Myocardial Tissue Characterization
Using Magnetic Resonance Imaging

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Cover: Thirteen short-axis slices T1 maps (left) and T2 maps (right) acquired with 3D-QALAS.

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Tove and Hjalmar,
I dedicate this thesis to you!

“Difficulties mastered are opportunities won”
- Winston Churchill
## CONTENTS

ABSTRACT .................................................................................................................. 3  
SVENSK SAMMANFATTNING .......................................................... 5  
LIST OF PAPERS ........................................................................... 7  
ABBREVIATIONS ........................................................................... 9  
ACKNOWLEDGEMENTS ............................................................... 11  
INTRODUCTION ............................................................................. 13  
AIM OF THESIS .............................................................................. 15  
THE HUMAN HEART ....................................................................... 17  
  Anatomy and physiology ............................................................. 17  
  Myocardial fibrosis ....................................................................... 19  
MAGNETIC RESONANCE IMAGING .................................................. 21  
  Basic principles of MRI ................................................................. 21  
  Image acquisition ......................................................................... 23  
  Contrast parameters ...................................................................... 23  
  Cardiovascular MRI ....................................................................... 28  
QUANTIFICATION ........................................................................... 29  
  Principles of relaxation times quantification .................................. 29  
  Myocardial relaxation times quantification ................................... 30  
3D-QALAS ..................................................................................... 35  
  Sequence design ........................................................................ 35  
  Data analysis ................................................................................ 37  
  In-vitro validation ......................................................................... 39  
  In-vivo validation ......................................................................... 42  
  Application of the method in patients .......................................... 45  
  Discussion .................................................................................... 54  
  Conclusion ................................................................................... 56  
REFERENCES ................................................................................ 57
In cardiovascular disease, which is the most common cause of death in the world, early diagnosis is crucial for disease outcome. Diagnosis of cardiovascular disease can be challenging, though. Quantification of myocardial T1 and T2 relaxation times with MRI has demonstrated to be a promising method for characterizing myocardial tissue, but long measurement times have hampered clinical use. The overall aim of this doctoral thesis was to develop, validate and, in patient studies, evaluate a very fast three-dimensional method for simultaneous quantification of myocardial T1 and T2 relaxation times with whole coverage of the left ventricle.

The 3D-QALAS method is presented in Paper I of this thesis. It is a method that simultaneously measures both T1 and T2 relaxation times in a three-dimensional volume of the heart. The method requires 15 heartbeats, to produce 13 short-axis slices of the left ventricle with voxel-wise information of both T1 and T2 relaxation times. The 3D-QALAS method was validated in phantoms and in 10 healthy volunteers by comparing the method with reference methods and demonstrated good accuracy and robustness both in-vitro and in-vivo.

In Paper II, the 3D-QALAS method was carefully validated in-vivo by investigating accuracy and precision in 10 healthy volunteers, while the clinical feasibility of the method was investigated in 23 patients with various cardiac pathologies. Repeated independent and dependent scans together with the intra-scan repeatability, demonstrated all a very good precision for the 3D-QALAS method in healthy volunteers.

In Paper III and IV, the 3D-QALAS method was applied and evaluated in patient cohorts where the heart muscle alters over time. In Paper III, patients with severe aortic stenosis underwent MRI examinations with 3D-QALAS before, 3 months after and 12 months after aortic valve surgery. Changes in T1 and T2 were observed, which might be used as markers of myocardial changes with respect to edema and fibrosis, which may develop due to increased workload over a long period of time.

In study IV, 3D-QALAS was used to investigate 10 breast cancer patients treated with radiation therapy prior to treatment, 2-3 weeks into treatment, and one and 6 months after completion of treatment, to investigate any changes in T1 and T2 and further if they can be correlated to unwanted irradiation of the heart during radiation therapy.
SVENSK SAMMANFATTNING

Hjärt- och kärlsjukdomar är idag den vanligaste dödsorsaken i Sverige. Att diagnosticera och bedöma sjukdomen på ett tidigt stadium kan vara avgörande för utfallet av en behandling. Att utveckla icke-invasiva kvantitativa metoder för att bedöma hjärtmuskels funktion är ett sätt att förbättra de diagnostiska metoderna. De flesta metoder som finns idag bygger på kvalitativa bedömningar och grundar sig på visuella intryck som varierar mellan olika observatörer. En kvantitativ metod där man tittar på absoluta värden av vävnadskarakteristiken i varje voxel i bilden, ger en mer objektiv bedömning och har visat sig öka säkerheten i den diagnostiska bedömningen. En kvantitativ metod möjliggör t.ex. diagnostik av diffusa sjukdomar och inlagringssjukdomar i hjärtmuskeln. Sådana sjukdomar påverkar inte enbart ett avgränsat område som tydligt kan bedömas visuellt, utan hela hjärtmuskeln påverkas, vilket då måste mätas kvantitativt. Kvantifiering av relaxationstider (T1 och T2) i hjärtmuskeln med hjälp av magnetresonanstromografi (MR) har visat sig vara en lovande metod för att kunna vävnadskarakterisera hjärtmuskeln. Det övergripande syftet med avhandlingen var att utveckla, validera och sedan i patientstudier utvärdera en mycket snabb metod för att kvantifiera både T1- och T2-relaxationstider samtidigt i en tredimensionell volym i hjärtat.

I delarbete I presenteras 3D-QALAS metoden, som är en metod för att samtidigt mäta T1- och T2-relaxationstider i en tredimensionell volym av hjärtat. Insamlingstiden på MR-kameran för 3D-QALAS metoden är 15 hjärtslag, ca 15 s, vilket producerar 13 kortaxelsnitt av vänster kammare med voxelvis information om både T1 och T2. Metoden valideras i fantom och 10 friska frivilliga genom att jämföra mätvärden mot existerande 2D metoders mätvärden och även genom att undersöka känslighet för yttre faktorers påverkan, såsom ändrad hjärtfrekvens och arytmier.

I delarbete II validerades 3D-QALAS noggrant in-vivo genom att bland annat undersöka metodens noggrannhet och precision hos 10 friska frivilliga och 23 patienter med olika typer av hjärtsjukdomar.

I delarbete III och IV appliceras 3D-QALAS i kliniska studier där hjärtmuskeln utsätts för förändringar över tid. I studie III undersöks
Svensk sammanfattning

Patienter med tät aortastenos där hjärtat har anpassat sig efter en ökad belastning under lång tid. Patienterna undersöks med 3D-QALAS före, 3månader efter och 12 månader efter aortaklaffoperation för att studera om T1 och T2 kan användas som markörer för att påvisa förändringar i myokardiet med fokus på ödem och fibros. I studie IV undersöks hjärt påverkan från strålbehandling hos 10 patienter med bröstcancer. Patienterna undersöks med MR och 3D-QALAS före påbörjad strålbehandling, 2-3 veckor in på behandlingen, en månad efter avslutad behandling och 6 månader efter avslutad behandling för att studera om förändringar i T1 och T2 kan ses, samt om de kan korreleras till oavsiktlig bestrålning av hjärtat under strålbehandlingen.
LIST OF PAPERS


