgery. The secondary endpoints are defined as:

1. Procedure related bleeding categorized as minor, major and life-threatening (BARC)
3. Procedure related vascular complications categorized as minor and major
4. Procedure related acute kidney injury categorized as stage one, two and three
5. Composite rate of all-cause mortality and disabling stroke
6. Cardiovascular mortality
7. Disabling stroke or transient ischemic attack
8. NYHA/functional class (Appendix 1)
9. Health-related quality of life will be assessed annularly using a generic measure (EQ-5D-3L) and temporal developments described. The youth version of the EQ-5D-3L (EQ-5D-Y) is used for patients aged 12-15 years and allows for later mapping onto the corresponding adult scale. The generic questionnaires are available in Danish.
10. New sustained supraventricular or ventricular tachyarrhythmia
11. Exercise capacity measured as peak-VO2 and exercise time (Appendix 1)
12. Right and left ventricular re-modelling (EDVi, ESVi, EF, myocardial mass) and fibrosis (assessed by CMR and analysis of endomyocardial biopsies (Appendix 1))
13. Levels of biochemical biomarkers of myocardial injury, myocardial stress, remodelling and fibrosis, as well as assessment of the correlation between biomarkers of fibrosis to fibrosis assessed by CMR and analysis of endomyocardial biopsies.
14. Time-related prosthetic valve safety (structural valve deterioration, infective endocarditis, prosthetic valve thrombosis)
15. Number of contacts to the Health System (hospital and general practitioner)
16. Number of children for female patients

Study population

The study will include Danish patients who give informed consent and fulfill the inclusion and exclusion criteria. Patients included in the cross-sectional study, who are interested in participating in the randomized study, will be included consecutively if suitability with regards to the inclusion and exclusion criteria for the randomized trial is fulfilled. Of the 1.170 living patients with ToF in Denmark, 707 have not yet undergone pulmonary valve replacement and are 12 years of age or older. As such 707 Danish patients

with ToF are possible candidates for the randomized clinical trial assuming all other conditions are satisfied. 360 (51 %) are male and 347 (49 %) are female. Median age is 30 years (range 12-82 years, IQR 20-46 years).

Inclusion criteria

1. ToF with pulmonary stenosis repaired with a TAP within the first two years of life.
2. RVOT anatomy is suitable for implantation of an adult sized conduit (≥ 18 mm homograft or Contegra graft) as assessed by CMR.

Exclusion criteria

1. ToF with pulmonary atresia, ToF with common atrioventricular canal, ToF with absent pulmonary valve syndrome, major aortopulmonary collateral arteries and other significant associated anomalies.
2. Palliation with a shunt (Blalock-Taussig or central) at any time.
3. The patient is symptomatic.
4. Sustained supraventricular or ventricular arrhythmia.
5. RVEDVi > 140 mL/m2 as assessed by CMR.*
6. RVESVi > 60 mL/m2 as assessed by CMR.
7. RVEF < 50 % as assessed by CMR.
8. Moderate or severe tricuspid regurgitation as assessed by echocardiography or CMR.
9. Significant residual lesions requiring intervention (e.g. ventricular septal defect, aortic regurgitation, branch pulmonary artery stenosis).
10. Co-morbidity preventing exercise testing (e.g. genetics, neuro-cognitive dysfunction, physical disability).
11. Contraindication for CMR (e.g. permanent pacemaker, intra-cardiac defibrillator, intracranial ferro-magnetic device).
12. Pregnancy at time of inclusion.
13. Age < 12 or unable to comply with instructions given during CMR or exercise testing.

* Patients who have an RVEDVi > 140 mL/m2 will not be included in the randomized trial, as these are expected to meet the guideline criteria for re-valving within few years, and thus the effect of early re-valving is expected to be limited.

Randomization

Participants will be randomized - stratified by gender (female or male) and age (<16 years or ≥16 years of age) - 1:1 to early or later re-valving. The randomization scheme will be unknown to the recruiters. Due to the nature of the study, masking after randomization is not possible. Concomitant surgery including prophylactic anti-arrhythmic surgery is allowed in both the early and later re-valving groups.

Early re-valving group

Patients will undergo surgical re-valving with an adult sized (≥ 18 mm) homograft or Contegra graft and open endomyocardial biopsy using standard institutional procedures within three months from randomization.

Later re-valving group

Patients will undergo surgical re-valving with an adult sized (≥ 18 mm) homograft or Contegra graft and open endomyocardial biopsy using standard institutional procedures, within three months from the point when they meet the current guideline criteria proposed by the European Society of Cardiology:

1. The patient is symptomatic.
2. RVEDVi > 160 mL/m2 as assessed by CMR (Appendix 1).
3. Progressive right ventricular dysfunction with an RVEF < 40 % as assessed by CMR.
4. Moderate or severe tricuspid regurgitation as assessed by echocardiography or CMR.
5. Decrease in exercise capacity (objective decrease of 30 % in peak VO2 from baseline test at randomization).
6. Sustained supraventricular or ventricular tachyarrhythmia.

Follow-up

All patients included in the randomized trial will be followed for at least ten years after randomization and the following examinations will be performed once every year:

1. Assessment of procedure-related complications, cardiovascular events, mortality, number of contacts to the health system and number of children for female patients using record data
2. NYHA/functional-class (Appendix 1) and occurrence of arrhythmias as assessed during interview
3. ECG
4. Blood samples (Appendix 1)
5. 24-hour Holter-monitoring
6. Transthoracic echocardiography (Appendix 1)
7. Cardiac CMR (Appendix 1)
8. Cardio-pulmonary exercise testing (Appendix 1)

Statistical considerations

Efficacy

Several studies report a reduction of 30-40 % in RVEDVi after re-valving (Table 2). We assume that RVEDVi will continue to increase over time in the later re-valving group, but not in the early re-valving group.

Statistical power

Based on the reported data, we assume that:

1. At the time of randomization patients have a median RVEDVi of 115 mL/m2.
2. Three years after randomization, RVEDVi will be reduced by 20 % (from 115 to 92 mL/m2) in the early re-valving group.
3. Three years after randomization, RVEDVi will be increased by 10 % (from 115 to 127 mL/m2) in the later re-valving group.
4. Conservation estimate of the standard deviation of 20 mL/m2.
5. 120 patients will be included (60 for early and 60 for later re-valving) with an expected loss to follow-up of 15 %, thus leaving 102 patients for end-point analyses.

