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Blood Flow Specific Assessment of Ventricular Function

Visualization and Quantification using 4D Flow CMR

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Front cover: Pathline visualization of right (blue) and left (red) intraventricular blood flow superimposed on a morphologic four chamber image.

Abstract

The spectrum of cardiovascular diseases is the leading cause of morbidity and mortality globally. Early assessment and treatment of these conditions, acquired as well as congenital, is therefore of paramount importance.

The human heart has a great ability to adapt to various hemodynamic conditions by cardiac remodeling. Pathologic cardiac remodeling can occur as a result of cardiovascular disease in an effort to maintain satisfactory cardiac function. With time, cardiac function diminishes leading to disease progression and subsequent heart failure, the end-point of many heart diseases, associated with very poor prognosis.

Within the normal cardiac ventricles blood flows in highly organized patterns, and changes in cardiac configuration or function will affect these flow patterns. Conversely, altered flows and pressures can bring about cardiac remodeling. In congenital heart disease, even after corrective surgery, cardiac anatomy and thereby intracardiac blood flow patterns are inherently altered. The clinically most available imaging technique, ultrasound with Doppler, allows only for one-directional flow assessment and is limited by the need of clear examination windows, thus failing to fully assess the complex three-dimensional blood flow within the beating heart. Cardiovascular magnetic resonance imaging (CMR) with phase-contrast has the ability to acquire three-dimensional (3D), three-directional time resolved velocity data (3D + time = 4D flow data) from which visualization and quantification of blood flow patterns over the complete cardiac cycle can be performed. Four functional blood flow components have previously been defined based on the blood route and distribution through the ventricle, where the inflowing blood that passes directly to the outflow is called *Direct flow*. From these components, various quantitative measures can be derived, such as component volumes and kinetic energy (KE) throughout the cardiac cycle. In addition, the 4D flow technique has the ability to quantify and visualize turbulent flow with increased velocity fluctuations in the heart and vessels, turbulent kinetic energy (TKE).

The technique has been developed and evaluated for assessment of left ventricular (LV) blood flow in healthy subjects and in patients with dilated dysfunctional left ventricles, showing significant changes in blood flow patterns and energetics with disease. There is however still no study addressing the gap in the spectrum from the healthy cohorts to patients with moderate to severe left ventricular remodeling. In **Paper III**, 4D flow CMR was utilized to assess LV blood flow in patients with subtle LV dysfunction, and a shift in blood flow component volumes and KE was seen from the *Direct flow* to the non-ejecting blood flow components.

In patients with both left- and right-sided acquired and congenital heart disease, right ventricular (RV) function is of great prognostic significance, however this ventricle has historically been somewhat overseen. With its complex geometry, advanced physiology and retrosternal location, assessment of the RV is still challenging and the right ventricular blood flow is still incompletely described. In **Paper I**, the RV blood flow in healthy subjects was assessed, and the proportionally larger *Direct flow* component was located in the most basal region of the ventricle and possessed higher levels of KE at end-diastole than the other flow components suggesting that this portion of

blood was prepared for efficient systolic ejection. In **Paper II**, the blood flow was assessed in the RV of patients with subtle primary LV disease, and even if conventional echocardiographic or CMR RV parameters did not show any RV dysfunction, alterations of flow patterns suggestive of RV impairment were found in the patients with the more remodeled LVs.

With improvements of the cardiovascular health care, including the surgical techniques, the number of adult patients with surgically corrected complex congenital heart diseases increases, one of which is tetralogy of Fallot (ToF). Surgical repair of ToF involves widening of the pulmonary stenosis, which postoperatively may cause pulmonary insufficiency and regurgitation (PR). Disturbed or turbulent flow patterns are rare in the healthy cardiovascular system. With pathological changes, such as valvular insufficiency, increased amounts of TKE have been demonstrated. Turbulence is known to be harmful to organic tissues and could be significant in the development of ventricular remodeling, such as dilation and other complications seen in Fallot patients. In **Paper IV**, the RV intraventricular TKE levels were assessed in relation to conventional measures of PR. Results showed that RV TKE was increased in ToF patients with PR compared to healthy controls, and that these 4D flow-specific measures related slightly stronger to indices of RV remodeling than the conventional measures of PR.

4D flow CMR analysis of the intracardiac blood flow has the potential of adding to pathophysiological understanding, and thereby provide useful diagnostic information and contribute to optimization of treatment of heart disease at earlier stages before irreversible and clinically noticeable changes occur. The flow specific measures used in this thesis could be utilized to detect these alterations of intracardiac blood flow and could thus act as potential markers of progressing ventricular dysfunction, pathological remodeling or used for risk stratification in adults with early repair tetralogy of Fallot. Visualizations of intracardiac flow patterns could provide useful information to cardiac/thoracic surgeons pre- and post-operatively.

Populärvetenskaplig sammanfattning

Hjärt-kärlsjukdomar fortsätter vara den vanligaste dödsorsaken i både Sverige och globalt, varför tidig upptäckt och behandling är viktigt.

Hjärtat har stor förmåga att ändra sin storlek och form för att möta nya krav på blodcirkulationen, en process som kallas remodellering. Vid hjärtsjukdom kan skadlig remodellering ske, initialt som en kompensationsmekanism för att upprätthålla blodcirkulationen till kroppens organ. Med tiden uttöms dock dessa kompensatoriska mekanismer och hjärtat sviktar. Hjärtsvikt representerar slutstadiet för många hjärt-kärlsjukdomar och har mycket dålig prognos, fullt jämförbar med flera vanliga cancerformer.

Inuti hjärtat flödar blodet för varje hjärtslag i komplexa mönster, och varje förändring i hjärtats form eller struktur påverkar detta flöde. På samma sätt kan förändrade blodflöden leda till remodellering. Hos patienter med medfödda hjärtfel är hjärtats struktur från födseln annorlunda vilket speglas i avvikande blodflöden, även efter kirurgisk korrigering av missbildningarna.

Den vanligast förekommande metoden att avbilda och mäta blodflöde baserar sig på ultraljud och Dopplertechnik. Denna teknik är dock begränsad till att endast kunna mäta blodflödet i en riktning över tid, och dessutom krävs bra undersökningsfönster mellan revbenen för att erhålla bra bildkvalitet. Ultraljud med Doppler kan således inte till fullo visualisera det komplexa tredimensionella blodflödet inuti det slående hjärtat.

Kardiovaskulär magnetresonanstomografi (eng. CMR) med faskontrastteknik gör det däremot möjligt att visualisera och kvantifiera hjärtats tredimensionella (3D) blodflödesmönster i alla tre rumsliga riktningar över tid (3D + tid = 4D). Metoden kallas 4D flödes MR (eller CMR).

Blodflödet i hjärtat har tidigare med fördel delats upp i fyra blodflödeskomponenter, baserat på dess flödesmönster inuti hjärtkammaren. Den flödeskomponent som kommer in i kammaren och sedan pumpas ut under samma hjärtslag kallas det direkta flödet (*Direct flow*). Utifrån denna komponentindelning kan olika kvantitativa mått beräknas över hela hjärtcykeln, såsom komponenternas volym och deras rörelseenergi. Dessutom kan 4D flödesmetoden kvantifiera och visualisera turbulenta blodflöden i hjärta och kärl, kallat turbulent kinetisk energi (TKE).

4D flödestekniken och analysmetoden har tidigare använts för att beskriva flödet i vänster hjärtkammare, både i friska hjärtan och hos patienter med måttligt till uttalat försämrade eller remodellerade vänsterkammare. I dessa patienter kunde signifikanta förändringar av vänster kammares blodflödesmönster ses jämfört med de friska försökspersonerna. I **Delarbete III** användes 4D flödes CMR för att beskriva blodflödet i vänster kammare hos patienter med endast diskret vänsterkammarsjukdom, och ett skifte i blodkomponenternas rörelseenergi och volym kunde ses från *Direct flow* till de blodflödeskomponenter som inte pumpas ut från kammaren.

Både hos primärt vänster- och högerkammarsjuka patienter är höger kammares funktion mycket viktig för prognosen. Dessvärre har höger kammare historisk hamnat något i skymundan, och

beroende på dess komplexa geometri och placering i bröstkorget är bedömning av dess funktion fortsatt svår. Blodflödet i höger kammare är ännu inte helt beskrivet. I **Delarbete I** studerades högerkammarens blodflödesmönster i friska försökspersoner, och den proportionellt större *Direct flow*-komponenten visade sig vara belägen närmast både inflöde- och utflödesregionerna och dessutom besitta störst rörelseenergi vid slutet av kammarfyllnaden. Detta antyder att en stor del av det inflödande blodet förbereds för effektiv utpumpning under nästa hjärtslag. I **Delarbete II** undersöktes högerkammarens blodflöde hos patienter med primär vänsterkammarsjukdom, och trots att de konventionella ultraljuds- och MR-parametrarna inte visade någon nedsatt högerkammarfunktion sågs förändrade blodflödesmönster i höger kammare hos patienter med mer remodellerade vänsterkammare.