IV. Kvernby S., Flejmer A., Dasu A., Ebbers T. and Engvall J. Myocardial T1- and T2-relaxation times indicate radiation induced effects on the myocardium – a pilot study on breast cancer patients. *In manuscript.*
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>2D</td>
<td>Two-dimensional</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>CMR</td>
<td>Cardiovascular magnetic resonance</td>
</tr>
<tr>
<td>IR</td>
<td>Inversion recovery</td>
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<td>LL</td>
<td>Look Locker</td>
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<tr>
<td>LV</td>
<td>Left ventricle</td>
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<td>ME</td>
<td>Multi echo</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>RF</td>
<td>Radio frequency</td>
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<tr>
<td>RT</td>
<td>Radiation therapy</td>
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<td>T1</td>
<td>Longitudinal relaxation time</td>
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<td>T2</td>
<td>Transverse relaxation time</td>
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Sofia Kvernby,
October 2018
INTRODUCTION

Cardiovascular disease is a serious health threat in developed countries, where ischemic heart disease is the number one cause of death. Medical investigations and diagnosis together with treatment of these patients are expensive and resource intensive for the health care system. Improvement of diagnostic procedures would imply a better and more cost-effective health care, for example due to the ability to choose a more suitable therapeutic approach. Assessment of myocardial function can be made non-invasive by various techniques. The first choice of diagnostic method depends on the medical issue that is of interest, but stress test with ECG and echocardiography are usually made in an early stage of diagnosis. Other techniques that can be used are myocardial scintigraphy, computed tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI).

Magnetic resonance imaging is to a large extent a qualitative tool that is based on visual analysis. Magnetic resonance imaging settings are generally chosen in a way that highlights intensity contrast between different tissues. The intensity in such images is defined on an arbitrary scale. Quantitative images where each pixel represents a value of the actual relaxation time, is of special interest in diffuse tissue changes where visual interpretation of images are difficult and in follow-up studies to assess disease progression. Development of quantitative methods for the assessment of myocardial function would minimize inter- and intra-observer variability, be beneficial for the diagnostic procedures and also increase the reliability of the clinical decision.

The length of magnetic resonance relaxation times, T1 and T2, is dependent on the characteristics of a tissue and can therefore be used as indicators of pathological tissue changes. Quantification of T1 and T2 relaxation times on a pixel-wise manner has demonstrated successful results in the assessment of several diseases, but it has shown to be challenging to obtain accurate measurements in clinically acceptable measurement times.
AIM OF THESIS

The overall aim of this thesis was to develop, validate and evaluate a quantitative approach for the assessment of myocardial function and myocardial tissue characteristics using magnetic resonance imaging.

More specifically, the aim for each study was to:

I. Develop a method for simultaneous 3D quantification of both myocardial T1 and T2 relaxation times in one breath hold (the 3D-QALAS method).

II. Validate the 3D-QALAS method by investigating the in-vivo precision and clinical feasibility.

III. Evaluate the 3D-QALAS method in patients and investigate whether myocardial relaxation times, measured with 3D-QALAS, can detect changes over time in patients with severe aortic stenosis, from before surgery to 12 months after aortic valve replacement.

IV. Investigate whether myocardial relaxation times can detect changes in myocardial tissue characteristics due to cardiac irradiation in breast cancer patients treated with radiation therapy.
THE HUMAN HEART

Figure 1: Illustration of the human heart anatomy.

Anatomy and physiology

The heart is a muscle, responsible for pumping blood through the vessels into the body via the circulatory system. The heart is located in the mediastinum, between the two lungs, and is asymmetrically located with approximately two-thirds of the heart on the left side of the body (1).

The heart is built from four chambers, the left and the right atrium together with the left and the right ventricle. Deoxygenated blood returns from peripheral parts of the body and enters the heart in the right atrium continuing to the right ventricle through the tricuspid valve. When the ventricle contracts, the blood exits via the pulmonary valve and...
The Human Heart

pulmonary artery to the lungs and the alveoli, where an exchange between carbon dioxide and oxygen occurs. The now oxygenated blood enters the heart in the left atrium, and then continues through the mitral valve to the left ventricle. The left ventricle contracts and pumps the oxygenated blood to the rest of the body through the aortic valve and the aorta (2).

The cardiac cycle consists of the systolic phase and the diastolic phase. The systolic phase is defined as the time from the closure of the mitral valve to the closure of the aortic valve, implying that the diastolic phase is defined as the rest of the cardiac cycle (3). In the systolic phase, the mitral and tricuspid valves are closed and the ventricular pressures are increasing. When the ventricular pressure exceeds the aortic pressure respectively the pulmonary artery pressure, the aortic and the pulmonary valves open and blood is pumped from the ventricles by the contraction of the myocardium. In the diastolic phase, the valves between the atria and the ventricles, i.e. the mitral valve and tricuspid valve, open and blood fills the ventricles. The ventricular filling phase can be divided into an early filling phase, where approximately 75% of the blood volume passively fills the ventricles, and the late filling phase, where the last 25% of the blood volume is actively pumped into the ventricles by atrial muscle contraction.

The left ventricular wall is comprised of three layers, epicardium, myocardium and endocardium. The epicardium is the outer layer, containing connective tissue, fat and the coronary arteries. The endocardium is the inner layer that is lined with endothelial cells and acts as a barrier between the blood pool and the myocardium. The thick muscular layer of the LV wall is the myocardium, whose function is to contract and relax the ventricles and the atria. The cardiac muscle cells, cardiomyocytes, are in many ways different from other muscle cells in the body. Cardiomyocytes have only one central nucleus per fiber, compared to skeletal muscle that has one or more nuclei. It is also an involuntary muscle, driven by an electrical stimulation in form of an action potential that leads to muscle contraction. The action potential in cardiomyocytes has a uniquely prolonged plateau that is important in preventing irregular heart beats, by rendering the muscle cell unresponsive a new contraction before relaxation has been achieved (4).
Myocardial fibrosis

Cardiac muscle cells are surrounded by extracellular matrix and cardiac fibroblasts that are important supporting cells. The fibroblasts are smaller in size but larger in number than the cardiomyocytes (5). Myocardial fibrosis is a scarring process that is characterized by accumulation of fibroblasts and increased deposition of extracellular matrix components including collagen. Myocardial fibrosis is either expressed as reactive interstitial fibrosis, which occurs without loss of cardiomyocytes, or as replacement fibrosis, which involves hypertrophy and necrosis of cardiomyocytes (6). Fibrosis is often seen as a final endpoint in the majority of the pathological processes in the heart. For example among aortic stenosis patients, presence of myocardial fibrosis has shown to dramatically increase the all-cause mortality risk (7), even after aortic valve replacement (8). An increased amount of interstitial fibrosis makes the left ventricle stiffer and may lead to diastolic dysfunction and increased filling pressure (9). Early cardiac filling is thus decreased, which causes a compensatory increase in the filling by atrial contraction (10). The left atrial muscle must thus contract against an increased filling pressure in the ventricle and, at the end stage of the disease, increased filling pressure throughout the entire cardiovascular system. However, fibrosis may be reversible (11) and it is thus of interest to both diagnose and quantify the disease at an early stage.
MAGNETIC RESONANCE IMAGING

Basic principles of MRI

Magnetic resonance imaging (MRI) is an imaging modality used for both anatomical and functional medical imaging. The MRI scanner consists of a large superconducting coil that creates a strong static magnetic field, $B_0$, usually 1.5 or 3 Tesla. The scanner also includes gradient coils, used to create magnetic field gradients for frequency, spatial and phase encoding during scanning. In the scanner ring, closest to the patient, there is also a radio frequency (RF) coil for creation of the time varying $B_1$ field.