Under these assumptions the study will be adequately powered and have > 90 % power to detect an absolute difference in RVEDVi of 35 mL/m2 between the two groups, corresponding to an effect size of 1.75 expressed as Cohen's d (Figure 5).

Figure 5. Post Hoc Calculation of the Achieved Power in the Randomized Trial with a Graphical Presentation of the Relationship Between Effect Size and Statistical Power Under the Abovementioned Assumptions.

Safety and ethical aspects

As with every type of cardiac surgery early re-valving has potential risks including minor procedure related complications such as bleeding, transient cardiac arrhythmia, pneumothorax, pleural effusion, transient renal impairment and surgical site infection which occur in about 10 % of patients and which can all be treated rather easily. Major procedure related complications include stroke, sternal infection and the risk of death, which are all very rare complications. In 16 studies published between 1985 and 2010 including a total of 1.035 patients with ToF and free PR, the procedure-related mortality was only 0,68 % (Table 3). The most recent studies from 2002-2010 report an operative mortality of 0,4 %, with 10 studies reporting no procedure-related mortality at all.

Table 3. Operative Mortality After Surgical Pulmonary Valve Replacement.

Institution	Year	Number of Patients	Operative Death	Average Length of Follow-Up (years)	Late Death or transplant
SUNY, Syracuse	1985	11	0	1	0
Children's Memorial Hospital, Chicago	1997	49	1		
University of Toronto	1997	85	1	5.8	3
Mayo Clinic	2001	42	1		
Children's Hospital, Atlanta	2002	100	1	4.9	1
Leiden University, The Netherlands	2002	26	0	1.5	1
New England Med Center, Boston	2003	36	0	5	1
University of Zurich, Switzerland	2005	39	0	1.25	0
Multicenter, The Netherlands	2006	158	0	4.2	2
University of Toronto	2007	82	0	8.8	2
University Medical Center, Rotterdam	2008	17	0	6.4	0
International Society of Congenital Heart Disease	2008	93	0	3	2
Great Ormond Street, London	2008	71	0	1	0
Emory University	2009	107	3		
Children's Hospital Boston	2009	77	0	2.8	6
Children's Hospital, Atlanta	2010	42	0	2.2	0
		1035	0.68%		2.2%

Adapted from "Repaired Tetralogy of Fallot: The Roles of Cardiovascular Magnetic Resonance in Evaluating Pathophysiology and for Pulmonary Valve Replacement Decision Support", Geva, Journal of Cardiovascular Magnetic Resonance, 2011, Volume 13).[20]

The overall incidence of sudden cardiac death (SCD) is 0.1-0.2 % annually, with an incremental increase to 4 % at 25 years and 6 % at 30 years of follow-up, thus suggesting a time-dependent risk of late mortality. In the present randomized trial, the incidence of SCD is expected to be 0.2 % per year for the group of patients undergoing later re-valving and 0.1 % per year for the group undergoing early re-valving. Assuming a perioperative mortality of 0.3 % in the early re-valving group, the cardiac mortality is expected to be approximately 0.6 % after three years in both the early and the later re-valving group. Other potential risks associated with early re-valving are altered ventricular geometry, infective endocarditis as well as prosthetic valve deterioration requiring re-intervention. The incidence of infective endocarditis is reported to be very low after surgical pulmonary valve replacement using homograft and Contegra grafts, and therefore not expected to be of concern in the early re-valving group. Studies have shown several factors affecting the longevity of right-ventricular-to-pulmonary-artery conduits, with smaller conduits being particularly prone to deterioration. Thus, patients will only be included in the randomized trial, if CMR and echocardiography ensure, that the patient's anatomy allows implantation of an adult sized conduit (≥ 18 mm). Most deteriorated conduits can be replaced by percutaneous techniques using transcatheter re-valving. Re-valving is performed as a routine procedure at Rigshospitalet by experienced cardiothoracic

surgeons and cardiologists, who are certified in the invasive treatment of congenital and structural heart abnormalities. Following surgery patients are admitted to the cardiothoracic intensive care unit for one to two days, where anaesthesiologists who are also experienced in congenital heart diseases, are focused on pain management as well as stabilization of patients, to ensure a safe transition from surgery to rehabilitation, starting at the cardiothoracic ward, where patients are discharged after an additional five to six days. All staff members involved in these processes are skilled in paediatric care and communication.

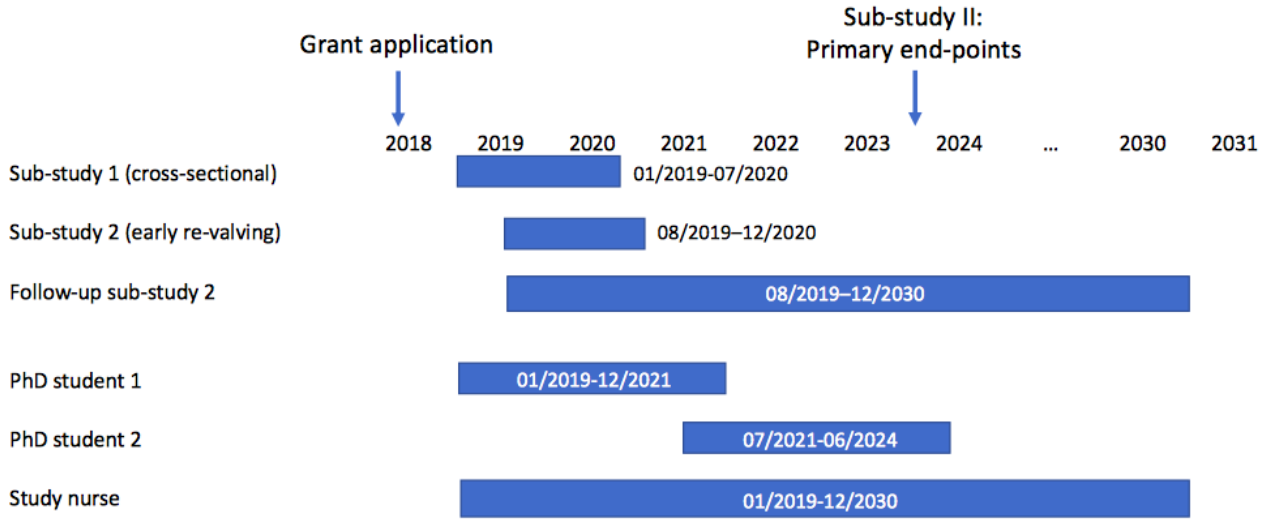
In conclusion, the risk of early re-valving is considered very low and to outweigh the potential adversities of later re-valving, which is associated with irreversible right ventricular dysfunction, exercise impairment, arrhythmia and SCD. Furthermore, the study has to include children in the specified age range (12 years of age or older) with Tetralogy of Fallot, as the potential benefits of the treatment sought to be evaluated cannot be extrapolated from older patients in whom the right ventricle is already irreversibly impaired due to the consequences of free/severe PR.