Med förbättrad hjärtsjukvård ökar antalet vuxna med kirurgiskt korrigerade medfödda hjärtfel, varav Fallots tetrad (ToF) utgör en betydande andel. Operation sker i tidig ålder med bland annat vidgning av den karaktäristiska förträngningen i högerkammarens utflödesregion. En vanlig komplikation är skadliga läckage i klaffen mellan hjärtat och lungartären.

Vanligtvis är turbulenta blodflöden sällsynta i det normala hjärt-kärlsystemet, men vid klaffläckage har ökade mängder TKE uppmätts. Turbulent blodflöde är skadligt för hjärtats vävnader och kan ha en betydande roll i utvecklingen av skadlig remodellering av höger kammare, en viktig komplikation efter korrigerande kirurgi hos Fallotpatienter. I **Delarbete IV** användes 4D flödes CMR för att mäta TKE-nivåerna i höger kammare hos patienter med kirurgiskt korrigerad ToF. Resultaten visade att TKE var förhöjt i ToF patienter med klaffläckage jämfört med friska försökspersoner, och att dessa 4D-flödesspecifika mått var något bättre på att förutsäga högerkammarrermodellering än de konventionella måtten på klaffläckage.

4D flödes CMR har således potential att öka vår förståelse för normal hjärtfunktion, men också kring sjukdomsmekanismer vid förvärvad och medfödd hjärtsjukdom. På detta sätt kan denna metod bidra med viktig diagnostisk information samt förbättra och optimera behandlingen av patienter med hjärtsjukdom så tidigt som möjligt i sjukdomsförloppet. De flödesspecifika mått som använts i denna avhandling skulle kunna användas för att upptäcka förändringar i kammarblodflöde, utgöra mått på försämrad hjärtfunktion samt inte minst bidra till bedömning av patienter med medfödda hjärtsjukdomar. I det senare fallet kan visualiseringar av det komplexa blodflödet i hjärtat bidra med nya möjligheter för kirurgen att studera resultatet av dagens operativa metoder.

List of Papers

This thesis is based on the following four original research papers, which will be referred to by their Roman numerals:

Paper I

Fredriksson AG, Zajac J, Eriksson J, Dyverfeldt P, Bolger AF, Ebbers T, Carlhäll CJ, **4-D blood flow in the human right ventricle**, *American journal of physiology Heart and circulatory physiology*, 2011, 301, 2344-2350, doi:10.1152/ajpheart.00622.2011.

Paper II

Fredriksson AG, Svalbring E, Eriksson J, Dyverfeldt P, Alehagen U, Engvall J, Ebbers T, Carlhäll CJ, **4D flow MRI can detect subtle right ventricular dysfunction in primary left ventricular disease**, *Journal of magnetic resonance imaging: JMRI* 2016, 43, 558-565, doi:10.1002/jmri.25015.

Paper III

Svalbring E, Fredriksson A, Eriksson J, Dyverfeldt P, Ebbers T, Bolger AF, Engvall J, Carlhäll CJ, **Altered Diastolic Flow Patterns and Kinetic Energy in Subtle Left Ventricular Remodeling and Dysfunction Detected by 4D Flow MRI**, *PloS one* 2016, doi:10.1371/journal.pone.0161391.

Paper IV

Fredriksson AG, Trzebiatowska-Krzynska A, Dyverfeldt P, Engvall J, Ebbers T, Carlhäll CJ, **Turbulent kinetic energy in the right ventricle: Potential MR marker for risk stratification of adults with repaired Tetralogy of Fallot**, *Journal of magnetic resonance imaging: JMRI* 2017, Aug 2, doi:10.1002/jmri.25830.

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Abbreviations and Nomenclature

2ch	Two-chamber view
3ch	Three-chamber view
3D	Three-dimensional
4ch	Four-chamber view
4D	Four-dimensional
Ao	Aorta
ATP	Adenosine triphosphate
AV	Atrioventricular
CHD	Congenital heart disease
CMR	Cardiovascular magnetic resonance
CT	Computed tomography
ECG	Electrocardiogram
ED	End-diastole
EDV	End-diastolic volume
EDPVR	End-diastolic pressure-volume relationship
EF	Ejection fraction
ES	End-systole
ESV	End-systolic volume
ESPVR	End-systolic pressure-volume relationship
FAC (RVFAC)	Right ventricular fractional area change
FID	Free induction decay
HF	Heart failure
HR	Heart rate
ICM/ICMP	Ischemic cardiomyopathy
IVC	Isovolumic contraction <i>or</i> inferior vena cava
IVR	Isovolumic relaxation
KE	Kinetic energy
LA	Left atrium
LGE	Late gadolinium enhancement
LV	Left ventricle
LVEDV	Left ventricular end-diastolic volume
LVEDVI	Left ventricular end-diastolic volume index
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume
LVESVI	Left ventricular end-systolic volume index
mJ	milli Joule
MRI	Magnetic resonance imaging
ms	millisecond
NYHA	New York Heart Association classification system for HF
PR	Pulmonary regurgitation
PVR	Pulmonary valve replacement

RA	Right atrium
RAAS	Renin-angiotensin-aldosterone system
Re	Reynolds number
RV	Right ventricle
RVEDV	Right ventricular end-diastolic volume
RVEDVI	Right ventricular end-diastolic volume index
RVEF	Right ventricular ejection fraction
RVESV	Right ventricular end-systolic volume
RVESVI	Right ventricular end-systolic volume index
RVOT	Right ventricular outflow tract
SA	Sinoatrial
SNR	Signal to noise ratio
SPECT	Single-photon emission computed tomography
SR	Sarcoplasmic reticulum
SV	Stroke volume
SVC	Superior vena cava
TAPSE	Tricuspid annular plane systolic excursion
TE	Echo time
TR	Repetition time
TKE	Turbulent kinetic energy
ToF	Tetralogy of Fallot
rToF	repaired Tetralogy of Fallot
TV	Tricuspid valve
VENC	Velocity encoding
VNR	Velocity to noise ratio

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Aims

The overall aim of this thesis was to utilize novel 4D flow CMR techniques to study intraventricular blood flow in healthy subjects and patients with both acquired and congenital left- and right-sided heart disease in order to gain incremental insights into ventricular function and dysfunction.

The specific aims of the included papers were as follows below:

Paper I

To investigate and assess the patterns and energetics of the blood flow through the human healthy right ventricle as well as compare to those of the blood flow through the healthy left ventricle.

Paper II

To investigate and assess right ventricular blood flow patterns and energetics in patients with primary left ventricular disease and compare to those of healthy subjects, as well as evaluate 4D flow parameters against conventional CMR and echocardiographic parameters.

Paper III

To investigate and assess left ventricular blood flow in patients with subtle left ventricular dysfunction, in order to address the knowledge gap in the spectrum of previously investigated healthy subjects and patients with moderately to severe left ventricular dysfunction.

Paper IV

To investigate and assess turbulent kinetic energy in the right ventricle of patients with surgically corrected Tetralogy of Fallot and pulmonary regurgitation as well as relate these flow specific markers to right ventricular remodeling.

1. The human heart

The heart is situated centrally inside the thoracic cavity (the chest) behind the sternum, enclosed in the **pericardial sac**. The pericardial sac consists of the non-distensible fibrous pericardium and the two-layered serous pericardium (visceral and parietal). Between the layers of the serous pericardium, a small amount of lubricating pericardial fluid allows the heart to move frictionless as it beats. The visceral pericardial layer constitutes the outer surface of the heart, also called the **epicardium**. The inside surface of the heart, including the heart valves, is lined by the **endocardium**, a layer of endothelial cells continuous with the endothelium of the great vessels leading to and from the heart. Between these layers is the **myocardium**, the heart muscle that generates the contractile work of the heart.

The heart consists of four distinct chambers, through which blood flows in highly organized complex patterns (1) (**Figure 1**). Deoxygenated blood from the systemic circulation returns to the heart by the caval veins, entering the **right atrium (RA)**, continuing into the **right ventricle (RV)** and is during systole ejected into the pulmonary artery and lungs for oxygenation. Oxygenated blood from the lungs returns to the **left atrium (LA)** through the four pulmonary veins and is ejected by the **left ventricle (LV)** into the aorta, which transports the blood to the systemic

Figure 1. Schematic image of the cardiac anatomy. The right side of the heart with deoxygenated blood is depicted in blue and the left side of the heart with oxygenated blood in red. Blood flow through the cardiac chambers is illustrated by the white arrows.

circulation once again. The cardiac chambers are separated by the atrioventricular valves, suspended by a fibrous ring structure between the atria and ventricles (annulus fibrosus): the mitral valve (MV) between the LA and the LV and the tricuspid valve (TV) between the RA and RV. Further, the ventricles are separated from their respective outgoing artery by the aortic and pulmonary semilunar valves.