![Figure 2. Illustration of the hydrogen atom in an external magnetic field $B_0$, precessing with the Larmor frequency $\nu_0$.](image)

The MRI technique is based on nuclear spin, $I$, which is an intrinsic quantum property that all nucleons experience. Magnetic resonance imaging generally utilizes information from the hydrogen atom (¹H), which is abundant in the body and has the spin quantum number $I=1/2$ and therefore the magnetic quantum number $m=±1/2$ (12). These states are normally degenerated with respect to energy, meaning that they have the same energy and the magnetic dipoles have a random spatial orientation. When an external magnetic field is applied, as in MRI, the degeneration is repealed and a splitting in $(2I+1)$ different energy states arises, Zeeman-effect for the nuclei (13). For the hydrogen atom, an
applied external magnetic field implies two energy states, \( m_I = -\frac{1}{2} \) and \( m_I = +\frac{1}{2} \).

![Figure 3. Illustration of the splitting of energy states when an external magnetic field is applied.](image)

The difference between the energy states at 1.5 T and 3 T is in the radiofrequency (RF) range of electromagnetic radiation and is proportional to the strength of the applied external magnetic field, \( B_0 \), according to

\[
\Delta E = 2 \cdot \mu_p \cdot B_0 = h \nu_0 \quad [1]
\]

\[
\nu_0 = \frac{2 \mu_p}{\hbar} \cdot B_0 \quad [2]
\]

where the magnetic dipole moment of the proton, \( \mu_p = 1,4104 \cdot 10^{-26} \text{ m}^2 \text{kg/s} \),

\[

\nu_0 = 42,6 \cdot 10^6 \left[ \text{MHz/T} \right] \cdot B_0 \quad [3]
\]

The constant 42,6 MHz/T is often mentioned as the gyromagnetic ratio \( \frac{\nu}{2\pi} \) of the proton, \(^1\text{H} \), which implies the Larmor equation

\[
\nu_0 = \frac{\nu}{2\pi} \cdot B_0 \quad [4]
\]

Spins will precess around the static magnetic field with the frequency, \( \nu_0 \), the Larmor frequency, meaning that the angular momentum is indefinite in the plane perpendicular to \( B_0 \).
In the presence of an external magnetic field, as in the MRI scanner, spins orient in the field according to an energy distribution determined by the Boltzmann distribution. The lower energy level is represented by spins aligned with the $B_0$ field. There is a small surplus of a few parts per million of spins oriented in this direction, implying that a net magnetization vector parallel to $B_0$ exists.

The orientation of the spins can be changed by induction due to the magnetic component of the RF waves that are on resonance, i.e. have the same frequency as the Larmor frequency of the spins. All spins will then continuously rotate from the lower energy level $m_I=+1/2$ to the higher energy level $m_I=-1/2$ and back until the RF waves are stopped. Therefore the combination of RF amplitude and time will determine the total flip angle of rotation. Common RF pulses are 90° pulses and 180° pulses which alters the direction of the net magnetization vector 90° respectively 180° from its original direction.

Applying an RF pulse to the system implies that a magnetization component will exist in the xy-plane, rotating with the Larmor frequency. Placing a receiver coil in the xy-plane will create an induction current in the coil and the MR signal can be detected (14).

**Contrast parameters**

The image contrast in an MRI image is mainly determined by the proton density and two different types of relaxation. The proton density varies between different tissues and is proportional to the water content in the tissue. The two types of relaxation are the spin-lattice relaxation, also referred to as longitudinal relaxation or T1 relaxation, and the spin-spin relaxation, also referred to as transverse relaxation or T2 relaxation. These two relaxations take various amount of time in different tissues and hence contribute creating image contrast (14).

**T1 relaxation time**

The longitudinal relaxation occurs when excited protons return to their initial state of energy and the magnetization vector in the $z$-direction increases. Excess energy is released as heat to the surroundings. The $T1$
relaxation time is defined as the time it takes for the longitudinal magnetization vector to recover to 63% of its initial value after being flipped into the transverse plane (14).

\[ M_x(t) = M_0 \cdot \left(1 - e^{-\frac{t}{T_2}}\right) \] \[ \text{(5)} \]

**T2 relaxation time**

The transverse relaxation is due to local magnetic field inhomogeneities in the \( B_0 \) field, \( \Delta B_0 \), which creates a reduction of the magnetization vector in the xy-direction. These inhomogeneities are partly due to the difficulties in creating a totally homogenous magnetic field inside the MR-scanner and partly that individual spin can be seen as small magnets affecting their neighbors and thus creates a locally varying \( B_0 \) field, which causes varying precession frequency of the protons in different locations and dephasing of spins in the xy-plane. Assuming perfectly homogeneous external magnetic field, this relaxation is referred to as T2 relaxation. When including effects of an inhomogeneous external magnetic field, this relaxation is referred to as \( T_2^* \) relaxation, and is thus faster than the T2 relaxation. The T2 relaxation time is defined as the time it takes for the transverse magnetization to decay to 37% of its initial value after being generated in the transverse plane (14).

\[ M_{xy}(t) = M_{xy,0} \cdot e^{-\frac{t}{T_2}} \] \[ \text{(6)} \]

**Image acquisition**

To create an MRI image, the spatial location of the tissue contributing to the detected MRI signal has to be known. For that purpose, spatially varying magnetic field gradients are used. The magnetic gradients are used to define the spatial location of protons by encoding the magnetization of the protons with a specific phase and a specific frequency. Protons located along a magnetic gradient experience slightly different magnetic field strengths and will precess with different Larmor frequencies depending on where along the gradient axis they are.

For formation of a 2D image, a slice selective gradient is applied in \( z \)-direction, along the \( B_0 \) field. The gradient is switched on in combination
with the RF-pulse. The transmitted RF-pulse has a well-defined frequency that is set to the resonance frequency of the protons within the selected slice, which means that only the protons within the slice are excited and rotated by the desired flip angle.

For the spatial resolution in the x- and y-directions, frequency encoding is used in one direction and phase encoding is used in the other direction. The frequency encoding gradient induces, in the same way as for the slice selective gradient, the protons to precess with different Larmor frequencies along the gradient. The currents induced in the receiver coil will therefore have different frequencies, depending on where along the gradient the spins that give rise to the signal are located. The frequency encoding gradient is applied during the signal readout.

The phase encoding gradient is directed perpendicular to the frequency encoding gradient and is applied for a short time somewhere between the RF-pulse and the signal readout. The gradient creates a phase shift between the spins that is retained until signal readout, and the size of the phase shift depends on the position along the gradient axis. The amplitude of the phase encoding gradient is altered between every repetition of the sequence, in as many steps as the image matrix consist of, for example 256.

Acquisition of the MRI signal in this way creates a signal matrix in the frequency domain, also referred to as k-space, that contains components with different frequencies and phases. To create the image matrix, an inverse Fourier transform is used, which transform the MRI signal from the frequency domain to the image domain (15).

Examples of pulse sequences
The pulse sequence describes how image data is acquired by describing the timing of radiofrequency pulses and gradient pulses. The signal echo in MRI can either be a gradient echo or a spin echo.

