PhD Protocol

Myocardial Remodelling due to Right Ventricular Dilatation in Congenital Heart Disease

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Setting the Scene

The overall objectives of this PhD study are to: 1) further validate an experimental large-animal model for right ventricular dilatation, and to 2) investigate the cardiophysiological consequences in this model, 3) to examine the myocardial rearrangement following right ventricular dilatation using MRI, and 4) to apply these methodologies to patients suffering from pulmonary regurgitation.

Background

Right ventricular dilatation (RVD) is a well known pathological consequence of congenital heart disease mainly caused by pulmonary regurgitation (PR)1. PR in its pure form is mainly seen following balloon dilatation of critical pulmonary stenosis or perforation of valvar pulmonary atresia [1]. The treatment of PR has improved dramatically over the past decades. However, complications like RVD as a result of longstanding PR still develop [2,3], resulting in an increased risk for severe arrhythmias and sudden death. [1, 3, 4] Very little is known about the aetiology of these complications and how to predict and avoid them. Today patients with severe PR are treated with valve substitution whenever cardiac symptoms occur, but in fact little is known about the optimal window for surgery. The keystone is the morphological changes occurring in this disease. Understanding these is a good starting point of investigation since they still remain poorly understood. The understanding of the correlates between morphology, anatomy and physiology will further enable us to better understand and treat the disease. Formerly, cardiac morphology was mainly evaluated by histological examinations of the myocardium [5–7]. However, a major drawback of this approach was that the myocardium could not be revealed in its three-dimensional entirety. Now, this limitation has been overcome by a new way of looking at the course and organization of the myocardial fibres: diffusion tensor magnetic resonance imaging (DTMRI) [8]. This technique measures the spontaneous self-diffusion of water in the tissue. Using this technique in fibrous tissue, the direction of water diffusion is a measure of fibre direction since water tends to diffuse parallel to the cell membranes. Originally, DTMRI was used to visualize the course of axons in the nervous system [9], but it has recently been introduced in cardiac research to portray myocardial muscle fibres in three dimensions [10–13]. Cardiac DTMRI is well tested ex-vivo where reliable and reproducible DTMRI of the myocardium can be performed, but it remains mainly a research tool due to its sensitivity to movements and contractions of the heart [14]. However, taking recent advances in MRI technology into consideration, we are confident that DTMRI may also become a potentially useful method in-vivo on beating heart. DTMRI studies have shown that the myocardium consists of an array of crossing helical fibre tracts, which evolve smoothly from a left-handed helix in the subepicardium to a right-handed helix in the subendocardium [12]. This structure contributes significantly to efficient ventricular function and is subject to remodelling and disarray in the presence of disease, such as myocardial infarction and cardiomyopathies The exact arrangement of myocardial fibres is unknown in patients with RVD, but it is likely that DTMRI may act for early registration of cardiac dysfunction. In theory deformation as in dilatation of the right ventricle will lead to remodelling of the ventricular myocardium in terms of changes in angulation of myocardial fibres. We have recently provided a mathematical model supporting this notion [15]. This, however, remains to be demonstrated experimentally. We have developed a large animal model for PR and RVD [16] relevant for further evaluation and use in this context. In this study, investigations of myocardial rearrangement correlated with anatomical and physiological measurements in a controlled animal model are paralleled with accurate assessment of right ventricular anatomy and physiology assessed with echocardiography, and conventional MRI in humans.

Hypotheses

- Physiological impairment can be detected in the dilated hearts by the use of MRI, conductance catheter measurements and echocardiography in our model of chronic PR and RVD in pigs.
- DTMRI can be used to visualize the right ventricular muscle fibre arrangement both in the normal and in the dilated state.
- Early signs of cardiac dysfunction can be detected in patients with pulmonary regurgitation using imaging techniques before clinical symptoms are seen.

To address these hypotheses, this PhD study is divided in the following 3 studies: Study 1: further evaluate our porcine model for RVD and elucidate the physiological consequences of PR by the use of MRI, conductance catheter measurements and echocardiography. Study 2: DTMRI is applied to examine the dilated porcine RV in terms of fibre organization and compare the results with a similar DTMRI examination on the normal porcine heart. Study 3: a clinical study in an adolescent group of patients with pulmonary insufficiency trying to detect early signs of cardiac dysfunction.

As such, the proposed Ph.D. study may provide valuable knowledge about the optimal timing of valve substitution in patients with PR and RVD. Secondly, the study may improve our knowledge about the pathophysiology of this condition and could result in better counselling of patients with PR and their families.