Anatomically, the LV is ellipsoid in shape while the RV presents a complex three-dimensional crescent shape as it wraps around the LV (2, 3). The LV can grossly be divided into three different regions: the basal, mid-ventricular and apical region. The inflow and outflow tracts of the LV are located in the basal region with their respective valves in close relation to each other. Similarly, the more complex and anteriorly oriented RV can also be divided using similar denominations, but is more often described from three functional regions: the inlet or inflow tract, the trabeculated apical portion and the infundibulum or conus leading to the outflow tract (3). Compared to the LV the angle between the RV inflow and outflow tracts is less acute, the two being separated by a muscular fold (3). The RV can thus be resembled to a U-bend.

The LV, sustaining the systemic circulation, is required to generate up to six times greater pressures than the RV. In order to cope with this increased wall stress, the LV myocardium is substantially thicker (6-10 mm) than the coarsely trabeculated RV free wall (<5 mm) (4). The LV and RV are divided by a convex muscular wall, the **interventricular septum**, that is structurally a part of the LV myocardium but also creates the inner wall of the RV. The interventricular septum is thus common for both ventricles.

2. Cardiac physiology

The main function of the human heart is to generate and maintain the blood flow through the cardiovascular system, providing oxygen and nutrients to the tissues of the body and removing carbon dioxide created by the cells. In order to do so, the myocardium rhythmically contracts and relaxes, throughout our lifetime more than 2.5 billion times!

The electrical conduction system

The contraction of the myocardium is initiated by the depolarization of cardiac muscle cells, **myocytes**, beginning in specialized myocardial pacing cells (pacemaker cells) in the **sinoatrial node** (SA-node), situated in the posterior wall of the right atrium. These cells generate regular spontaneous action potentials that spread throughout the atria, depolarizing the atrial myocardium (5).

As the action potential progresses, there is only one pathway through the non-conductive annulus fibrosus in order to reach the ventricles, the **atrioventricular node** (AV-node). The AV-node is situated in the inferior posterior region of the atrial septum, and the specialized cells here will slow down the impulse (6). This delay in conduction is physiologically important as it allows for the late ventricular filling through atrial contraction before ventricular depolarization and contraction.

Cardiac myocytes are joined together by gap junctions, allowing continuous flow of ions from one myocyte to another (7), propagating the action potential from cell to cell through the myocardium and allowing the heart to contract as a unity, a **functional syncytium**. The conduction of action potentials between cells in this manner is however usually slow, why ventricular depolarization occurs via a specialized high-speed conduction system (6). Action potentials leaving the AV-node enter this ventricular conduction system by the bundle of His, dividing into left and right bundle branches. These branches further divide into an extensive system of fibers that conduct the impulses at high velocities, the Purkinje fibers, that in turn are in contact with the myocardium (6). This permits the impulse to reach the whole ventricular myocardium almost simultaneously creating a rapid, organized, near-synchronous depolarization and contraction of the ventricles.

The cardiac cycle

The cardiac cycle comprises a series of physiologic events from a ventricular point of view, that can be illustrated using a diagram as presented in **Figure 2**, conceived by Carl J Wiggers in 1915 (8), and refined by Thomas Lewis a few years later (9). In the Wiggers diagram, the LV/RV pressures as well as aortic/pulmonary and atrial pressures are depicted as a function of time. The cardiac cycle is divided into ventricular systolic and diastolic phases, and throughout this thesis, systole will be defined as starting at mitral valve closure when the ventricle has reached its maximum volume and ending when the aortic valve closes and the ventricle has reached its minimum volume. When heart rates increase, it is mainly the diastolic phase that shortens.

Figure 2. The Wigger diagram. (Top) The pressure variations of the left ventricle (red line), aorta (corresponding dotted line) and left atrium (finely dotted line), as well as left ventricular volume changes (green line) are depicted throughout two consecutive heart beats. (Middle) The pressure variations in the right ventricle (blue line), pulmonary artery (corresponding dotted line) and right atrium (corresponding finely dotted line). The coinciding aortic and pulmonary flows are also presented (red and blue lines, respectively) as well as the electrocardiographic events (Bottom). AoC, aortic valve closure; AoO, aortic valve opening; ECG, electrocardiogram; EDV, end-diastolic volume; ESV, end-systolic volume; IVC, isovolumic contraction; IVR, isovolumic relaxation; MC, mitral valve closure; MO, mitral valve opening; LV, left ventricle.

The description of the cardiac cycle is generally based on the LV, however similar events take place in the RV, with the main differences related to the lower RV pressures. Throughout the following discourse, these differences will be commented upon where indicated. Following the ECG at the bottom of **Figure 2**, the initial P-wave represents the depolarization of the atria following an impulse from the SA-node, resulting in atrial contraction (atrial systole). At rest in the young individual, the atrial contraction contributes to a minor part of the ventricular filling, however this proportion increases with age.

Ventricular contraction and isovolumic contraction (IVC)

At the end of atrial systole, the ventricular volume is maximal and represents the **end diastolic volume** (EDV). After a slight delay produced by the AV-node (PQ-interval in the ECG), the depolarization signal propagates, inducing ventricular contraction (QRS-complex of the ECG). The early ventricular contraction produces a rapid increase in ventricular pressure, that together with atrial relaxation reverses the atrioventricular pressure gradient, closing the atrioventricular valves. The mitral valve closes first, followed by the tricuspid valve. This slight delay is due to lower RV pressure, allowing inflow of blood to the RV for a slightly longer period (3). The brief time period between the closure of the mitral valve and the subsequent opening of the aortic valve, when LV pressure increases due to myocyte tensioning without any change in the ventricular volume is called **isovolumic contraction** (IVC). When the ventricular pressure exceeds the aortic pressure, the aortic valve opens and ejection of blood begins. RV ejection begins before and ends after LV ejection. The intraventricular pressure buildup needed to eject blood from the RV is quickly achieved due to the lower resistance of the pulmonary circulation, why the IVC phase is very short or even absent in the RV (10). Interventricular interdependence may further assist in explaining this phenomenon: during LV and septal IVC, RV pressures increase due to interventricular interaction, initiating RV ejection before LV pressures are high enough to produce LV ejection (11, 12).

Ventricular relaxation and isovolumic relaxation (IVR)

The ventricular ejection rate peaks and then falls as the myocardium relaxes (as shown by the flow curves in **Figure 2** at the T-wave in the ECG). As a result, the LV ventricular pressure falls below the aortic pressure, as shown by the intersection of these curves in the diagram (red line for LV pressure and corresponding dotted line for aortic pressure). The flow continues into the outflow tract due to the momentum (kinetic energy) of the ejecting blood (13). As LV pressure drops further below the aortic, the pressure gradient is reversed, closing the aortic valve. Once again, the lower RV pressure differences allows the pulmonary valve to remain open slightly longer for flow, termed the RV hang-out period (2). The blood volume remaining after ejection is termed the ventricular **end-systolic volume** (ESV). The volume difference between the EDV and ESV is the **stroke volume** of the ventricle (SV), representing the volume of blood that is ejected into the aorta/pulmonary artery during a heartbeat.

LV pressures continues to fall during the relaxation of the myocardium, however the mitral valve remains closed until the left atrial pressure exceeds the ventricular. The time period when no volume change occurs despite relaxation, is called the **isovolumic relaxation** (IVR). None or little isovolumic relaxation occurs in the RV.

Ventricular filling

The subsequent ventricular filling is composed of three phases, the **early rapid filling phase**, the **diastasis or reduced filling** and the **late filling phase** (atrial systole). As the atrioventricular valves open, rapid inflow of blood from the atria occurs during the early filling phase, in the young individual at rest contributing to a major part of the total ventricular filling volume. The inflow of

blood equalizes the atrioventricular pressure gradient and the blood flow slows/stops during the diastasis. The subsequent atrial contraction increases atrial pressures, simultaneously shifting the AV-plane towards the base, providing the late diastolic filling. Due to lower pressure levels, the breathing impacts RV filling: during inspiration and thoracic expansion, intrathoracic pressures fall and blood is suctioned into the thoracic cavity, increasing the venous return and the RV filling.

The cardiac contractile cell

The phenomenon when electrical depolarization of the cardiac myocyte leads to myocardial contraction is called the **excitation-contraction coupling**, and occurs when myofilaments inside the cardiac myocyte move in relation to each other (14). The cardiac myocyte is roughly cylindrical and composed of myofibril bundles. Each myofibril is further composed of **sarcomeres** arranged in series. The sarcomere is the basic contractile unit of the myocyte, measuring approximately 1.8 to 2.2 μm in physiological conditions and is limited on either side by the Z-lines (**Figure 3**) (15). The sarcomere itself contains the myofilaments, **actin** and **myosin**. The thin actin filaments are attached to the Z-line at either end of the sarcomere. Coupled to the actin at regular intervals are additional protein complexes, tropomyosin and troponin. The thick filaments are composed by hundreds of myosin molecules, each with two heads, and are suspended between the actin strands centrally in the sarcomere. When the myocardium is in the resting state, the tropomyosin-troponin complex on the actin strand inhibits the binding of myosin. The troponin complex is however

Figure 3. The sarcomere. Schematic image of the contractile unit of the myocyte and its components. During contraction, actin-myosin interaction produces shortening of the sarcomere (dashed arrows at bottom).

sensitive to differences in intracellular calcium concentrations.