Time schedule, study organization, research staff, participant recruitment and PhD studies

Time schedule

The study is planned to start in April 2019 and the last patient in the randomized clinical trial to pass the 10-year follow-up by December 2031 (Figure 6).

Figure 6. Expected time frame for the study.



Study organization

Follow-up of complex congenital heart diseases in Denmark is centralized at Rigshospitalet, Odense University Hospital and Aarhus University Hospital Skejby and the study will be a close collaboration between these three centres. The study organization will include paediatric cardiologists and adult cardiologists from the three involved centres, and cardiac surgeons from Rigshospitalet who are specialized in congenital heart diseases. Physicians in the study organization will participate in the recruitment, treatment and follow-up of the study patients. Furthermore, public involvement is important in order to ensure that the study design, conduction and evaluation meet the users' perspectives. Therefore, one

patient and one patient relative will be involved in these processes as members of the study organization. Particular contributions from the two members include evaluation of whether exercise tolerance, quality of life, absence from education/work, number of contacts to the health care system (hospital and general practitioner) and counselling regarding pregnancy will be different in the two groups. Due to practical issues the study will be coordinated from the Department of Cardiology, Rigshospitalet in Copenhagen, where the principal investigators Lars Søndergaard and Morten Smerup as well as the PhD students and study nurse will be based.

Rigshospitalet, Copenhagen
Henrik Ørbæk Andersen, cardiac surgeon
Morten Helvind, cardiac surgeon
Morten Holdgaard Smerup, cardiac surgeon
Klaus Juul, paediatric cardiologist
Lars Søndergaard, adult cardiologist

Odense University Hospital
Helle Andersen, paediatric cardiologist
Henrik Nissen, adult cardiologist

Aarhus University Hospital Skejby
Dorte Guldbrand Nielsen, adult cardiologist
Kim Munk, adult cardiologist

Public involvement
Relative - mother of two children with ToF
Patient - patient with ToF repaired with a TAP and later re-valved.

Research staff

In addition to the study group, one study nurse and two PhD students (with different starting points due to the time frame of the study) will be involved and based at Rigshospitalet, as well as one part time study nurse at Odense University Hospital and Aarhus University Hospital. Mathis Gröning, MD, is expected to start in as the first PhD student in April 2019. The study nurses and the PhD students will coordinate the work, participate and supervise parts of the examinations and ensure proper data collection at all three study sites. Furthermore, they will continuously update the study organization regarding the study progress and potential issues.

Participant recruitment

As soon as the study is approved by the Danish Ethics Committee, all Danish patients with ToF will be identified through the Danish National Patient Registry. Data extracted from the registry will be civil names and addresses. In patients under the age of 18 years, the names and addresses of patients' parents or legal guardians will be extracted as well. Letters containing information about the study, consent forms and medical power of attorney forms are sent to all patients or patients' parents in patients under the age of 18 years. Patients who are interested in participating are encouraged to contact the study group as stated in the information letter, in order to set up an appointment at the nearest hospital. The PhD student will inform patients about the studies in Copenhagen, whereas this task will be taken care of by the recruiting physicians in Odense and Aarhus. An appropriate amount of time will be scheduled for every appointment, in order to make sure, that all possible questions can be clarified before consent forms are signed. Information will be presented in an easily understandable manner, especially if the patient is a child and be

based on the written information in the information letter including information about risks, complications, handling of personal data. All recruiters are skilled in communication with the paediatric population and have detailed knowledge about the study and congenital heart diseases in general. Patients who need further time for consideration are offered at least 24 hours to make up their minds, and are encouraged to contact one of the recruiting physicians, the PhD student or the study nurse when a conclusion is reached, in order to set up a new appointment, where consent forms can be signed. In patients who are not of legal age (under 18 years of age) consent from is needed from every person who has custody of the child. New consent will be achieved for enrolled patients who reach legal age during their participation in the study. Whether or not patients want to receive information regarding their health status acquired through examinations conducted as part of the study is optional. Patients will be informed if novel knowledge regarding risks and complications associated with their participation in the study is achieved during the study period, as well as if the design of the study is changed at any point, or if the information achieved during the study changes recommendations with regards to the most appropriate treatment of the individual patient, provided patients did not decide to stay uninformed in these cases. In case of discontinuation of the study enrolled patients will be informed about the reason for this decision.

PhD studies

There will be two PhD studies attached to the project (PhD 1: January 2019 - December 2021; PhD 2: July 2021 - June 2014). The time frames of the students' employment will include six months of overlay, in order to secure a smooth hand-over of the project from the first to the second PhD student, as well as to ensure sufficient time for the first student to finish the thesis.