Materials and methods

Study 1:
Further evaluation of our Animal Model of Right Ventricular Dilatation.

This study will be conducted as a prospective randomized intervention-control study. It will consist of two groups (group a and group b) of 5 kg female piglets. In group a) (n=12) pulmonary valve insufficiency will be induced as described below. Group b) (n=8) a sham-operation will be performed.

Surgery description:
After appropriate administration of sedatives and analgesics the animals will be placed on a ventilator. The pulmonary artery will be exposed through a left lateral thoracotomy in the fifth intercostal space. In order to create pulmonary valve insufficiency, four to six single sutures (5-0 polyprolene) will be placed through the wall of the pulmonary trunk and around the pulmonary valve leaflets. The pericardium will then be closed and subsequently, the incision will be closed in three layers.

Pulmonary insufficiency is confirmed by echocardiography postoperatively. The hearts will be assessed in vivo after 8 weeks. The anatomy in terms of volumes, diameters and wall thickness will be assessed along with the degree of pulmonary insufficiency using MRI and the physiology will be assessed in terms of contractile parameters, systolic and diastolic function and impairment using conductance technique. Moreover, we will introduce a non-invasive in-vivo DTMRI approach: a diffusion-weighted STEAM-based single-shot echo-planar imaging sequence, implemented on a clinical scanner. Motion artefacts are reduced by simultaneous navigator-gating and ECG triggering.

Study 2:
Changes in Myocardial Morphology due to Right Ventricular Dilatation.

This study will be conducted as a prospective randomized intervention-control study. Post mortem DTMRI will be performed on porcine hearts with induced RV dilatation.

Excised porcine hearts (group a and b, Study 1), are fixated in formalin overnight immediately after excision and filled with plastic polymer to keep the hearts in diastolic state. Then, the hearts will be placed in a 9.4 Tesla MRI scanner, and a dedicated radiofrequency coil is used for optimal signalto-noise. A diffusion-weighted spin-echo imaging sequence is employed for DTMRI, using 20-32 diffusion-encoding directions, with an expected acquisition time of 12-16 hours to allow a very high spatial resolution (400 ?m3 per voxel). Afterwards, the data will be transferred to our custom made software for analyses of fibre angulations. In short, the fibre-tracking algorithm is based on the deterministic assignment method that operates under the assumption that the primary eigenvector indicates the orientation of myocardial bundles and follows the primary eigenvector from voxel to voxel in 3D.

Following the calculated fibre map of the heart, we will derive the angulation of the myocardial muscle fibres relative to the horizontal plane (helix angle) and relative to the epicardium (intrusion). Primary endpoints will be compared between group a and group b.

Study 3:
Cardiac Morphological and Physiological Changes in Patients with Pulmonary Insufficiency and Right Ventricular Dilatation.

This study is conducted as a matched case-control study. The morphological and physiological knowledge from study 1 and 2 will be utilized in a clinical setting. We will examine a group of adolescent patients earlier treated with balloon dilatation of pulmonary stenosis now suffering from pulmonary insufficiency. The study will comprise 20 patients (n=20) and 20 age and sex matched controls. They will be selected from our local database based on ICD-10 diagnosis and treatment codes.

Inclusion criteria include: pulmonary insufficiency; balloon dilatation of isolated pulmonary stenosis before the age of 16 years; older than 10 years of age at the time of follow-up; and understanding spoken and written Danish. Exclusion criteria: other cardiac diseases congenital or acquired; pulmonary conditions requiring treatment; missing patients charts; metallic implants or similar; and pregnancy.

Cardiac anatomy will be evaluated with MRI assessing the same primary endpoints as in study 1 including in-vivo DTMRI. Here the image resolution will be as high as possible without compromising the generally accepted patient time in the MRI system.
Cardiac physiology will be evaluated with echocardiography using a standard protocol according to the principles of the Danish Society of Cardiology expanded with tissue doppler measurements. TAPSE (*T*ricuspid *A*nnulus *P*lane *S*ystolic *E*xcursion) [17–19] and TASM (*T*ricuspid *A*nnular Peak *S*ystolic *M*otion) [19] are used as a measure of right ventricular systolic

function. Echocardiography is chosen as assessment method since it is the most widely used in the clinical setting and it is non-invasive as opposed to conductance catheter technique. Identical measurements will be done in rest and during exercise. Study subjects will be placed on a custom-made ergometer-bed reducing body movement enabling simultaneous echocardiography and exercise. The exercise protocol is constructed with gradual increase in performance starting with a load of 50 watt increasing gradually in steps of 25 watt until reaching 80% of maximum heart rate defined as 220 beats per minute minus age. 30 sec. after increase in workload echocardiographic measurements are done. A total exercise time of approx. 6-12 minutes is expected.