Surrounding the myofibril bundles the myocyte cell membrane forms deep invaginations permitting ion exchanges deep within the myocyte during depolarization. In close relationship, an extensive branching system called the sarcoplasmic reticulum (SR) extends in between the myofilaments. Inside the SR, calcium concentrations are high, and its primary function is to regulate the intracellular concentrations of calcium ions (Ca^{2+}). Depolarization of the myocyte triggers the release of Ca^{2+} stored in the SR, vastly increasing its intracellular concentration and changing the configuration of the troponin. By moving the tropomyosin away from the myosin-binding site on the actin, the myosin heads can bind to the actin, so called **cross-bridge formation**. As the myosin heads bind to the actin, ATP (an energy-carrying molecule) is hydrolyzed causing the myosin head to pull itself along the actin strand. The sliding of the myosin and actin in response to changes in intracellular calcium concentrations causes the sarcomere to shorten and is called the **sliding filament theory** (16). This process is repeated as long as intracellular Ca^{2+} levels are increased. Removing intracellular Ca^{2+} by ATP-dependent calcium pumps reverses this process, again inhibiting cross-binding, allowing relaxation. Thus, not only the contraction of the myocardium but also relaxation is dependent on energy in order to function optimally.

The myosin filament is suspended from the Z-line by a giant distensible but non-contractile protein called **titin** (**Figure 3**). Titin has been suggested to act as a bi-directional spring providing elasticity to the sarcomere (17). During forceful sarcomere shortening titin gradually compresses, producing a restoring force during the subsequent relaxation of the sarcomere. Conversely, during lengthening of the sarcomere, e.g. during diastolic ventricular filling, the titin molecule stretches, producing passive tension. As the titin spring retracts during systole it enhances the ventricular contraction. This elastic restoring force is thus present whenever the myocardium stretches or contracts out of its resting equilibrium, and may explain some of the elastic characteristics of the cardiac muscle (15).

Normal ventricular function

Systolic function

The performance of the heart can be described in terms of the **cardiac output** (CO). CO represents how much blood gets pumped out to the body every minute and is the product of the SV and the heart rate (HR):

$$CO = SV \times HR$$

In the normal resting state, the cardiac output ranges from 4-6 l/min. Both changes in HR and SV, and factors that influence them, can alter the CO. The heart rate is proportionally more influential in changing the CO, since it can increase dramatically during strenuous exercise. As the cardiac SV is the difference between the EDV and ESV, it is dependent on the filling of the ventricle (**preload**), the resistance of the peripheral circulation (**afterload**) and the contractility of the myocardium (**inotropy**) (18).

Figure 4. Pressure-volume loop for the left ventricle at rest. AoC, aortic valve closure; AoO, aortic valve opening; EDV, end-diastolic volume; EDPVR, end-diastolic pressure-volume relationship; ESV, end-systolic volume; ESPVR, end-systolic volume-pressure relationship; IVC, isovolumic contraction; IVR, isovolumic relaxation; MC, mitral valve closure; MO, mitral valve opening; LV, left ventricle; SV, stroke volume.

The pressure-volume loop

The cardiac cycle can be illustrated and analyzed by plotting the ventricular pressure against the volume throughout the cycle, in this case for the LV (**Figure 4**) (18). This diagram represents a **pressure-volume loop** (PV loop), where the different events of cardiac cycle are represented in relation to preload, afterload and inotropic state. These determinants of cardiac function will alter this pressure-volume relationship, why the pressure-volume loop is essential for the understanding of the cardiac pumping physiology. Further, from the pressure-volume loop, several physiological measurements can be deduced, such as EDV, ESV, SV, systolic and diastolic blood pressures as well as the work performed by the heart.

The pressure-volume relationships, preload and Frank-Starling mechanisms

The ventricular preload is defined as the degree of sarcomere distention before contraction, i.e. the length of the sarcomeres at end-diastole. The contractile performance of the sarcomeres changes with their length, a relationship called the **length-tension relationship**. Thus, preload will impact ventricular performance as described below.

The **passive length-tension relationship**, reflecting the elastic sarcomere elements, e.g. titin, can be measured by suspending a piece of relaxed myocardium and measuring the tension as it is stretched. As the length of individual sarcomeres can be considered proportional to ventricular volume (i.e. size of the ventricle, EDV and ESV) and tension is proportional to pressure, the passive

length tension relationship of the whole ventricular myocardium can be plotted in the PV loop as the **end diastolic pressure-volume relationship** (EDPVR). The EDPVR represents how pressure increases during filling of the completely relaxed ventricle and is non-linear, meaning that during filling the pressure inside the ventricle will increase faster and faster. The ventricle becomes progressively stiffer and the rate at which this occurs is defined by the ventricular **compliance**.

Measuring the tension at each length during stimulation of the muscle gives the **active length-tension relationship**. With muscle stretch, both the passive tension and the active tension will increase, increasing the contractile force developed by the myocytes (**length-dependent activation**). The optimal overlap of actin and myosin has previously been thought to be the main mechanism explaining this phenomenon, however the increase in force is primarily a result of increased troponin sensitivity to Ca^{2+} as the sarcomere stretches (19, 20). More recent research has proposed that with sarcomere stretching, actin and myosin filaments come closer, increasing Ca^{2+} sensitivity but also facilitating cross-binding (21). For the whole ventricle, this increase in contractile force translates to the maximal pressure that the ventricle can generate at any given volume. As this occurs at maximal contraction, i.e. at end-systole, the relationship is termed the **end systolic pressure-volume relationship** (ESPVR). At this point, the myocardium is fully contracted. Besides being dependent on myocardial distention, the ESPVR shifts with intrinsic changes in cardiac contractility (**length-independent activation**), described below.

Given the above, an increase in preload, thus an increased EDV will also increase the SV. This phenomenon is at the level of the ventricle called the **Frank-Starling mechanism** or the Frank-Starling law of the heart, i.e. the heart pumps what it receives! The Frank-Starling mechanism is important for regulation of ventricular function and for balancing the output of the two ventricles (15). In the pressure-volume loop, an increase in venous return and ventricular filling (preload) occurs along the EDPVR line, leading to higher EDV and a widening of the pressure volume loop, increasing the SV.

Afterload

Afterload can be described as the **wall stress** during systolic ejection. Wall stress can also be thought of as the tension that the myocardium must generate in order to shorten against the intraventricular pressure during systolic ejection. Therefore, afterload is more generally defined as the load the ventricle has to overcome in order to eject blood during systole, the main component of which is the arterial pressure in the aorta or pulmonary artery. As arterial pressures increase, the afterload on the ventricle increases. Wall stress is proportional to the intraventricular pressure (P), ventricular radius (r) and myocardial thickness (h) according to the following modified La Place equation:

$$\sigma \propto \frac{P \times r}{2h}$$

As the above equation shows, factors pertaining to ventricular morphology become important when the ventricle is subjected to altered loads. In patients with elevated blood pressure the afterload is increased and thus the wall stress. Hypertrophy of the ventricular wall (increase in wall thickness) as a compensatory mechanism mathematically reduces the wall stress on individual fibers.

In the pressure-volume loop, an increased afterload (e.g. increased arterial pressure) at a given preload (a given EDV) will shift the point of aortic valve opening upwards. With no change in

ESPVR (inotropy) the pressure-volume loop will be narrowed, resulting in a SV decrease. With lower SV, an increase of the preload by increased EDV and filling pressures will compensate this effect by again widening the loop along the EDPVR line.

Contractility

The SV is further altered by the inherent contractility of the myocardium, independent of changes in preload or afterload, also called ventricular inotropy (14). The change in inotropy is caused by cellular mechanisms that alter the interaction between actin and myosin, regulating the force of myocyte contraction. An increase in inotropy, increasing the pressure that the ventricle can generate at any given volume, will shift the ESPVR line to the left and increase its slope. The change of the ESPVR line means that the PV loop widens and the SV is greatly increased (EDV remains constant, but ESV decreases), meaning that the ejected fraction of the EDV is increased. **Ejection fraction**, (EF), defined as the percentage of the EDV that is ejected during ventricular contraction according to:

$$EF(\%) = \frac{SV}{EDV} \times 100$$

is widely used to assess the systolic function of the ventricle. While this may be true when no other factor than inotropy changes, it is important to remember that the EF also changes with respect to preload (Frank-Starling mechanism) and afterload (blood pressure) and the use of EF as a measure of the ventricular performance is debated (22). Increased inotropy also independently increases the myocardial relaxation, lusitropy. This combined with lower resulting ESV may augment ventricular suction during e.g. exercise (23).