PhD 1 will focus on the data from the cross-sectional study (sub-study 1). This may include, but not be limited to:

1. To compare right ventricular dimensions and function, functional class, exercise capacity, presence of sustained arrhythmia, biochemical biomarkers of myocardial injury, myocardial stress, remodelling and fibrosis as well as other measures at different ages in patients with ToF repaired using a TAP compared to patients with ToF who have undergone valve-sparing repair in the Danish cohort of patients with ToF.
2. To evaluate whether the patient age (early: 10-18 years of age, intermediate 19-27 years of age, late: > 27 years of age) at re-valving in patients with ToF repaired with a TAP affects right ventricular remodelling, functional class, exercise capacity, burden of arrhythmia, biochemical biomarkers of myocardial injury, myocardial stress, remodelling and fibrosis and other measures compared with patients repaired with a valve-sparing approach.
3. To evaluate the safety of re-valving in patients with ToF repaired with a TAP with regards to post-operative mortality, stroke, acute kidney impairment, endocarditis etc.
4. To evaluate the durability of the bioprosthetic pulmonary valve using time to re-intervention as end-point. Furthermore, to assess whether the patients' age (early: 10-18 years of age, intermediate 19-27 years of age, late: > 27 years of age) at the time of re-valving affects the durability of the bioprosthesis.

PhD 2 will focus on the outcome of the randomized clinical trial (sub-study 2). This may include, but not limited to:

1. Primary efficacy endpoint: RVEDVi is 35 mL/m2 less in the early re-valving group, compared to the later re-valving group three years after randomization
2. Primary safety endpoint: All-cause mortality within three years after randomization is less than 0.6 % in the early re-valving group

3. Procedure related bleeding categorized as minor, major and life-threatening (BARC)
4. Procedure related vascular complications categorized as minor and major
5. Procedure related acute kidney injury categorized as stage one, two and three
6. Composite rate of all-cause mortality and disabling stroke
7. Cardiovascular mortality
8. Disabling stroke or transient ischemic attack
9. NYHA/functional class (Appendix 1)
10. Health-related quality of life using a generic measure (EQ-5D-3L) will be assessed annularly and temporal developments described. The youth version of the EQ-5D-3L (EQ-5D-Y) is used for patients aged 12-15 years and allows for later mapping onto the corresponding adult scale. The generic questionnaires are available in Danish.
11. New sustained supraventricular or ventricular tachyarrhythmia
12. Exercise capacity measured as peak-VO2 and exercise time (Appendix 1)
13. Right and left ventricular re-modelling (EDVi, ESVi, EF, myocardial mass) and fibrosis (assessed by CMR and analysis of endomyocardial biopsies (Appendix))
14. Time-related prosthetic valve safety (structural valve deterioration, infective endocarditis, prosthetic valve thrombosis)
15. Number of contacts to the Health System (hospital and general practitioner)
16. Number of children for female patient

Permissions, equipment, reimbursement, budget and funding

Permissions

The 4th version of the study protocol has been approved by the Danish Ethical Committee and the Danish Data Protection Agency. The Danish Ethical Comittee and the Danish Data protection agency are applied for approval of the 5th version of the study protocol. Important amendments in the 5th version include sections regarding laboratory tests, endomyocardial biopsies, the establishment of a biobank as well as an update of the budget and funding sections.

Equipment

Echocardiographic systems, CMR scanners and ECG machines will be available at all three participating hospitals. Expenses for equipment for cardio-pulmonary exercise testing and Holter monitoring will partly be covered by the study and located at Rigshospitalet, Odense University Hospital, and Aarhus University Hospital. Expenses for paraclinical tests will be considered as standard of care.

Reimbursement

Additional travel expenses arising due to appointments in addition to regular clinical follow-up at the hospital during the study period will be reimbursed. In total, 120 patients in the randomized trial are expected to have 1.200 additional travels.

Budget

2 PhD students	(2 x 3 years) x 600.00 DKK/year	3.600.000 DKK
1 full-time study nurse	10 years x 460.000 DKK/year	4.600.000 DKK
2 part-time study nurses	10 years x 230.000 DKK/year	2.300.000 DKK
Travel expenses staff	10 years x 25.000/year	450.000 DKK

Travel expenses patients	1.200 travels x 500 DKK	500.000 DKK
Exercise equipment	1 x 200.000 DKK	200.000 DKK
Total expenses		11.200.000 DKK

Funding

5.000.000 DKK have been granted by the Danish Heart Foundation and 5.055.000 DKK have been granted by the Novo Nordisk Foundation. The remaining amount will be applied for at a later stage. The Danish Ethical Committee as well as patients enrolled in the study will be informed if other funding is achieved. Grants will be administered by the accounting service of Rigshospitalet, Copenhagen.

Implementation

Optimal timing of re-valving in patient with ToF and free PR is unknown. Although treatment according to the current guidelines results in good short and intermediate term outcomes, there are major concerns regarding the long-term perspective for the commonly applied re-valving strategy. This includes the lack of full remodelling of the right ventricle, permanent impairment of exercise capacity, arrhythmia and the risk of sudden cardiac death. Robust data and randomized trials in particular are needed. This study addresses these issues and the outcomes may have an impact on clinical practice and guidelines regarding the optimal timing of re-valving in a national as well as international scale. The study results will be published in international peer-reviewed journal in order to ensure dissemination of the acquired knowledge.

Summary

About 25 children are born with Tetralogy of Fallot in Denmark every year. Due to improvements in diagnosis, management and care most of these children now reach adulthood. Despite an excellent outcome, the long-term mortality rate is still increased compared to the general population. Free pulmonary regurgitation as a consequence of right ventricular outflow tract repair with a transannular patch, is the main reason for higher mortality rates, as long-standing severe pulmonary regurgitation is associated with chronic volume overload on the right ventricle leading to right ventricular dysfunction, impaired exercise capacity, arrhythmia and increased risk of sudden cardiac death. Current guidelines for re-valving are based solely on retrospective studies, and as such, optimal timing remains controversial. The current recommendations suggest re-valving when right ventricular size has almost doubled or when symptoms arise. However, after this conservative approach to re-valving patients do not experience significant improvement in exercise capacity or burden of arrhythmia, and in the majority of cases the right ventricle does not reverse remodel to normal size. As operative mortality for surgical pulmonary valve replacement is very low, a more favourable approach might be to strive for implantation of a valved conduit as soon as the child's anatomy allows for the use of an adult-sized prosthesis, which has an increased longevity compared to smaller conduits.
The aim of this study is to evaluate the benefits and drawbacks of an early versus later approach to re-valving and it will be divided in two sub-studies. The first part will be a cross-sectional evaluation of approximately 500 Danish patients with Tetralogy of Fallot, with tests including echocardiography, cardiac MRI, Holter-monitoring, exercise testing and blood samples. The study will provide information about the long-term outcomes after initial repair of Tetralogy of Fallot, as well as suggestions about the optimal timing for re-valving. Among patients included in the cross-sectional study, 120 patients with free pulmonary regurgitation, will be randomized evenly for early or later re-valving with at least 10-years of follow-up, in order to evaluate long-term efficacy and safety of early re-valving. Novel insights regarding the optimal timing of re-valving in patients with Tetralogy of Fallot and pulmonary regurgitation after initial

repair with a transannular patch will be achieved. If this study can prove, that early re-valving is more advantageous than the current approach, it can contribute to a change of recommendations in favour of early re-valving on an international scale.