The gradient echo sequence
The radiofrequency pulse ($\alpha<90^\circ$) creates a magnetic component in the transverse plane and is applied together with the slice selective gradient. Immediately after the RF pulse is switched off, a short negative gradient is
applied in the frequency direction with the aim to pre-compensate for the dephasing of the transverse magnetization that will occur when the positive frequency encoding gradient will be switched on during signal readout. The phase encoding gradient, whose amplitude changes between each repetition of the sequence, is also applied immediately after the RF pulse. The positive frequency encoding gradient is applied at the same time as the gradient recalled echo is formed, during signal readout. The time between the RF pulse and the echo is the echo time (TE), while the time between one RF pulse and the next is the repetition time (TR). The signal amplitude of the gradient echo is dependent on the $T_2^*$ relaxation and decay according to $e^{-\frac{TE}{T_2^*}}$. The gradient echo sequence has short TE and TR and allows for fast imaging since there is no waiting time for a refocusing RF pulse as in the spin echo sequence. The gradient echo sequence is dependent on $T_2^*$ relaxation instead of $T_2$ relaxation, which may lead to issues with susceptibility and chemical shift artifacts in the images (16).

Figure 4: Schematic illustration of the gradient echo sequence.
The spin echo sequence

For the spin echo sequence, two radiofrequency pulses are applied, one 90° excitation pulse and one 180° refocusing pulse. Directly after the 90° pulse, the spins will start to dephase due to inhomogeneity in the static magnetic field. After a time, TE/2, the 180° refocusing pulse is applied and mirror the dephasing of spins, giving that spins are in phase after an other TE/2 after the 180° pulse, where the signal readout occurs and the echo is acquired. The slice selective gradient is switched on when the RF pulses are applied. The phase encoding gradient is applied sometime between the 90° RF pulse and the 180° RF pulse and the frequency encoding gradient is applied during signal readout. The time between one 90° pulse and the next 90° pulse is the TR. The signal amplitude of the spin echo is dependent on the T2 relaxation and decay according to $e^{-\frac{TE}{T2}}$. The spin echo sequence is slower than the gradient echo sequence but provides better signal (16).

Figure 5. Schematic illustration of the spin echo sequence.
Cardiovascular MRI

Cardiovascular magnetic resonance (CMR) has several clinically important applications (17). In the assessment of left ventricular function, CMR is a very accurate method for measurement of ventricular volumes, ventricular wall mass and ejection fraction (18). After myocardial infarction, contrast enhanced CMR can be used for the assessment of myocardial viability, which is of major importance for the ability to predict functional recovery (19). Other examples of clinically important CMR applications are flow assessment (20), perfusion imaging (21), evaluation of congenital heart diseases (22) and assessment of cardiomyopathy (23).

Most often, cardiac triggering using ECG is used to sample image data in a specific phase of the cardiac cycle. To avoid motion artifacts from the heart itself, the data acquisition window is often restricted to a short time period within the cardiac cycle, or divided into several time periods/phases to sample time resolved dynamic data. Motion artifacts can also arise due to breathing motion and breath holds can therefore be used to keep motion at a minimum during data acquisition.
Quantification

Principles of relaxation times quantification

In daily clinical practice MRI settings are generally chosen to highlight intensity contrast between tissues. By varying parameters such as the echo time, repetition time and radiofrequency flip angle it is possible to create different contrast weighted images. The absolute intensity in contrast weighted images is defined on an arbitrary scale that differs from one examination to another. Image interpretation thus relies on visual comparisons with surrounding tissue. Another approach of MRI is to use quantitative images, where each pixel value represents the actual value of the relaxation time, also referred to as relaxation times mapping. Relaxation times mapping enables numerical comparison between different tissue compositions and hence the objective detection of pathological processes by for example measuring a deviation from the range of normal values.

The gold standard approach for relaxation times quantification is to use an inversion recovery sequence with several different inversion times to quantify T1 and to use a multi echo sequence with several different echo times to quantify T2 (24).

Inversion recovery sequence (IR)

The inversion recovery sequence is based on a prepulse of 180° that is applied an inversion time, TI, before the 90° excitation pulse. The prepulse inverts the direction of the magnetization vector from $M_z$ to $-M_z$. Directly after the RF prepulse is switched off, the magnetization vector starts to relax back to equilibrium according to the T1 relaxation of the tissue. Since different tissues experience different T1 relaxation times, the inversion time can be set in a way that suppresses the signal from a specific tissue, for example fat or fluid, by applying the 90° pulse when net magnetization of that tissue is zero.
For quantification of the T1 relaxation time, the signal, S, is measured, i.e. images are acquired, several times after different inversion times, TI. The T1 relaxation time can be estimated according to

\[ S = S_0 \cdot (1 - 2e^{-\frac{TI}{T_1}}) \]  

[7]

To allow full magnetization recovery between the radio frequency inversion pulses a relaxation period of four to five times T1 is necessary. For cardiac applications, the clinical applicability of the inversion recovery sequence for T1 quantification is limited since T1 often has a value exceeding the duration of the cardiac cycle and the acquisition would be very time consuming.

**Multi echo sequence (ME)**

The multi-echo sequence consists of a 90° excitation pulse, which transfers the magnetization vector into the xy-plane. The signal, S, is then measured several times after different echo times, TE, and a value of the T2-relaxation time can be calculated according to

\[ S = S_0 \cdot e^{-\frac{TE}{T_2}} \]  

[8]

The multi echo sequence requires several echo time points and thus several repetitions to sample enough data and to get a good signal to noise ratio. A multi echo sequence therefore becomes time consuming and difficult to use for in-vivo cardiac T2 measurements.

**Myocardial relaxation times quantification**

**General principles and challenges**

Quantification of relaxation times in myocardial tissue is connected to several difficulties. Motion of the heart, both from breathing motion but also from the heart beating, has to be taken care of. Time constraints due to the length of the cardiac cycle, further complicate data collection. To avoid motion artifacts, the signal readout is timed to always be in the same phase of the cardiac cycle. It is usually performed at the end of diastole when the heart is moving least, giving a short window of
maximum 300 ms to collect the required data. Since relaxation times mapping requires several data points along the relaxation curve to estimate the relaxation time accurate, a large amount of data is often necessary and accelerated imaging techniques are therefore frequently used.

**Look Locker inversion recovery**

*Original Look Locker*

The traditional inversion recovery sequence is not applicable for myocardial T1 relaxation times mapping due to the extremely long imaging time. Look and Locker described a multi point approach (25), where the relaxation curve is sampled multiple times after the initial inversion pulse, which contributes to accelerate the acquisition. The magnetization vector is hence measured several times during its return to equilibrium after the 180° pulse. The drawback with the original Look Locker (LL) -method is that it uses continuous data acquisition and not selective data acquisition in a specific time frame of the cardiac cycle.

*Modified Look Locker inversion recovery*

Several modified versions of the Look Locker method exist for myocardial T1 mapping. Messroghli et al presented, a now established, modified LL inversion recovery sequence (MOLLI) (26) which uses selective ECG-triggered data acquisition and multiple LL experiments. Balanced steady-state free precession readout is used. In the original format, three sets of LL experiments are performed successively with an additional 100 ms inversion time for each set. The LL sets consists of three, three and five single shot image readouts respectively in end-diastole. A waiting period of three heartbeats between each set is used to ensure undisturbed magnetization recovery before the following inversion pulse. The MOLLI sequence acquires in its original format one single 2D slice of the heart in 17 (3+3+3+3+5) heartbeats. The T1 relaxation time is estimated from the 11 different inversion times (3+3+5).
Other fast T1-mapping sequences

The original MOLLI sequence requires relatively long breath hold to quantify T1 in one 2D-slice of the heart, which has led to the development of several faster T1 mapping methods. A shortened version of the MOLLI sequence exists, ShMOLLI (27), which has been validated and used in several studies with good agreement to the original version. The ShMOLLI method is based on three IR experiments, in the same way as MOLLI, but requires only 9 heartbeats to quantify T1 in one 2D-slice of the heart. While the MOLLI sequence has demonstrated a heart rate dependence and tends to underestimate T1 for low heart rates and for long T1 relaxation times or very short T1 relaxation times (28), the ShMOLLI method mitigates the heart rate dependence by performing a conditional analysis, where only the data points after the first inversion recovery experiment are used for long T1 times and for high heart rates (29).