Inotropy is influenced by multiple factors, however the majority of them involve intracellular Ca^{2+} levels (14). Sympathetic nerve activation and increases in circulating levels of catecholamines (epinephrine and norepinephrine) have positive inotropic effects by e.g. changing intracellular Ca^{2+} concentrations (increased Ca^{2+} influx during depolarization) and sensitizing the troponin molecule to Ca^{2+} . Inotropy is also dependent on the number of functional myocytes in the myocardium; a larger number of contractile units renders a higher contractile force at the level of the ventricle. This is illustrated by the drop in ventricular contractile performance following a loss of myocytes after a myocardial infarction.

Cardiac work

The term work in its simplest definition is the product of the force applied to an object and the distance this object moves, however the heart performs its systolic work by ejecting a blood volume (SV) against a resistance, in this case the blood pressure. The formulation can be translated into the product of the mean arterial blood pressure and the SV:

$$W = P \times \Delta V \quad \Rightarrow \quad \mathbf{W = P \times SV}$$

Besides creating the pressure work needed for blood ejection, the ventricle also imparts velocity to the ejected blood volume, **kinetic energy** (KE),

$$KE = \frac{1}{2} mV^2$$

where m is the mass and V is the velocity. Together, the work performed is called the **external work** of the heart (18). The external work of the heart can be plotted as the area inside the pressure-volume loop (blue in **Figure 4**). The amount of KE is less than 1% of the external cardiac work at rest, however this proportion increases significantly during exercise (24). Therefore, the KE is not an insignificant part of the total external cardiac work:

$$W = P \times \Delta V + \frac{1}{2}mV^2$$

The external work performed (on the blood) by the heart occurs during systolic ejection. This is only a small part of the total energy consumed by the heart. The absolute major part of the energy required by the heart is used during ventricular isovolumic contraction to develop and sustain pressure without ejection of any blood, called **internal work** or potential energy and is used to overcome the myocardial internal viscosity, rearrange the muscular architecture and stretch the elastic myocardial components (18). The amount of energy needed is proportional to the wall tension and the time that the heart needs to keep this tension up, much like compressing a spring for a period of time. The wall tension is related to the pressure that needs to be overcome, which means that the higher afterload is, the higher the energy demands. Further, the higher the HR, the shorter the relative time of diastolic relaxation to systolic tension becomes. Lowering the systolic blood pressure and HR will have tremendous effect on lowering the energy demands of the pumping heart, e.g. in patients with ischemic heart disease (18). The ratio of the external work to the total energy is termed the **mechanical efficiency** of the heart.

Diastolic function

To this point much of the ventricular function has been described from a systolic point of view, however, normal ventricular relaxation and filling during diastole is of great importance for optimal cardiac performance. Besides atrial function during the late diastolic filling, the ventricular diastolic function is determined by a seamless interplay of active **myocardial relaxation** and **elastic recoil**. The elastic recoil forces of the ventricle could be explained by the elastic properties of titin (17). With increased contractility and compression of the titin molecule, the released elastic recoil force also increases during the subsequent diastolic phase. The elastic recoil is also related to myocardial stiffness, affected by the composition of the extracellular matrix containing elastin and fibrous collagen fibers. Fibrosis in pathologic hypertrophic states increases myocardial stiffness, contributing to diastolic dysfunction even if systolic function may be preserved (25).

Myocardial relaxation involves deactivation and detachment of myosin/actin cross-bridges as a result of Ca^{2+} reuptake into the SR (18). IVR is generally regarded as a pure relaxation dependent process, however, shape changes of the ventricle during IVR have been observed, implying that elastic recoil forces simultaneously distract the myocardium as the cross-bridge uncoupling occurs (26). Similarly, during and throughout the early rapid filling phase, viewed as a process determined by suction from the elastic recoil, the cross-bridge uncoupling continues, acting as a resistive force (a break) to the ventricular distention produced by the elastic recoil forces (26). When relaxation occurs faster than ventricular filling, the negative atrioventricular pressure gradient created produces the diastolic ventricular suction (26). The ventricular suction is made possible by optimal and fast active relaxation of the myocardium, a process that requires energy. Significant diastolic suction has also been observed in the RV despite the thin walls and short IVR of this ventricle (23,

27), and it has been proposed important for driving the central venous return (28). Any disturbance of myocyte metabolism (e.g. ischemia) can result in impaired relaxation and diastolic dysfunction. Diastolic dysfunction is a substantial cause of cardiac morbidity and a common early finding in heart failure patients with normal or nearly normal systolic function (preserved ejection fraction), in many cases detectable before systolic dysfunction is evident (29).

Myocardial structure

Besides the properties of the myocardial contractile cells and the relationship between the loading conditions, the importance of the cardiac muscular architecture for optimal mechanical efficiency has been recognized (30). The structural organization of the myocardial fibers has been long studied, including early measurements of local myofiber direction and contraction patterns using implanted markers to more recent whole heart analyses using MRI (31). After simple hand dissection of the heart, Torrent-Guasp proposed that the ventricles were composed of a single myocardial band twisting around to form the two ventricles, creating a crossed oblique helical LV myocardial structure (32). While the exact structure of the myocardium is still debated (33), the overall helical nature of the LV myocardium has never been contradicted.

The LV free wall is mainly composed by crossing oblique oriented fibers at approximately 60 degree angles as described by Torrent-Guasp (34), with a circumferential transverse fiber layer in the mid-wall between the oblique layers (35-37). The fiber orientations thus present a smooth fan-shaped distribution from the subepicardial to subendocardial fibers, proposing a continuous muscular organization (33). Combined, this helical oblique myocardial structure creates the characteristic “wringing” contraction of the LV, involving both twisting and long-axis shortening as well as circumferential squeezing (34, 38). The oblique helical myocardial structure has been shown to optimize the systolic ejection of LV blood (39). As sarcomere contraction only yields an approximative 15% shortening of the myofibers, early mathematical experiments confirmed that the contractile performance of transverse circular or longitudinal fiber patterns, in regard to volume change and pressure generation, were vastly outperformed by an oblique fiber architecture. In order to achieve ejection fractions >60%, a fiber angle of approximately 60 degrees from horizontal was needed (39, 40).

The RV free wall is mainly composed of transverse fibers. In addition to this, oblique fibers from the LV extend past the septum to envelop the RV free wall, further connecting the ventricles (41). The RV muscular structure creates the characteristic bellows-like motion of the free wall towards the interventricular septum (3).

Interventricular interaction

The two cardiac ventricles constitute very different but at the same time interconnected units of the same heart. Their interaction is termed **interventricular interaction** (or sometimes ventricular interdependence), defined as the forces transmitted between the ventricles through direct myocardial cross-talk by the shared myocardial fibers, or by geometric ventricular changes inside the pericardial sac inducing septal shifts (11). Besides this, circulatory changes by way of the pulmonary circulation constitutes a more indirect communication between the ventricles, the

effects however needing multiple heart beats to propagate (11, 28). Interventricular interaction is important in both the healthy heart and in different disease states (42, 43).

Since many early experiments demonstrated that the RV could be compromised without significant cardiac dysfunction, RV function has historically been considered rather unimportant (2). Consequently, cardiologic research has historically mostly revolved around LV function and failure, as the higher demands posed on this systemic ventricle means that heart disease and failure is more prevalent in the LV than in the RV.

When discussing the systolic interventricular interaction, the high-pressure thick-walled LV contribution to RV function is usually in focus. Indeed, up to 20-40% of the RV pressure and flow generation and as much as 50% of the RV mechanical work result from LV contraction by way of direct interventricular interdependence (11). By separating the LV and RV electrically, but not mechanically, isolated LV contraction was shown to produce almost normal RV pressures and flow (12), however isolated RV contraction did not produce any significant LV pressure. The common myocardial fibers of the interventricular septum are central for this interdependence between the RV and LV (11, 41). These relationships have also been shown in human hearts (44), and RVEF is substantially lowered in patients with infarction of the septum (45). Also the LV free wall performance will impact RV function by pulling at the RV free wall junctions to the septum (11). In both acquired and congenital cardiac disease, RV function will be of great prognostic importance, affecting the LV function (42).

Interventricular interaction is not only present during systolic contraction, but highly relevant for ventricular diastolic function (diastolic interventricular interaction) (11, 43). RV loading conditions will affect LV diastolic function (43): being enclosed in the pericardium, altered RV load will cause the interventricular septum to bulge into the opposite ventricle, impacting its filling (11). Acute dilation of the RV also leads to decreased LV contractility (46). Releasing the pericardium, LV volumes normalized however the contractility did not, reflecting back to disturbances in the direct myocardial cross-talk. Further, acute RV ischemia likewise was shown to impact LV contractility negatively (43).