Conflicts of interest
None of the staff members has any affiliation to the grant givers or other financial interests in the conduction of the study.

References

1. Bernier PL, Stefanescu A, Samoukovic G, Tchervenkov CI. The challenge of congenital heart disease worldwide: Epidemiologic and demographic facts. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu*. 2010;13(1):26-34. doi:10.1053/j.pcsu.2010.02.005.
2. Calzolari E, Barisic I, Loane M, et al. Epidemiology of multiple congenital anomalies in Europe: A EUROCAT population-based registry study. *Birth Defects Res Part A - Clin Mol Teratol*. 2014;100(4):270-276. doi:10.1002/bdra.23240.
3. Dolk H, Loane M, Garne E. Congenital heart defects in Europe: Prevalence and perinatal mortality, 2000 to 2005. *Circulation*. 2011;123(8):841-849. doi:10.1161/CIRCULATIONAHA.110.958405.
4. Van Der Linde D, Konings EEM, Slager MA, et al. Birth prevalence of congenital heart disease worldwide: A systematic review and meta-analysis. *J Am Coll Cardiol*. 2011;58(21):2241-2247. doi:10.1016/j.jacc.2011.08.025.
5. Bhatt AB, Foster E, Kuehl K, et al. *Congenital Heart Disease in the Older Adult: A Scientific Statement From the American Heart Association*. Vol 131.; 2015. doi:10.1161/CIR.0000000000000204.
6. Warnes CA, Liberthson R, Danielson GK, et al. Task Force 1: the changing profile of congenital heart disease in adult life. *J Am Coll Cardiol*. 2001;37(5):1170-1175. doi:10.1016/S0735-1097(01)01272-4.
7. Baird PA, Sadovnick AD, Yee IML. Maternal age and birth defects: a population study. *Lancet*. 1991;337:527-530.
8. Van Der Bom T, Zomer AC, Zwinderman AH, Meijboom FJ, Bouma BJ, Mulder BJM. The changing epidemiology of congenital heart disease. *Nat Rev Cardiol*. 2011;8(1):50-60. doi:10.1038/nrcardio.2010.166.
9. Donofrio MT, Moon-Grady AJ, Hornberger LK, et al. Diagnosis and treatment of fetal cardiac disease: A scientific statement from the american heart association. *Circulation*. 2014;129(21):2183-2242. doi:10.1161/01.cir.0000437597.44550.5d.
10. Al Habib HF, Jacobs JP, Mavroudis C, et al. Contemporary patterns of management of tetralogy of fallot: Data from the society of thoracic surgeons database. *Ann Thorac Surg*. 2010;90(3):813-819. doi:10.1016/j.athoracsur.2010.03.110.
11. Sen DG, Najjar M, Yimaz B, et al. Aiming to Preserve Pulmonary Valve Function in Tetralogy of Fallot Repair: Comparing a New Approach to Traditional Management. *Pediatr Cardiol*. 2016;37(5):818-825. doi:10.1007/s00246-016-1355-1.
12. Tornos P, Brecker S, Dean V, et al. ESC Guidelines for the management of grown-up congenital heart disease (new version 2010): The Task Force on the Management of Grown-up Congenital Heart Disease of the European Society of Cardiology (ESC). *Eur Heart J*. 2010;31(23):2915-2957. doi:10.1093/eurheartj/ehq249.
13. Videbæk J, Laursen HB, Olsen M, Høfsten DE, Johnsen SP. Long-term nationwide follow-up study of simple congenital heart disease diagnosed in otherwise healthy children. *Circulation*. 2016;133(5):474-483. doi:10.1161/CIRCULATIONAHA.115.017226.
14. Marelli AJ, Ionescu-Ittu R, Mackie AS, Guo L, Dendukuri N, Kaouache M. Lifetime prevalence of congenital heart disease in the general population from 2000 to 2010. *Circulation*. 2014;130(9):749-756. doi:10.1161/CIRCULATIONAHA.113.008396.