Saturation recovery sequences have also been used for T1-mapping. The magnetization vector in such sequences is measured several times during its return to equilibrium after a 90° RF pulse, instead of a 180° RF-pulse
as used in the inversion recovery based sequences. Saturation recovery single shot acquisition, SASHA (30), is an example of a saturation recovery based T1 mapping sequence that provides a 2D slice of the heart in a breath hold of 10 heartbeats. Another T1 mapping method is the SAPPHIRE sequence (31), which uses a combined inversion and saturation recovery sequence. The SAPPHIRE sequence takes slightly longer time than the MOLLI-sequence but is, like the saturation recovery based SASHA sequence, not heart rate dependent.

Inversion recovery based sequences have a larger dynamic range than the saturation recovery based sequences, and the MOLLI and ShMOLLI methods have in a comparison demonstrated higher precision but lower accuracy than SASHA and SAPPHIRE (32).

**T2 preparation pulse**
The traditional multi echo sequence becomes very time consuming in-vivo and is not applicable for myocardial T2 mapping. An approach that is more suitable for cardiac imaging is to use a method based on T2 preparation pulses (33), which has shown to be both robust and fast (34). The T2 prepared sequence is used to generate two or more T2 weighted images, each with different T2 preparation times. The T2 preparation pulse consists of a 90° RF pulse, a delay time (T2 preparation time) and a -90° RF pulse. After the reversed 90° RF pulse, a signal readout is performed and the signal intensity in each image represent a specific echo time (TE) along the T2 decay curve.

**Clinical application of myocardial relaxation time mapping**
Magnetic resonance imaging utilizes information from the hydrogen atom within water molecules in the body. The T1 and T2 relaxation time are therefore dependent on how the water molecules are structured in the tissue. The water content together with the composition and solidity of a tissue thus determine how fast the relaxations of the spins occur. Since T1 and T2 relaxation time are dependent on these tissue parameters, myocardial relaxation times mapping can be used to characterize myocardial tissue composition and diseases related to intracellular and/or extracellular changes (35-37).
Myocardial relaxation time mapping has evolved to be a successful clinical tool in the assessment of several different heart diseases and gives the opportunity to separate different tissue compositions (38). Both T1- and T2 maps of the myocardial tissue have shown to provide important information in the assessment of acute and chronic myocardial infarction (39), acute myocarditis (40,41), inflammation and myocardial edema (42-44). However, the largest potential for myocardial relaxation time mapping lies in the ability to detect diffuse tissue changes, for example diffuse fibrosis (45,46), lipid storage (47) and iron overload (48,49), where no remote or healthy myocardium is accessible for visual comparison, and other non-invasive methods are lacking.
3D-QALAS

The 3D-QALAS (3D-quantification using an interleaved Look-Locker acquisition sequence with T2 preparation pulse) method is a combination of an iterative analysis approach and a relaxation times mapping sequence that enables left ventricular wall quantification of both myocardial T1 and T2 relaxation times in one breath hold. The 3D-QALAS method is presented in Paper I of this theses (50).

**Sequence design**

The 3D-QALAS sequence block is based on an inversion recovery sequence with an interleaved T2 preparation pulse, giving the sequence one T1 sensitizing phase and one T2 sensitizing phase.

![Figure 7: Schematic overview of the 3D-QALAS sequence design.](image)

**T2-sensitizing phase**

The T2-sensitizing phase consist of a T2 preparation pulse that aims to encode the T2 relaxation time on the longitudinal, $M_z$, magnetization vector. The T2 preparation pulse starts with a 90° RF pulse, which tilts the magnetization vector into the x-y-plane, and is followed by two 180°
RF refocusing pulses. After a waiting time of 50 ms, an inverse -90° RF pulse is applied, which tilts the magnetization vector back into the M<sub>c</sub>-direction. The magnetization vector has during these 50 ms decreased according to the tissues T2 relaxation time. A 3D turbo field echo data acquisition is performed after the T2-sensitizing phase.

**T1-sensitizing phase**
The T1-sensitizing phase starts in the next heartbeat with an adiabatic 180° inversion pulse. During the following T1 relaxation, the magnetization vector is measured four times during the return to its initial state. The second data acquisition in the 3D-QALAS sequence block is performed 100 ms after the inversion pulse, and is followed by three more data acquisitions in the consecutive heartbeats. For example, giving a heart rate of 60 beats per minute, the T1 sensitizing phase will have the following inversion times: 100 ms, 1100 ms, 2100 ms and 3100 ms.

**Data acquisition and k-space trajectory**
The data acquisition is performed once after the T2-preparation pulse and four times after the T1 sensitizing inversion pulse (five heartbeats). The data acquisition has to be fast and short in comparison with the length of the cardiac cycle. The 3D-QALAS sequence uses an ultrafast spoiled gradient echo readout, also referred to as turbo field echo, for image readout. The turbo field echo has a low RF flip angle (α=5°) and both short echo time (1.2 ms) and repetition time (2.6 ms). The number of RF pulses during one data acquisition, the TFE-factor, is 90, which implies that 90 k-space phase encoding steps are acquired during each TFE-acquisition. The acquisition window becomes 2.6 ms × 90 ≈ 230 ms, and aims to be short enough to minimize artifacts from cardiac motion. Subsequent spoiling of any remaining magnetization in the x-y-plane follows each TFE readout. Spoiling is performed both as gradient spoiling, where any remaining magnetization in the x-y-plane is transferred to the far corner of k-space, and as RF spoiling, where the phase of the flip angel α alters for every repetition.

The data acquisitions are segmented, which means that only a set of the required k-space data lines are acquired during readout. The data acquisition of the 3D-QALAS sequence is divided into three segments, which means that the sequence is repeated three times to sample all the k-
space data needed. The total acquisition time of the 3D-QALAS sequence is therefore 15 ($5 \times 3$) heartbeats.

K-space is sampled with a radial trajectory, also referred to as low-high order, which means that the center of k-space is sampled first and the periphery last. In order to sample the required data during the end diastolic phase of the cardiac cycle, accelerated acquisition techniques are used. Elliptical k-space filling is used, which means that the corners of k-space are not sampled, which reduces the required number of data points. Parallel imaging is applied, by the use of sensitivity encoding (SENSE) in both y- and z-direction. Sensitivity encoding reduces the scan time by reducing the number of required phase encoding steps and utilizes the sensitivity from an array of multiple receiver coils (51). The 3D-QALAS sequence use a SENSE factor of 2.0 in phase encoding y-direction and a SENSE factor of 1.2 in slice encoding z-direction. The image resolution of 3D-QALAS is 2 mm in the frequency direction, 2 mm in phase direction and 12 mm in the slice direction. The data were reconstructed into 13 short axis slices with a resolution of 2 mm in plane and a slice thickness of 6 mm.