In recent years, the role of the RV in cardiovascular disease has thus been acknowledged and progressively more research is now conducted on RV dysfunction and pathophysiology as well as its impact on LV disease. Reduced RV systolic function (RVEF) has in multiple studies been found to be a strong negative prognostic predictor in heart failure patients (47, 48) and preserved RV function has been shown to be a strong predictor for survival in patients with primary LV failure (49). Accurate assessment of RV function is therefore important, and in **Paper I** we sought to add to the understanding of RV physiology and pathophysiology by studying the RV intraventricular blood flow. Further, in **Paper II** we aimed at elucidating the impact of LV dysfunction on RV flow patterns and energetics.

3. Heart failure

The spectrum of cardiovascular disease is the leading cause of morbidity and mortality globally (50), and presents a substantial health care burden (51). **Heart failure** (HF) represents the end-point of this cardiovascular disease continuum (acquired and congenital) and is associated with very poor prognosis, the 5-year survival rates being worse than many of the most common cancer forms (52). Heart failure is a complex syndrome, most commonly described as an inability of the heart to sustain adequate blood flow (SV and CO) to the tissues of the body while maintaining normal filling pressures. This inability results from two major mechanisms: impaired ventricular ejection of blood (**systolic dysfunction**), impaired ventricular filling (**diastolic dysfunction**), or a combination of the two (14, 53).

Many conditions, comorbidities and risk factors are associated with the development of HF. Among these, hypertension could be the most important risk factor (but also cause), followed by diabetes, metabolic syndrome and atherosclerotic disease (54), leading to **ischemic cardiomyopathy (ICM or ICMP)**. Further, any abnormality in cardiac structure or function, such as valvular disease, congenital heart disease or different primary cardiomyopathies can also lead to heart failure (55).

Based on the two main mechanisms, the terms systolic heart failure and diastolic heart failure have previously been used. The systolic dysfunction results from a decreased contractile function of the myocardium (56), most commonly associated with ischemic heart disease or ICMP, when reduced myocardial perfusion causes hypoxia and dysfunction or in the case of acute myocardial infarction, localized myocyte death. In the pressure-volume loop, the decreased systolic contractility can be visualized as a decreased slope of the ESPVR (14). The diastolic dysfunction results from impairment of myocardial relaxation or a decrease in myocardial compliance e.g. in the setting of pressure overload (see section on **Cardiac remodeling and hypertrophy** below). This phenotype is more commonly seen in older women with a history of hypertension (57). The reduced ventricular compliance will in the pressure-volume loop shift the EDPVR upwards and to the left, meaning that EDV is lowered and LV filling pressures are increased, leading to congestion of pulmonary and peripheral circulation. Depending on the EDV and SV, EF may be unchanged.

Due to overlaps of systolic and diastolic dysfunction in many heart failure patients, today measurements of LV ejection fraction (LVEF) are used to describe different types of failure (53), even if this surrogate marker is associated with significant pitfalls (22). Diagnosis of HF is usually based on a combination of typical symptoms as well as clinical signs, laboratory results and cardiac imaging revealing objective evidence of ventricular dysfunction (e.g. lowered EF) (53, 54). The most commonly used classification of HF is the New York Heart Association functional classification system (NYHA) (58). The **NYHA classification** consist of four stages (I – IV) based on patient symptomatology, and was used in **Papers II and III** to characterize the included patients.

In the setting of heart disease, several initially beneficial and compensatory mechanisms are triggered in order to restore normal hemodynamics. With time, the compensatory mechanisms are however slowly exhausted and decompensation and HF ensues.

Neurohumoral activation

One of the most important compensatory mechanisms is the activation of **neurohumoral systems**. The falling CO leads to a fall in arterial blood pressure and simultaneous increase in venous pressures, decreasing tissue perfusion. This leads to activation of the sympathetic nervous system and release of catecholamines (57). The sympathetic and adrenergic systems will increase cardiac performance by stimulating contractility and heart rate, increasing blood pressure. In the peripheral vasculature, increased levels of catecholamines induce vasoconstriction, sustaining arterial pressure for perfusion of vital organs.

In response to the initial decreases in blood pressure and ensuing reduced renal blood flow as well as the increased sympathetic state, the kidneys secrete renin, activating the **renin-angiotensin-aldosterone system (RAAS)** (18). The net result is retention of fluid and salt in order to further compensate for the lowered arterial blood pressure. The increase in circulating volume and venous pressure will also augment the generation of preload. This increased preload initially functions as a compensatory mechanism in the early stages of heart failure, as it provokes increased inotropy by way of the Frank-Starling mechanism (57, 59).

With chronic neurohumoral activation in heart failure, the attempts of the compensatory systems to increase CO of the failing heart will however rather exacerbate the condition than aid. By i.e. increasing arterial blood pressure, afterload increases, with further depression of SV and CO, leading to decompensation.

Cardiac remodeling and hypertrophy

The human heart has a great ability to adapt in various conditions both in health and disease by **remodeling** (60, 61). Remodeling increases or decreases the myocardial mass and changes its structure in response to altered demands. Remodeling generally refers to the growth of myocardial mass (**hypertrophy**) and subsequent conformational changes (62). Cardiac hypertrophy can be either **physiological** or **pathological**, sometimes further classified as adaptive or maladaptive (60).

Physiologic cardiac remodeling results from adaptation in response to increased physiologic demands (i.e. during development, physical exercise or pregnancy). Physiologic hypertrophy usually presents balanced increases in both LV wall thickness and LV volumes (63) as well as structurally normal myocardial growth. Cardiac performance is normal or enhanced (63-65). In HF, both systolic and diastolic dysfunction involve alterations of myocardial structure. The pathologic remodeling occurs both due to increased hemodynamic load on the heart, such as increased pressure and volume loads or following myocardial injury (60) as well as activation of the neurohumoral pathways described (55). Despite important differences between the mechanisms of physiologic and pathologic remodeling, they represent the extremes of a continuous spectrum of cardiac plasticity, sharing certain signaling and mechanical pathways.

Cardiac hypertrophy can present three major patterns: **concentric**, **eccentric** (65) or a combination of both (mainly after myocardial injury) (64). As proliferative activity in the myocardium is limited, remodeling is largely based on conformational changes of the myocyte (66). The process also

involves changes of the extracellular matrix due to fibroblast activity as well as changes in cellular homeostasis (67). The mechanisms of cellular growth are not completely clear, but it is believed that aspects of mechanotransduction, when myocytes and extracellular matrix stretch, are involved (66).

The impact of wall stress on development of hypertrophy was studied by Grossmann in 1975 (68). Utilizing the formulation of wall stress and afterload, Grossmann concluded that concentric hypertrophy generally is a result of pressure overload and eccentric hypertrophy follows volume overload. As the ventricular wall stress increases following pressure overload, concentric wall thickening would reduce this wall stress, compensating the heart (64, 68). Concentric hypertrophy mainly results from thickening of myocytes due to the addition of new sarcomeres in parallel. This response can be seen with hypertensive heart disease, resulting in thickened LV myocardium but fairly normal ventricular volume, reflecting the diastolic dysfunction pattern. With sustained stress on the myocardium, decompensation and dilation however occurs.

In LV systolic dysfunction, less blood is ejected during systole and the ESV increases, leading to an increase in preload, EDV/end diastolic pressure. With increased preload, increases in diastolic wall stress will induce eccentric ventricular enlargement. As the process progresses the sarcomeres stretch to their maximal length. In order to accommodate the increased preload, the ventricle generally dilates as a result of myocyte lengthening as new sarcomeres are added in series. In this way, eccentric hypertrophy allows normalization of filling pressures even if the ventricle is volume overloaded, initially maintaining forward flow (69). This morphology represents the eccentric hypertrophy pattern (64). The increase in EDV does however not fully compensate the increase in ESV, why EF is significantly reduced (56), potentially leading to pulmonary congestion due to progressively increasing filling pressures (14). Ventricular dilation increases wall tension and thereby also oxygen consumption of the already compromised myocardium, leading into a vicious cycle of decompensation. Besides macroscopic alterations such as dilation, hypertrophy, increased transversing of oblique myocardial fibers has been seen in patients with heart failure (30, 70) as well as increased sphericity of the ventricle.

The hypertrophic response of the myocardium in pathological conditions has been considered initially adaptive, aiming at maintaining a satisfactory cardiac output while normalizing the load on the heart (68). It is well known that with time, progression of pathological remodeling will lead to deterioration and decompensated heart failure (61). The hypertrophic response to mechanical stress involves multiple interconnected pathways, both adaptive and maladaptive, and while the interplay between these signaling pathways is not completely understood, it is believed that the hypertrophic response may be adaptive or maladaptive depending on which of these cellular pathways are activated (61, 66). Factors such as myocyte necrosis and/or apoptosis, activation of myocardial fibroblasts resulting in fibrosis (or scarring) as well as disturbances in Ca^{2+} -metabolism have been proposed to play important roles (54).