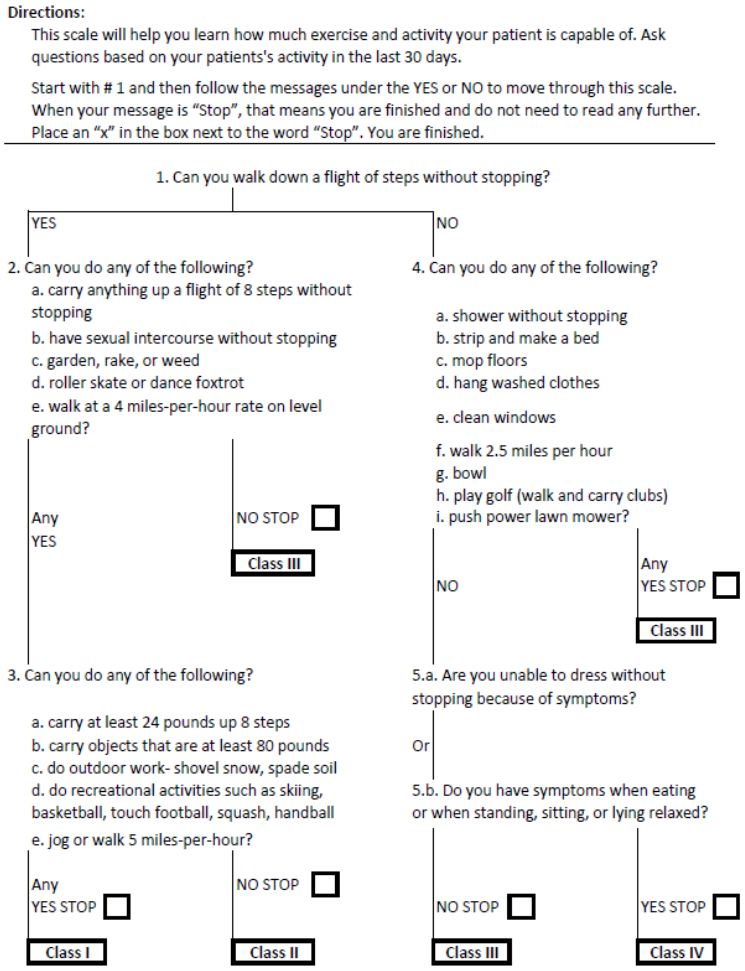
15. Verheugt CL, Uiterwaal CSPM, Van Der Velde ET, et al. Mortality in adult congenital heart disease. *Eur Heart J*. 2010;31(10):1220-1229. doi:10.1093/eurheartj/ehq032.
16. Gatzoulis M a, Balaji S, Webber S a, et al. Risk factors for arrhythmia and sudden cardiac death late after repair of tetralogy of Fallot: a multicentre study. *Lancet*. 2000;356(9234):975-981. doi:10.1016/S0140-6736(00)02714-8.
17. Therrien J, Siu SC, McLaughlin PR, Liu PP, Williams WG, Webb GD. Pulmonary valve replacement in adults late after repair of tetralogy of Fallot: Are we operating too late? *J Am Coll Cardiol*. 2000;36(5):1670-1675. doi:10.1016/S0735-1097(00)00930-X.
18. Therrien J, Provost Y, Merchant N, Williams W, Colman J, Webb G. Optimal timing for pulmonary valve replacement in adults after tetralogy of Fallot repair. *Am J Cardiol*. 2005;95(6):779-782. doi:10.1016/j.amjcard.2004.11.037.
19. Legendre A, Richard R, Pontnau F, et al. Usefulness of maximal oxygen pulse in timing of pulmonary valve replacement in patients with isolated pulmonary regurgitation. *Cardiol Young*. 2015;(2016):1-9. doi:10.1017/S1047951115002504.
20. Geva T. Repaired tetralogy of Fallot: the roles of cardiovascular magnetic resonance in evaluating pathophysiology and for pulmonary valve replacement decision support. *J Cardiovasc Magn Reson*. 2011;13(1):9. doi:10.1186/1532-429X-13-9.

Appendix 1

NYHA functional class assessment

It is well described, that patients with congenital heart diseases adapt their physical activities to their condition and thus underreport symptoms. A specific activity is used in this study, to allow for a more objective assessment of patients' functional class (figure 6).

Figure 6. Specific Acitivity Scale Which Will Be Used for Assessment of Patients' Functional Class During Interviews.
Modified from "Comparative Reproducibility and Validity of Systems for Assessing Cardiovascular Functional Class: Advantages of a New Specific Activity Scale", Goldman et al., Circulation, 1981, Volume 64, page 1227.[21]



ECG

A standard 12-lead surface ECG is obtained for assessment of:

- Heart rhythm
- Ventricular rate
- Intervals
- Conduction disturbances
- Ischemia

Echocardiography

In addition to CMR TTE is performed in all patients for assessment of valvar regurgitation and pressure gradients, as well as residual defects using the following protocol:

All B-mode loops will include a minimum of 2 beats. All Spectral Doppler traces are optimized with sweep setting allowing 3 beats.

- Subxiphoid: optimized for IVC with and without inspiration
- PLAX: RV linear dimension, LV linear dimension
- PLAX color: MV and AV interrogation of valve regurgitation
- SAX RVOT level: Color interrogation of RVOT, PW spectral Doppler below PV/conduit for PA acceleration time, CW Doppler optimized for MPA antegrade flow velocity, CW Doppler optimized for RVOT regurgitant flow profile. PW interrogation of LPA and RPA
- 4C: LV and RV dimensions and function, 4C loop optimized for GLS calculation,
- 4C color: MV interrogation for MI estimation
- 2C: LV dimension and function, 2C loops optimized for GLS calculation
- 2C color: MV interrogation for MI estimation
- APLAX: LV dimension and function, APLAX loops optimized for GLS calculation
- APLAX color: MV and AV interrogation for MI and AI/AS estimation
- 4C RV optimized for free wall and septal GLS calculation

Cardiovascular magnetic resonance (CMR) imaging

CMR is performed for assessment of:

1. Ventricular size and function
2. Characterization of pulmonary outflow at three levels
3. Right and left ventricular scar tissue and localized fibrosis
4. Quantification of right and left ventricular diffuse fibrosis

<u>Ventricular size and function</u>

Right ventricular end systolic and end diastolic volumes are measured from a transverse stack of images without gaps using steady-state free precession cine sequences. Left ventricular volumes are measured using a short axis stack through the left ventricle. The following measurements will be obtained:

- Right ventricular end-systolic and end-diastolic volume index (RVESVi and RVEDVi)
- Right ventricular ejection fraction (RVEF)
- Left ventricular end-systolic and end-diastolic volume index (LVESVi and LVEDVi)
- Left ventricular ejection fraction (LVEF)
- Septal and left ventricular free wall extracellular volume

<u>Characterization of pulmonary outflow at three levels</u>

Pulmonary blood flow is measured as forward flow, regurgitant volume and fraction. Velocity encoding is performed in the main pulmonary artery.

Right and left ventricular scar tissue and localized fibrosis

A 3D whole heart, inversion-recovery gradient echo pulse sequence with a respiratory navigator pulse placed over the dome of the right hemidiaphragm is used to obtain both an early (coronary enhanced) and late (scar enhanced) dataset (voxel size 1.3 x 1.3 x 1.3 mm^3 with interpolation to 0.65 mm for reconstruction, echo time 1.3 ms, flip angle 20 degrees, integrated parallel acquisition technique 2, end-expiratory respiratory navigator acceptance window 3.5 mm, phase encoding direction anterior-posterior). Fat saturation is employed to supress pericardial fat signal. Imaging volumes are prescribed in the transverse plane from the aortic arch to below the most inferior aspect of the heart (slab thickness 120-144 slices) based on multiplanar scout images. Adjustment of trigger delay and number of segments is performed to maintain image acquisition between the onset and termination of cardiac standstill and determined from the 4-chamber cine.