**Data analysis**

**Calculation of parameters**

The T1 and T2 relaxation times are calculated from the five measured data points, acquisition 1-5, by using an iterative analysis approach based on simulations of the evolution of the longitudinal magnetization vector $M_Z$. During the T1-sensitizing phase, the longitudinal magnetization vector is inverted. During the delay time between the acquisitions, in the absence of RF pulses, the longitudinal magnetization vector relaxes with a T1 relaxation time, approaching the unsaturated magnetization $M_0$. According to the schematic overview of the 3D-QALAS sequence in figure 7, T1 relaxation occurs during $M_3$–$M_4$, $M_5$–$M_6$, $M_7$–$M_8$, $M_9$–$M_{10}$, $M_{11}$–$M_{12}$ and $M_{13}$–$M_1$. During these intervals, the magnetization, $M_{n+1}$, after a time interval $\Delta t$ can be calculated from the previous magnetization $M_n$, according to:
During acquisition readout, the T1 relaxation time is affected by the RF pulses with a flip angle of $\alpha$, which is repeated every TR, with subsequent spoiling. During readout, the magnetization vector $M_Z$ relaxes with an altered T1 relaxation time, an effective T1* relaxation, that approaches the saturated magnetization $M_0^*$ instead of the unsaturated magnetization $M_0$. In figure 7, the effective T1* relaxation occurs during M2 - M3, M6 - M7, M8 - M9, M10 - M11 and M12 - M13. Since the acquisition time is 230 ms and the cardiac cycle length is approximately 1000 ms, the proportion of T1* relaxation and T1 relaxation is approximately 25% T1* and 75% T1 during the T1-sensitizing phase. The connection between the unsaturated magnetization $M_0$ and the saturated magnetization vector $M_0^*$ can be described by:

$$\frac{T_{1^*}}{T_1} = \frac{M_0^*}{M_0} = \frac{1-e^{-\frac{TR}{T_1}}}{1-\cos(\alpha)e^{-\frac{TR}{T_1}}}$$  \[10\]

Now, each magnetization, $M_{n+1}$, after a time interval $\Delta t$ can be calculated from the previous magnetization $M_n$, according to:

$$M_{n+1} = M_0^* - (M_0^* - M_n) \cdot e^{-\frac{\Delta t}{\tau_{1^*}}}$$  \[11\]

During the T2-sensitizing phase, the longitudinal magnetization $M_Z$ decreases by a factor of:

$$e^{-\frac{TE_{T2prep}}{T_2}}$$  \[12\]

The $TE_{T2prep}$ is the time of the T2-preparation pulse, which means the time between the 90° tip-down RF pulse into the x-y-plane and the -90° tip-up pulse back along the z-axis.

The total acquisition sequence block results in five measured signals for each image voxel that is proportional to the magnetization $M_2$, $M_6$, $M_8$, $M_{10}$ and $M_{12}$ described in figure 7.
By using equation 9-11 in an iterative analysis method, the values of $M_0$ and $T_1$ are found by minimizing the squared difference between the expected magnetization $M_2$ and the five measured signal data points. For calculation of the $T_2$ relaxation time, the extrapolated magnetization just prior to the $T_2$-preparation pulse, $M_1$, and right after the $T_2$-preparation pulse, $M_2$, are used according to:

$$T_2 = \frac{T_{E2prep}}{\ln \left( \frac{M_1}{M_2} \right)}$$

[13]

Myocardial T1 and T2 maps were generated by implementation of the method on a standalone version of the Synthetic MRI software.

**In-vitro validation**

The 3D-QALAS sequence was validated in-vitro with the aim to investigate the robustness and the accuracy of the method (50). Phantoms were manufactured with different amount of gadolinium and different concentrations of agarose, giving phantoms with $T_1$ relaxation times in the range ~ 200 – 1800 ms and $T_2$ relaxation times in the range ~ 35 – 170 ms.

Parameters that potentially could affect the relaxation times quantification were intentionally varied. Heart rates between 40 and 120 beats per minute were simulated to investigate any heart rate dependency. The flip angle, $\alpha$, during data acquisition was varied between 4° and 8° to investigate the method’s sensitivity to $B_1$ inhomogeneity. Variations in the length of the cardiac cycle were deliberately applied to simulate cardiac arrhythmias or atrial fibrillation and to investigate any effect on the relaxation times measurements. The length of the cardiac cycle was varied by applying three different widths of Gaussian noise distributions (5%, 10% and 15%) to a standard cardiac cycle length of 1000 ms.
To investigate the accuracy of the 3D-QALAS method, T1 and T2 reference values were established. For T1 reference measurements, an inversion recovery turbo spin echo sequence was used, with varying inversion times between 100 ms - 5000 ms and a repetition time of 10000 ms. For T2 reference measurements, a multi echo sequence was used with echo times varying from 10 ms – 200 ms and a repetition time of 3000 ms.

Results
Both T1 and T2 relaxation times measured with 3D-QALAS had a very good correlation with reference methods. The Pearson correlation coefficient was R=0.998 for inversion recovery T1 measurements and R=0.996 for multi echo T2 measurements. The 3D-QALAS method did not show any dependency on heart rate within the tested range of heart rates (see figure 8). The relaxation times quantification was not affected by small changes in B1, since no dependency on RF flip angle could be seen within the tested range of α, and was also robust for light (5 and 10% width of Gaussian noise distribution) cardiac cycle length variations that aims to simulate arrhythmia.
Figure 8: Heart rate dependency in T1 (upper) and T2 (lower) measurements with 3D-QALAS.
Discussion
The in-vitro validation of the 3D-QALAS method demonstrated highly accurate measurement results over a large range of both T1 and T2 relaxation times. The tested range of relaxation times cover both contrast enhanced and native myocardial tissue. Heart rate dependency is a known problem among other myocardial T1-mapping sequences and the T1 mapping consensus statement recommends to investigate heart rate dependency in novel mapping methods within the range of 40-120 beats per minute (52), which is the range that was used for validation of 3D-QALAS. The 3D-QALAS sequence appeared to be robust for changes in heart rate, flip angle and arrhythmia during the measurement.

In-vivo validation
The 3D-QALAS method was in Paper I applied in healthy volunteers to demonstrate the function of the method in-vivo, and to make a comparison with existing 2D myocardial mapping methods. For T1 reference measurements, a 3-3-5 MOLLI sequence was used, and for T2 reference measurements, a dual echo T2 GraSE sequence was used. The method was further carefully validated in a separate article, Paper II, to investigate the in-vivo precision and clinical feasibility of 3D-QALAS (53).

In Paper I, ten healthy volunteers underwent three 3D-QALAS acquisitions, three mid-ventricular T1 MOLLI acquisitions and three mid-ventricular T2-GraSE acquisitions. Relaxation times measurements with 3D-QALAS were compared with measurements from reference methods.

In Paper II, ten healthy subjects underwent MRI examinations including 3D-QALAS, MOLLI and multi echo T2-GraSE acquisitions. Precision was investigated in the healthy subjects as standard deviation of eight consecutive scans. An intraclass correlation analysis was also performed to measure how strongly the eight repeated acquisitions within a group, represented by each mapping method, resemble each other. Precision was assessed by the Pearson correlation coefficient as agreement between two measurements, both under repeatability conditions in independent scan
situations, where the subject was removed from the scanner between scans, and under intra-scan conditions in dependent scan situations.

In Paper II, the clinical feasibility of 3D-QALAS was investigated from 23 patients with different cardiac pathologies by acquiring 3D-QALAS, MOLLI and T2-GraSE data both before and after injection of gadolinium based contrast agent. A Pearson correlation analysis was performed between patient data obtained with 3D-QALAS and the reference methods.