Despite many advances in the assessment and treatment of cardiac conditions, heart failure still presents major clinical challenges with high incidence and poor outcome. Early detection and treatment is of paramount importance, a concern addressed in **Paper III**.

4. Congenital heart disease

Congenital heart disease (CHD) is defined as an abnormality of the hearts structure or function resulting from either altered or incomplete early embryonic or fetal development present at birth, even if it is discovered in adult life (71).

The spectrum of CHD is vast and can involve basically any anatomic portion of the heart, ranging from anomalies causing death to mild lesions that produce only minimal symptoms. Patients thus present significant heterogeneity, not the least within a specific disease group, why diagnosis is sometimes complicated (72). Up to 1% of newborns present with some form of cardiac malformation, and approximately 0.3% present moderate or severe forms (73). Altered hemodynamics and blood flow patterns due to structural abnormalities can further impact development of the rest of the cardiovascular system, contributing to the development of complex CHD. CHD may present with or without cyanosis at the time of birth, with left-to-right ventricular shunts representing the most common type of acyanotic disorders (e.g. ventricular or atrial septal defects). Among the cyanotic CHD, also termed right-to-left shunts, **Tetralogy of Fallot** is the most common (74).

Tetralogy of Fallot

The Tetralogy of Fallot syndrome was first described by Nils Stensen in 1671 and further refined by Etienne-Louis Arthur Fallot in 1888 as comprising four distinct, characteristic morphologic and structural abnormalities: **ventricular septal defect (VSD)**, **overriding of the aorta**, **RV outflow obstruction** and subsequent **RV hypertrophy (Figure 5A)** (74).

The ventricular septal defect in ToF is usually large, permitting equalization of left and right ventricular pressures (74), and the intracardiac flow is characterized by a right-to-left shunting of deoxygenated blood into the systemic circulation, leading to peripheral desaturation (**cyanosis**). The magnitude of blood flow through the defect is mainly defined by the severity of the pulmonary obstruction, why like in many other CHD, ToF patients presents highly variable symptoms. Most patients have adequate pulmonary flow at birth, but as pulmonary vasculature resistance increases, they develop cyanosis during the first post-natal period (74). Left uncorrected, severe pulmonary obstruction will lead to a significant decrease in exercise tolerance, fatigue and profound cyanotic episodes (blue-spells) (75).

The first successful operative intervention was performed by Blalock and Taussing in 1944, aiming at palliation by connecting the subclavian artery to the pulmonary artery. By bypassing the obstruction in the RV outflow tract, pulmonary blood flow increased, enabling survival of the patients into adolescence and adulthood.

With the invention of the cardiopulmonary machine, ToF was one of the first CHD to be successfully surgically repaired. The complete surgical repair, first performed by Lillehei in 1954 (76), involved closure of the VSD with a patch and relieving of the characteristic pulmonary stenosis, thereby restoring normal pulmonary circulation. This was sometimes achieved by the

placement of a transannular patch in the RV outflow tract (**Figure 5B**). Uncorrected ToF is today a rare occurrence, as most patients are surgically corrected at 3-6 months after birth (early repair ToF, rToF) (77).

The recent advances made in the diagnosis and surgical treatment of these patients have significantly improved survival and also the post-repair prognosis (74). Transannular patching results in a widening of the pulmonary valve, in some cases leading to pulmonary valve insufficiency and subsequent varying degrees of **pulmonary regurgitation** (PR) (78, 79). PR and the following RV volume overload is usually well tolerated during adolescence, but left uncorrected, volume overload will induce progressive RV remodeling, fibrosis and subsequent RV failure with significant limitation of exercise tolerance (80-82). Irreversible remodeling will progressively ensue and in these settings, arising ventricular and atrial arrhythmias constitute common causes of symptoms and can also lead to sudden unexpected death (82). Historically considered unimportant, more recent studies have shown a clear relation between the PR and many of these late complications (80). Besides the development of PR, transannular patching may predispose to RV outflow tract aneurysms, why a shift in operative techniques is now seen (83).

By replacing the pulmonary valve, beneficial effects on symptomatology in rToF patients with significant PR have been shown (84-86). **Pulmonary valve replacement** (PVR) is generally associated with low risks, and survival is excellent (80). As the pulmonary valve prostheses have a limited lifespan, the timing of PVR is still discussed in the light of potential need for additional reoperations in these patients (78, 84, 85, 87-89). Patients with signs of RV failure and significant PR will be candidates for reoperation, however it has been suggested that surgery should be performed in asymptomatic patients before irreversible RV remodeling and dysfunction occur (87, 90). Generally, in patients with moderate to severe PR, a variety of objective parameters and

Figure 5. Tetralogy of Fallot. (A) Schematic diagram of the four distinct morphological abnormalities seen in Tetralogy of Fallot: ventricular septal defect (VSD), overriding aorta, right ventricular obstruction and right ventricular hypertrophy and (B) operative implantation of septal and transannular patches, the latter leading to varying degrees of pulmonary regurgitation (yellow arrow).

clinical criteria are recommended for assessment of the PVR indication: RV size (RV end diastolic and systolic volume indexes, RVEDVI and RVESVI, respectively), RV ejection fraction (RVEF), QRS-duration on electrocardiogram, ventricular arrhythmias and exercise intolerance. Many studies have shown improvement of these objective parameters after PVR, albeit in varying degrees, and guideline documents have been published based on these studies (91-93).

Even though management of patients with ToF has evolved greatly, the number of patients now reaching adulthood with rToF increase and consequently, new challenges associated with the long-term complications of early repair emerge. Despite identification of the PR as a main driving force in the development of late complications, the hemodynamic alterations brought on by the PR are not fully explored. Consequently, in **Paper IV**, we aimed at investigating the hemodynamic effects of the PR in terms of turbulence intensity in patients with rToF.

5. Cardiac blood flow

As the main function of the human heart is to generate and maintain the blood flow through the cardiovascular system, it can be argued that “cardiology is about flow” (94).

Laminar and turbulent flows

Whenever a fluid is steadily flowing along a solid surface, such as blood flowing through a straight, smooth walled, cylindrical blood vessel, it flows in an infinite number of concentric well-ordered and non-intersecting layers (laminae). This separation of the fluid is termed **laminar flow**, and presents a parabolic velocity profile (**Figure 6A**). The fluid layer closest to the vessel wall is near stationary, being slowed down by frictional forces, while the next layer slips over this fluid-fluid interface. As the frictional forces decline with the distance from the walls, the flow velocity of the layers increase towards the center of the stream, where the flow velocity is highest. Laminar flow helps reducing the energy losses by minimizing the viscous interference between the fluid layers and the vessel wall.

When the rate of blood flow becomes too high, or an obstruction is present in the vessel, the laminar flow may become disrupted and chaotic. This is called **turbulent flow** and is characterized by rapidly and randomly varying flow properties, such as flow velocity, pressure and direction in space and time (**Figure 6B**) (95). Turbulent flow is inherently ineffective and associated with energy losses due to internal friction forces (96, 97). In the human cardiovascular system turbulence has been shown to be harmful (98, 99).

The shift between laminar and turbulent flow can be predicted by the dimensionless quantity called **Reynolds number** according to:

$$Re = \frac{2rv\rho}{\eta}$$

where r is the radius of the vessel, v is the flow velocity, ρ is the density of the fluid and η is the viscosity of the fluid. Assuming the smooth walled, long and cylindrical vessel, there will be a critical Reynolds number, below which the flow is laminar, usually $Re < 2000$. In the aorta, turbulent flow generally occurs at $Re > 2000$ -2500 (100). As is evident from the equation, the probability of turbulent flow increases with vessel radius and flow velocity. This is important in stenotic blood vessels or at stenotic valve orifices, as the velocity increases as the radius (diameter)

Figure 6. Laminar and turbulent flow. (A) Laminar flow, the flow laminae illustrated by the straight arrows, present a parabolic flow velocity profile. (B) Turbulent flow, characterized by fluctuating flow velocities and directions.

of the vessel/valve decreases.

The Reynolds number is however dependent on the configuration of the hemodynamic system, and when arteries branch or obstruction occurs (i.e. atherosclerosis) the critical Reynolds number may be substantially lower, turbulent flow occurring even at low and physiologic flow velocities. Obviously, the cardiovascular system and the heart in particular does not completely fulfill the criteria for laminar flow, however flow through the normal cardiovascular system is highly organized and turbulence is not very common.