For coronary-enhanced imaging, an intravenous infusion of gadolinium contrast (0.15 mmol/kg) is infused. Imaging is initiated 25 seconds following infusion onset. A repeat scar-enhanced dataset is acquired 20-25 minutes after infusion onset with adjustment of the inversion time to provide optimal myocardial signal suppression. The inversion-time is set at 200 ms for scar-enhanced imaging (typical range 240-270 ms). These adjustments are performed using a test-image slab (10 mm thickness) acquired over the mid-ventricle. The acceptance rate of the respiratory navigator is recorded for both full-volume acquisitions.

Feasibility, accuracy and reproducibility of the abovementioned methods for assessment of ventricular scar tissue and localized fibrosis have been demonstrated by other researchers.[2]

Quantification of right and left ventricular diffuse fibrosis

Equilibrium contrast cardiovascular magnetic resonance (CMR) is used in this setting. T1 mapping is performed in three short axis slices through the heart with modified Look-Locker inversion recovery (MOLLI) sequences. Nine minutes after infusion of gadolinium contrast T1 mapping is repeated in the same 3 slices. Using the patient's haematocrit, the extracellular volume (ECV) in the interventricular septum and left ventricular free wall is calculated using the following equation:

$$ECV = (1\text{-haematocrit}) \times (1/T1_{\text{myocardium post-contrast}} - 1/T1_{\text{myocardium pre-contrast}})/1/T1_{\text{blood post-contrast}} - 1/T1_{\text{blood pre-contrast}})$$

As the right ventricular free wall is thin and trabeculated, it is not suited for T1 mapping.

Post-processing

Imaging is performed using a 1.5 Tesla Siemens scanner. Post-processing of the MRI measurements is performed using CVI42 (Circle of Cardiovascular Imaging, Calgary, Canada) on Macintosh computers (Apple Inc., Cupertino, USA).

Safety

Pregnancy will be ruled out for all female patients of childbearing age, prior to infusion of gadolinium contrast and CMR, using a urine pregnancy test.

Exercise testing

Cardio-pulmonary exercise testing (CPET) will be performed on a bicycle ergometer. Each test starts with a two-minute warm-up. With the aim of reaching maximal effort within 6-12 minutes after the warm-up, an appropriate incremental load protocol starting at either 20 W with a stepwise increase of 20 W per minute, or starting at 25 W with an increase of 25 W per minute will be chosen for each participant, based on expected fitness. Both CPET at baseline and CPET during follow-up will be performed after the same protocol. ECG and oxygen saturation will be measured continuously during the test. Respiratory volumes as well as O2 and CO2 concentrations will be measured breath by breath through a mask and a connecting tube, worn by the patient. Every patient is encouraged to continue the exercise until exhaustion. A respiratory exchange ratio > 1.1 will be considered as an indicator of exhaustion. Measurements will be given as mean of 15 second intervals, by subtraction of the highest from the lowest value. Peak-VO2 will be defined as the 15 second interval with the highest VO2. The anaerobic threshold will be defined as the first 15-second interval during which the respiratory exchange ratio exceeds 1.0 without a subsequent reduction < 1.0. The maximum work-load will be defined as the highest watt load achieved and held for at least 15 seconds.

Laboratory tests

Laboratory tests are obtained from all patients included in sub-study I (the cross-sectional study) and sub-study II (the randomized clinical trial).

Sub-study I

<u>One set of laboratory tests are obtained from all 500 patients including:</u>

B-haemoglobin, B-erythrocyte count, B-mean corpuscular volume, B-mean corpuscular haemoglobin concentration, B-reticulocyte count, B-haematocrit, B-leukocyte count with differential counts (B-neutrophils, B-eosinophils, B-basophils, B-lymphocytes, B-monocytes), B-platelet count, P-ferritin, P-CRP, P-albumin, P-potassium, P-sodium, P-creatinine, eGFR*, P, P-aPTT, P-prothrombin time, P-international normalized ratio, P-alanine transaminase, P-alkaline phosphatase, P-bilirubin, P-lactate dehydrogenase, P-glucose, P-haemoglobin A1C, P-cholesterol (total, HDL, LDL, VLDL), P-triglycerides, P-creatine kinase, P-creatine kinase MB, P-troponin T, P-NT-proBNP and blood samples for storage in a biobank** (3.5 mL in a sodium-citrate tube, 12 mL in an EDTA tube and 4 mL in a serum tube with no anticoagulant).

*eGFR is calculated using the CKD-EPI equation.
** Will only be obtained from patients scheduled for visits at Rigshospitalet, Copenhagen.

Sub-study II

The same laboratory tests obtained as part of sub-study I are obtained at the 10 annual follow-up visits for the 120 patients included in substudy II apart from whole blood which is only obtained as part of substudy I.

Furthermore, laboratory tests are obtained before surgical revalving as well as 6, 12 and 24 hours after the surgical procedure. 80 patients are expected to undergo revalving during the study period (60 early revalving procedures and 20 later revalving procedures).

<u>Laboratory tests obtained before surgery include</u>:

B-haemoglobin, B-erythrocyte count, B-mean corpuscular volume, B-mean corpuscular haemoglobin concentration, B-reticulocyte count, B-haematocrit, B-leukocyte count with differential counts (B-neutrophils, B-eosinophils, B-basophils, B-lymphocytes, B-monocytes), B-platelet count, P-ferritin, P-CRP, P-albumin, P-potassium, P-sodium, P-creatinine, eGFR*, P-aPTT, P-prothrombin time, P-international normalized ratio, P-alanine transaminase, P-amylase, P-alkaline phosphatase, P-bilirubin, P-lactate dehydrogenase, P-glucose, P-creatine kinase, P-creatine kinase MB, P-troponin T, P-NT-proBNP and blood samples for storage in a biobank (3.5 mL in a sodium-citrate tube, 4 mL in an EDTA tube and 4 mL in a serum tube with no anticoagulant).