**Results**

In-vivo relaxation times data acquired in Paper I demonstrated a good agreement between 3D-QALAS and the reference methods. Average T1 value and standard deviation was $1083 \pm 43$ ms for 3D-QALAS and $1089 \pm 54$ ms for MOLLI. Average T2 value and standard deviation was $50.4 \pm 3.6$ ms for 3D-QALAS and $50.3 \pm 3.5$ ms for T2 GraSE. An example of the 13 slices T1 and T2 maps, obtained with 3D-QALAS, from a healthy volunteer is demonstrated in figure 9.
Precision measurement results from the eight repeated acquisitions within the group of healthy subjects in Paper II, demonstrated average myocardial relaxation time values and SD of $1178 \pm 18.5$ ms (1.6%) for T1 with 3D-QALAS, $52.7 \pm 1.2$ ms (2.3%) for T2 with 3D-QALAS, $1145 \pm 10.0$ ms (0.9%) for T1 with MOLLI and $49.2 \pm 0.8$ ms (1.6%) for T2 with GraSE. Myocardial relaxation times from the two independent scans demonstrated a less good correlation than relaxation times from the two dependent scans, for all mapping methods. Myocardial relaxation times data from 23 patients with different cardiac pathologies obtained with 3D-QALAS, correlated very well with reference methods (MOLLI for T1, $r=0.994$, and T2-GraSE for T2, $r=0.818$). Average myocardial T1 relaxation times in the patient group were in native/contrast enhanced myocardium 1166.2 ms/411.8 ms with 3D-QALAS and 1174.4 ms/438.9 ms with MOLLI. Corresponding native myocardial T2 values were 53.2 ms with 3D-QALAS and 54.4 ms with T2-GraSE.

Figure 9. 3D-QALAS images from a healthy volunteer (50). The 13 short axis slices T1 maps (left) and T2 maps (right).
Discussion
Results from the in-vivo validation performed in Paper I and paper II demonstrated a very good correlation to existing 2D mapping methods. Since the range of normal myocardial relaxation times values differ depending on for example mapping method and scanner (54,55), the in-vivo accuracy may be problematic to establish. For most clinical applications, in-vivo precision is of even greater importance to investigate. The in-vivo precision of 3D-QALAS was investigated between independent scans, between dependent scans and as intra-scan repeatability and demonstrated a very good precision for the method in healthy volunteers.

The clinical feasibility of 3D-QALAS were evaluated in patients, pre- and post-contrast, to create a broad range of relaxation times values from both healthy and disease affected myocardium. In conclusion, Paper II demonstrated that 3D relaxation times mapping of both T1 and T2 in one breath hold is feasible with 3D-QALAS in patients with different cardiac pathologies.

Application of the method in patient studies

Investigate diffuse fibrosis in severe aortic stenosis patients
In Paper III of this thesis (56), the 3D-QALAS method was applied in a clinical study to investigate diffuse fibrosis in patients with severe aortic stenosis.

Background
In aortic stenosis, the left ventricular outflow is obstructed because of a narrowing of the aortic valve, creating a pressure gradient across the aortic valve. This often leads to concentric hypertrophy and increased amounts of interstitial fibrosis (4) which is associated with increased mortality risk (57). It is of interest to quantify the amount of diffuse fibrosis in the myocardium, but unfortunately there is a lack of non-invasive methods for quantification of diffuse myocardial fibrosis.
Myocardial relaxation times mapping with MRI has demonstrated promising results, however (58,59). Increased amounts of interstitial fibrosis have been associated with elevated T1 relaxation times (60). The purpose of this study, Paper III in this thesis, was to investigate if myocardial T1 and T2 relaxation times can detect longitudinal changes in myocardial tissue composition related to diffuse fibrosis in patients with severe aortic stenosis prior to and 3 and 12 months after aortic valve replacement.

**Method**

Fifteen patients (see flow chart of recruitment in figure 10) with severe aortic stenosis referred for surgical aortic valve replacement were included in this study. The patients underwent MRI examinations including multiple cine short axis balanced steady-state free precession acquisition together with 3D-QALAS, MOLLI and T2-GraSE at three different time points, prior to surgery and 3 and 12 months after surgery. Left ventricular myocardial contours were manually drawn and data were analyzed using Segment v 1.9 (http://segment.heiberg.se), as 16 myocardial segments according to the 16 segments model recommended by the American Heart Association (61). For statistical analysis, a linear mixed model for repeated measures were used for segment-wise data, where each individual myocardial segment was followed over time.
Results

The results from this study demonstrated that T1 relaxation times were significantly shorter 3 and 12 months after surgery compared to before surgery, for both 3D-QALAS and MOLLI. The T2 relaxation time was significantly longer 3 and 12 months after surgery compared to the value before surgery, for both 3D-QALAS and GraSE. No significant difference was found between 3 and 12 months after surgery for either T1 or T2 relaxation times. Results are presented in figure 11. Average percentage differences in relaxation times for each myocardial segment between 3 respectively 12 months follow up and before surgery, are presented and color coded in figure 12.
Figure 11. Average ± standard deviation myocardial T1 and T2 relaxation times at the three different timepoints; preoperatively, 3 and 12 months postoperatively for T1 values with 3D-QALAS and MOLLI (upper) and T2 values with 3D-QALAS and GraSE. The P-values are from the linear mixed model test and represent changes in each individual segment over the three timepoints.
Figure 12. Average percentage differences in relaxation times for each myocardial segment. Blue indicates a decrease in relaxation time, while red indicates an increase between preoperative relaxation time and 3 respectively 12 months postoperative relaxation time.
Discussion
Relaxation times mapping was in this study performed in a way where every patient was followed over time, from before aortic valve replacement to 12 months after aortic valve replacement. This study aimed to study the association between myocardial relaxation times and reverse positive remodeling related to aortic stenosis and fibrosis after surgical intervention.

Based on earlier studies, there is an association between interstitial fibrosis and increased T1 relaxation time (62). For T2 relaxation and the association to fibrosis, studies demonstrate contradictory results where both longer and shorter T2 times have been connected to increased amounts of fibrosis (63-65). In this study, significant changes in relaxation times were found over the study period, expressed as a decrease in T1 and an increase in T2 relaxation time. The decrease in T1 may be interpreted as a reduction of the amount of interstitial fibrosis from before surgery to after surgery. Since the T2 relaxation time is closely connected to the water content in the tissue, an increased T2 value after surgery may indicate increased water content and thus a less stiff tissue with less amount of interstitial fibrosis.

In conclusion, changes in myocardial relaxation times could in this study be seen already early after surgery in patients with severe aortic stenosis, within the first three months after aortic valve replacement. The relaxation times changes seen may be interpreted as a reduction of interstitial fibrosis in the myocardial tissue.

Detection of myocardial tissue changes related to irradiation
In Paper IV of this thesis, the 3D-QALAS method was applied in a clinical pilot study to investigate whether myocardial tissue changes related to ionizing radiation can be detected with relaxation times mapping.

Background
A large proportion of breast cancer patients, especially left-sided patients, treated with external radiation therapy are at risk for unwanted cardiac irradiation during treatment (66). Ionizing radiation can cause damage to healthy heart tissue in several ways (67-70). Early effects of irradiation,
visible already early after treatment start, are often microvascular changes, inflammation and edema, while examples of late effects are reduced perfusion and increased amounts of fibrosis (71). The aim of this pilot study was to investigate whether myocardial relaxation times mapping can detect changes in tissue characteristics related to unwanted cardiac irradiation in breast cancer patients treated with radiation therapy.