Applicant's part in the project

- Elaboration of research protocol and application for approval for animal and humane procedures.
- All surgical procedures incl. anaesthesia (Study 1)
- All MR procedures (Study 1, 2 and 3)
- All echocardiography (Study 3)
- All data collection and analyses.
- Writing of manuscripts.

Statistical Considerations

Study 1:
All primary endpoints will be compared between groups using ANOVA. In case of statistical significant difference post hoc testing will be done using students' t-test.

Study 2:
Angulations will be compared between the two groups using students' t-test.

Study 3:
All primary endpoints will be compared between groups using ANOVA on data following the normal distribution. Kruskall-Wallis test will be used as a non-parametric alternative on data not following the normal distribution.

Feasibility

All animal experiments will be performed at the Institute of Clinical Medicine, Aarhus University Hospital Skejby, facilitating necessary equipment for animal handling and experimental procedures. Conductance measurements will be done by Janus Adler Hyldebrandt, MD assisted by the project leader. MRI procedures in both animals and humans will be performed at the MR Center, Aarhus University Hospital Skejby, using state-of-the-art MRI systems. Usage of MR equipment will be supervised by prof. Michael Pedersen and Steffen Ringgaard, MSc PhD.

Clinical Perspectives

Surgical correction of the lethal congenital heart diseases has evolved over the last 60 years. The focus of research has until recently been on postoperative survival. Improvements in surgical methods have shifted the focus towards issues such as cardiac function, co-morbidities and quality of life. This study will help us improve these factors along with the quality of information given to patients with pulmonary regurgitation and dilated right ventricle regarding prognosis and physical abilities. Moreover, it will help us selecting the optimal time frame for valve substitution.

Ethical Considerations

The animal experimental procedures will be conducted after approval from the Danish Inspectorate of Animal Experimentation. It is of serious concern for us that no suffering occur among the animals, therefore the animal will be anaesthetized during the entire procedure and they will be closely observed after arousal to avoid suffering and any sign of pain will be treated immediately. The veterinary technicians are instructed in the management of drugs to the animals and will administer analgesics according to specific guidelines and whenever animals show any sign of postoperative pain. Furthermore the animal caretakers will stay in close contact with the study leader during the postoperative period, and any sign of poor thriving, pain or infection will be dealt with immediately. In case of poor thriving the animals will be euthanized.

When conducting animal experiments we always consider whether the sacrifice of the animals is acceptable compared to the future benefits of this project to humans. By reference to the 'Clinical Perspectives' part above we consider this justified.

After a thorough risk assessment the clinical part of this study is also considered ethical acceptable. The study will be conducted after approval from the regional ethical committee. All assessment methods involved are non-

invasive and without any risks, discomforts or disadvantages for the patients and the risk of unforeseen complications is minimal. The patients are familiar with all assessment methods from earlier clinical examinations apart from the exercise test. This test, however, is very similar to what is known from ordinary bicycle rides and is therefore not expected to cause any problems or discomforts in it self.
Finally the members of the research group have thorough experience in working and communicating with children and adolescents.

Funding

Application will be submitted for a Ph.D. scholarship at Aarhus University. Furthermore the project will be partly financed by assignment as clinical assistant at the dept. of cardiothoracic and vascular surgery, Aarhus University Hospital. Finally, funding will be applied for through various private foundations.

Time Table

This 3-year project will be conducted in the period 2013-2016 and is planed as follows:

<table>
<tr><td></td><td colspan="4">2013</td><td colspan="2">2014</td><td colspan="2">2015</td></tr>
<tr><td>Study 1</td><td colspan="3">Data Collection</td><td colspan="2">Article</td><td></td><td colspan="2"></td></tr>
<tr><td>Study 2</td><td colspan="2"></td><td colspan="3">Scanning</td><td>Data Analysis</td><td>Article</td><td></td></tr>
<tr><td>Study 3</td><td></td><td colspan="3">Inclusion</td><td colspan="3">Data Collection and Analysis</td><td>Article</td></tr>
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<tr><td>PhD Thesis</td><td colspan="4"></td><td colspan="2"></td><td></td><td>Writing</td></tr>
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