Laboratory tests obtained 6, 12 and 24 hours after surgery include:

B-haemoglobin, B-erythrocyte count, B-mean corpuscular volume, B-mean corpuscular haemoglobin concentration, B-reticulocyte count, B-haematocrit, B-leukocyte count with differential counts (B-neutrophils, B-eosinophils, B-basophils, B-lymphocytes, B-monocytes), B-platelet count, P-CRP, P-potassium, P-sodium, P-creatinine, eGFR*, P-aPTT, P-prothrombin time, P-international normalized ratio, P-alanine transaminase, P-creatine kinase, P-creatine kinase MB, P-troponin T, P-NT-proBNP and blood samples for storage in a biobank (3.5 mL in a sodium-citrate tube, 6 mL in an EDTA tube and 4 mL in a serum tube with no anticoagulant).

*eGFR is calculated using the CKD-EPI equation.

Postprocessing of blood samples for biobank storage

Centrifugation of the 3.5 mL of blood in a sodium-citrate tube is performed for 10 minutes at a speed of 2200 G and a temperature of 20-24 degrees Celsius. The plasma is dispensed into 4 pieces of 2 mL cryotubes and frozen for storage in the biobank.

Centrifugation of the 6 mL of blood in an EDTA tube is performed for 10 minutes at a speed of 2200 G and a temperature of 20-24 degrees Celsius. The plasma is dispensed into 4 pieces of 2 mL cryotubes and frozen for storage in the biobank. The remaining 6 mL of blood in EDTA will be stored as whole blood (only as part of sub-study 1 and only for patients scheduled for visits at Rigshospitalet, Copenhagen).

Centrifugation of the 4 mL of blood in a serum tube is performed for 10 minutes at a speed of 2200 G and a temperature of 20-24 degrees Celsius. The serum is dispensed into 4 pieces of 2 mL cryotubes and frozen for storage in the biobank.

Utilization of blood samples stored in the biobank

Blood samples stored in the biobank will be used for later analysis of biochemical biomarkers of myocardial injury, myocardial stress, remodelling and fibrosis. Furthermore, excess material will be stored in the biobank for future, yet undefined research (The Danish Data Protection Agency will be applied for approval of this biobank for future, yet undefined research). If further analysis of the extra material is planned at a later stage, the Ethics Committee will be applied for approval of this new project and new written consent will be obtained from the patients, unless a waiver of consent is considered appropriate by the Ethics Committee depending on the circumstances of this new project. Patients will be informed that analysis performed at a later stage might include genetic research.

Endomyocardial biopsies

5 endomyocardial fragments, each 1-2 mm^3 in size, are obtained from the right ventricular inlet portion, the right ventricular outflow tract and the left ventricular lateral wall during surgical revalving, resulting in a total of 15 specimens. Much of the specimens can be obtained from tissue, which is resected as part of the revalving procedure in any case, irrespective of the desire to obtain biopsies.

Storage and processing of endomyocardial biopsies

The specimens are stored, processed and evaulated as follows:

9 specimens (three from each biopsy site) are fixed in 10 % buffered formalin and stored at room temperature for staining and light microscopy. A haematoxylin and eosin stain is applied to each of these specimens at 2 levels of the biopsy. A Masson trichrome collagen stain is applied to each specimen in order to determine the degree of endomyocardial fibrosis, a Verhoeff-Van Gieson stain is applied to each specimen for evaluation of endomyocardial fibroelastosis and a phosphotungstic acid haematoxylin stain is applied to each specimen for identification of myocyte disarray.

3 specimens (one from each biopsy site) are placed in glutaraldehyde fixative for electron microscopy.

3 specimens (one from each biopsy site) are immediately snap frozen in liquid nitrogen and stored at minus 80 degrees Celsius for later molecular tests and stains.

Techniques used for preparation and processing of the endomyocardial biopsies are in line with the consensus statement on endomyocardial biopsy from the Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology and the recommendations for processing cardiovascular surgical pathology specimen from the Standards and Definitions Committee of the Society for Cardiovascular Pathology and the Association for European Cardiovascular Pathology.[22,23]

Utilization of endomyocardial biopsies stored in the biobank

Endomyocardial biopsies stored in the biobank will be used for later analysis of myocardial injury, myocardial stress, remodelling and fibrosis. Furthermore, excess material will be stored in the biobank for future, yet undefined research (The Danish Data Protection Agency will be applied for approval of this biobank for future, yet undefined research). If further analysis of the extra material is planned at a later stage, the Ethics Committee will be applied for approval of this new project and new written consent will be obtained from the patients, unless a waiver of consent is considered appropriate by the Ethics Committee depending on the circumstances of this new project. Patients will be informed that analysis performed at a later stage might include genetic research.

Safety

Endomyocardial biopsy is a commonly performed procedure for the evaluation of cardiac tissue for transplant monitoring, myocarditis, drug toxicity, cardiomyopathy, arrhythmia, secondary cardiac involvement in systemic diseases and for the diagnosis of cardiac masses. Endomyocardial biopsies are usually obtained percutaneously. As such, data regarding the risks accompanying open chest endomyocardial biopsy is limited. Percutaneous endomyocardial biopsy is generally considered a safe procedure. Possible complications of percutaneous endomyocardial biopsy include bleeding or hematoma at the access sites, arteriovenous fistula, vasovagal reactions, pneumothorax, arrhythmia or conduction disturbances, pulmonary embolism during right ventricular biopsy or systemic embolism during left

ventricular biopsy and cardiac chamber perforation with possible haemopericardium and cardiac tamponade. The overall complication rate of percutaneous endomyocardial biopsy using a flouroscopy or ultrasound guided transvenous or transarterial sheath with insertion of a biotome is reported to be 1-2 %. In particular the risk of cardiac peforation was estimated to be 0.4 % in the largest case records in the world published in 1980 and 0.12 % in a more recent report based on a large number of cases. (reference, [24]). The risk of complications related to endomyocadial biopsy during open heart surgery is likely to be considerably lower, as catheter-related adverse events are precluded (bleeding or hematoma at the access sites, arteriovenous fistula, vasovagal reactions, pneumothorax and pulmonary or systemic embolism) and as visual instead of flouroscopy or ultrasound guidance of the biotome reduces the risk of cardiac chamber perforation and associated complications (haemopericardium and cardiac tamponade).