Method
Ten breast cancer patients, seven left-sided and three right-sided, treated with external radiation therapy were included in this longitudinal pilot study. The patients underwent MRI and echocardiography examinations at four different time points; within one week before radiation therapy start, between two and three weeks after radiation therapy start, one month after radiation therapy was completed and the fourth examination was performed six months after radiation therapy was completed.

The MRI examination included a multiple cine short axis balanced steady-state free precession acquisition and a 3D-QALAS acquisition. The echocardiography examinations included speckle tracking for longitudinal strain measurements and tissue Doppler for E/é ratio calculation as a measure of diastolic function. Data were analyzed in the same way as in Paper III, using a mixed linear model with repeated measures that follows changes in data values from the same patient over time. As a basis for discussion and data visualization, not for statistical analysis, patients were divided into subgroups with respect to right-sided or left-sided breast cancer, chemotherapy or not, as well as Herceptin treatment or not, to investigate affecting factors that are of interest for a larger study in the future.

Results
The results demonstrated a significant increase in the T1 relaxation time late after radiation therapy, between measurement 1 (before treatment start) and measurement 4 (six months after radiation therapy treatment was completed). For the T2 relaxation time, a significant decrease was found early, already after 3 weeks into radiation therapy. In figure 13, the average myocardial T1 and T2 relaxation times, for the whole group of subjects, at the four different time points are illustrated.
Measurements of global longitudinal strain, left ventricular mass and E/é ratio demonstrated a significant difference from before radiation therapy start to 6 months after radiation therapy was completed (mean difference: LVM: 2.1 g; GLS: -3.0 %; E/é: 0.8). No significant difference was found in left ventricular ejection fraction between these time points.

Figure 13. Average myocardial T1 (left) and T2 (right) relaxation times at the four different time points.

Patients treated for left-sided breast cancer received higher absorbed dose to the heart than the right-sided breast cancer patients. Visualization of data separated into right-sided and left-sided breast cancer patients were performed and can be seen in figure 14 for T1 relaxation time, T2 relaxation time, left ventricular mass, ejection fraction, global longitudinal strain and E/é ratio.
Figure 14. Longitudinal evolution of T1 relaxation time (upper left), T2 relaxation time (upper right), left ventricular mass (mid left), ejection fraction (mid right), global longitudinal strain (lower left) and E/é ratio (lower right), separated into patients treated for left-sided (red) and right-sided (blue) breast cancer.

Discussion
In this pilot study, significant changes in T1 and T2 relaxation times, left ventricular mass, E/é ratio and global longitudinal strain were found over time, from before radiation therapy start to 6 months after radiation therapy was completed.
The separation of data into left-sided and right-sided patients aims to make a separation of patients into high and low absorbed dose to the heart. By doing that, the early significant decrease in T2 is not visible for the group of left-sided patients but only for the group of right-sided patients, and may therefore be predominately caused by other factors than irradiation, such as inflammation or edema due to for example Herceptin treatment or chemotherapy. However, differences in other parameters indicate a difference between left-sided and right-sided patients that may be connected to irradiation. The significant increase in T1, indicating increased fibrotic tissue, is supported by increased left ventricular mass and E/é ratio together with a significant decrease in global longitudinal strain from before radiation therapy to 6 months after radiation therapy. Due to the small number of patients in this pilot study, statistical analysis of subgroup could not be made, but a larger study including subgroup analysis of parameters that were found interesting in this pilot study would be of great interest for the future.

Discussion

The 3D-QALAS sequence provides three-dimensional quantification of both T1 and T2 relaxation times in one breath hold with whole coverage of the left ventricular wall. In Paper I of this thesis, the 3D-QALAS method was presented and validated in vitro by deliberately vary parameters that might affect the relaxation times quantification. A comparison against in-vitro reference methods, inversion recovery for T1 and multi echo for T2, was also performed together with in-vivo measurements in ten healthy volunteers where 3D-QALAS was compared with conventional 2D mapping methods, MOLLI for T1 and GraSE for T2. The 3D-QALAS method demonstrated good agreement with reference methods, both in-vitro and in-vivo. In comparison with other inversion recovery based T1-mapping methods, for example MOLLI, the 3D-QALAS method appeared to be robust for changes in heart rates within the range of 40-120 beats per minute. Regarding the dynamic range of 3D-QALAS in comparison with other T1-mapping methods, the dynamic range should be somewhere between that of inversion recovery sequences and saturation recovery sequences.
In Paper II of this thesis, the 3D-QALAS method was further validated in-vivo by investigating the in-vivo precision and clinical feasibility. The in-vivo precision was validated as standard deviation of eight consecutive scans and as repeatability between independent scans and between dependent scans. The 3D-QALAS method demonstrated to be a robust and precise 3D-mapping method that correlate well with existing 2D-mapping methods. In comparison with the 2D-methods, precision investigated as standard deviation of eight consecutive scans, was in this study slightly better for MOLLI and GraSE, while the correlation between two independent scans was slightly better for 3D-QALAS.

In Paper III and IV of this thesis, the 3D-QALAS method was applied to specific patient groups in a longitudinal manner, where the same patient was followed over time with repeated measures. Since myocardial relaxation times differ fairly much between individuals and the range of normal values in a healthy cohort may be large and even larger in a patient cohort, the focus in these studies was not to differentiate between patients and healthy subjects, but instead to follow the same patient and focus on changes in myocardial T1 and T2 values on a longitudinal manner. In Paper III, patients with severe aortic stenosis were followed from before the aortic valve replacement to 6 months after, and in Paper IV, patients with breast cancer who received external radiation therapy were followed from before treatment start to 6 months after treatment was completed, with the aim to investigate any changes in myocardial T1 or T2 during the study time. In both of these studies, significant changes were found in myocardial relaxation times, detected with 3D-QALAS.

In the development and evaluation of the 3D-QALAS method, we aimed for a method that could quantify myocardial T1 and T2 relaxation times simultaneously in a fast way with large myocardial coverage, without compromising with precision or robustness of the method. Performing relaxation times mapping of both T1 and T2 in a 3D volume instead of in a 2D slice, is followed by the requirement to collect more k-space lines and therefore compromises have to be done. Since the acquisition window within the cardiac cycle is limited due to cardiac motion, and the length of the total acquisition block is restricted to a reasonable length of the breath hold, it implies that only a limited number of data acquisition points can be acquired within the sequence block. Acquiring more data points would
either require a longer breath hold or it would reduce the signal to noise ratio per data point. To compensate for the fewer data points used in 3D-QALAS in comparison to for example the MOLLI sequence, the 3D-QALAS method utilizes an iterative analysis approach, based on a simulation of the magnetization vector over the acquisition block, instead of an exponential fit that is commonly used in the conventional 2D T1-mapping methods. The 3D-QALAS sequence also requires a slightly longer acquisition window together with a little bit larger slice thickness compared to the existing 2D-mapping methods.

Conclusion

The 3D-QALAS method allows for rapid quantification of both T1 and T2 relaxation times within one breath hold of 15 heartbeats and with full coverage of the left ventricular wall. 3D-QALAS has demonstrated to be a robust method with good accuracy and precision. The method has also demonstrated to be feasible for use in patients with different cardiac pathologies and has in longitudinal patient studies demonstrated an ability to detect significant changes in myocardial relaxation times over time.
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References


Papers

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