Hemodynamic principles

The “physical study of flowing blood and of all the solid structures through which it flows” is termed **hemodynamics** and comprises multiple concepts and principles (101), such as the **Bernoulli equation**. Bernoulli found that the total mechanical energy (TE) per unit volume of a fluid flowing inside a tube can exist in two main different forms: **potential** (or pressure, P) **energy** and **kinetic energy**,

$$TE = P + KE$$

The kinetic energy can also be defined as the energy an object possesses due to its movement and is thus proportional to its mass and velocity. The kinetic energy of the blood per unit volume is a function of its density (ρ), volume (V) and its velocity (v):

$$TE = P + \frac{\rho V v^2}{2}$$

The basis of the Bernoulli equation reflects the law of conservation of energy, and states that the sum of the pressure energy (P) and kinetic energy is the same at any point in an ideal rigid tube, thus relating pressure and velocity to energy. This means that for two points in a hemodynamic system:

$$P_1 + \frac{\rho V v_1^2}{2} = P_2 + \frac{\rho V v_2^2}{2}$$

The continuity equation is similarly based on the principle that inside a closed system with no losses, flow (Q) in one point will be equal to the flow at any other point, meaning that flow velocity (v) increases as the cross-sectional area (A) of the tube decreases in order to maintain flow (97):

$$Q = A_1 \times v_1 = A_2 \times v_2$$

The principle of Bernoulli, the continuity equation and the importance of kinetic energy can be illustrated by the flow inside a tube that suddenly is narrowed and then returned to its normal diameter, e.g. a stenosis in a blood vessel or stenosis of a valve. When blood flowing in a laminar fashion enters a narrowing in the vessel, the flow velocity will increase in accordance to the continuity equation, with a four-fold increase in velocity if the radius of the vessel is halved. This implies an interconversion between pressure and kinetic energy, the latter increasing 16-fold. As

the total energy remains the same, even if a proportional decrease in pressure energy will occur in the stenotic segment, flow still persists.

The flow through the cardiovascular system can be described as being mainly driven by pressure gradients, as flow (Q) results when a pressure gradient (ΔP) overcomes a resistance (R), described by Ohm's law (Bulk Flow Law) (96),

$$Q = \frac{\Delta P}{R}$$

however, flow in reality results from a difference in total energy between two points (flowing from a higher total energy to a lower total energy). In the cardiovascular system, the heart provides the blood with both pressure energy and kinetic energy during ejection, thus creating flow. The reason for the simplification above is that the kinetic energy content of the blood throughout the cardiovascular system is relatively low (18). During the late ejection phase (mainly in the LV), the intraventricular pressure falls below the aortic pressure (see Wiggers diagram on page 6), however flow continues through the aortic valve as the high blood velocity and KE keeps the total energy higher than the energy of the blood more distal in the ascending aorta.

As the flow exits the stenotic segment, the converged flow will continue as a jet, the narrowest portion called the **vena contracta**, where the highest velocities can be measured. After the stenosis, as the vessel radius is restored, the kinetic energy will revert back to pressure energy, with flow velocity equal to the pre-stenotic (pressure however being slightly lower than before the stenosis, reflecting the total energy gradient inducing the flow). High fluid velocities in a tube of increasing radius predisposes for turbulence and further energy losses occur as heat and fluid friction, a major point in the discussion of **Paper IV**.

Ventricular blood flow

The intracardiac blood flow is closely interconnected with the morphology and function of the cardiac chambers; structural changes of ventricular geometry alter the flow patterns and altered flow conditions in turn can initiate remodeling, not the least during development (102, 103). Within the normal human heart, blood flows in highly organized and three-dimensionally complex patterns, characteristic of the human looped heart (1, 104). It has been suggested that the asymmetric curvatures of the cardiac chambers provide functional advantages. By avoiding energy losses through development of turbulence, redirecting both atrial and ventricular inflow towards their respective outflows, the resulting asymmetrical flow patterns may facilitate energy efficient redirection of blood from diastolic filling to systolic ejection (1, 102). Blood flowing from the inferior and superior vena cava do not collide on their way into the RA, but rather contribute to a forward rotation of flow towards the RV inlet. Similarly, in the LA, the anatomic location of the pulmonary veins contribute to an anti-clockwise rotation of the blood towards the mitral valve. Further, inflow through the atrioventricular valves give rise to vortex formations beneath the valve leaflets, the main direction being towards the ensuing outflow tract (1). The asymmetric flow patterns through the heart are characterized by the occurrence of **vortical flows** (105). The formation of vortices is related to the hemodynamic principles of laminar flow, as the central portion of the fluid moves faster than the outer layers. When the constraints of the vessel walls disappear or the vessel dilates, e.g. when blood enters a cardiac chamber from a vessel or through

a valve orifice, the friction forces between the fluid layers generates a swirling motion, spinning the peripheral fluid layers into vortices. It has been proposed that vortices preserve blood momentum and kinetic energy, augmenting ventricular filling and constituting an important role in the diastolic-systolic coupling (106, 107). Vortex size, intensity and position have been related to LV function and dysfunction (105).

Kinetic energy

The term kinetic energy has been defined previously as the energy an object possesses due to its movement, but can also be translated as the energy required to accelerate a certain object to a certain velocity. Preservation of kinetic energy in the blood may thus be of importance in order to achieve an efficient diastolic-systolic coupling.

As discussed, the majority of the energy used by the heart is utilized to generate pressure gradients in order to achieve flow, and only a smaller portion is imparted to the blood as kinetic energy. This fact has been raised in the debate concerning the importance of blood flow momentum for the preservation of energy in the heart (1, 108-110). Studies have indicated that less than 1% of the total energy constitutes kinetic energy at rest, suggesting that it may be of small importance (24). The same study, however notes that during strenuous exercise, kinetic energy of LV blood increased 57 times compared with a 7-fold increase in pressure energy, concluding that kinetic energy may be of importance during exercise. An efficient diastolic-systolic coupling with preserved KE may thus add to systolic ejection. The KE of the intraventricular blood was studied in both the RV and LV in **Papers I, II and III**.

6. Cardiovascular imaging

Today, fast non-invasive imaging of the cardiovascular system is of great value in clinical practice and different modalities are used daily, providing both morphological information as well as functional parameters for assessment of cardiac function. Some of the modalities employed include **cardiac computed tomography (CT)** (71). Data is acquired by rotating a thin x-ray beam around the patient at multiple angles, producing signals that are detected by an opposing detector array. Generally, intravenous contrast agents are needed for cardiovascular CT imaging, and scans imply exposure to ionizing radiation. **Radionuclide imaging** is another important modality for assessment of patients with coronary arterial disease, and the most common procedure is the single-proton emission computed tomography (SPECT) (71). Intravenously injected radioactive isotopes are absorbed and retained by viable myocytes and gamma radiation is emitted in proportion to the amount of absorbed isotope, reflecting the perfusion of the myocardium.

In this thesis, the main modalities used were **cardiac magnetic resonance imaging** and **echocardiography**. Both these imaging techniques offer non-invasive imaging of the heart, vessels and blood flow without exposing the patient to any ionizing radiation or necessitating contrast agents, albeit with their respective inherent limitations.

Echocardiography

Echocardiography remains the clinically most widely used imaging modality. Constant technological improvements render this modality reliable and inexpensive. Assessment and treatment of almost all cardiovascular diseases are today being based on bedside echocardiographic examinations. The clinical echocardiographic examination usually involves the simple two-dimensional imaging described below, however three-dimensional echocardiography is increasingly used.

General principles of echocardiography

Echocardiographic images are created by the emission of **ultrasound** waves from oscillating piezoelectric crystals in a handheld probe placed against the body. These ultrasound waves are reflected by the tissues of the body, creating a returning **echo** that is received by the crystals inside the probe. As the transmission velocity of sound through the tissues of the body is fairly constant, the time until the echo returns provides information on the position or depth of the reflecting structure. As the ultrasound echoes return to the probe, the digitalized electronic input from the receiving crystals is proportional to the amplitude (intensity) of the sound waves, and is presented in the image as different shades of grey. Highly reflective surfaces render higher amplitudes in the echo signal and are depicted as lighter areas in the image. The sequence of emission and receiving of soundwaves and echoes is repeated to achieve real-time imaging of the moving heart.

Flow measurement with echocardiography - Doppler

The most clinically used flow visualization in the heart is based on echocardiographic **Doppler** technique. This flow measurement relies on the frequency shift of the returning echo when it is reflected by moving blood that will be proportional to the speed of these structures. Blood flow velocities are inherently higher than the movement velocities of tissues, why color-coded Doppler using a reduced speed interval is used in the analysis of tissue movements, called tissue Doppler.

The assessment of blood flow with Doppler permits registration of velocities only in one spatial dimension (to and from the probe) over time, potentially overlooking complex three-dimensional flow structures. In addition to this, the frequency shift and thus the flow velocity will be underestimated when the probe is angulated to the flow direction. Further, echocardiography is dependent on satisfactory examination windows through the thoracic cage. These aspects limits the echocardiographic ability to measure intraventricular blood flow.

Assessment of cardiac function using echocardiography

The clinical assessment of cardiac function involves the integration of many different systolic and diastolic measures of ventricular function. Generally, four standard examination windows are used (parasternal, apical, subcostal and suprasternal) minimizing the acquisition variation and facilitating analysis (71). Besides dimensions and volumes, global LV systolic function is most commonly assessed using measures of LVEF (4). Both LV systolic and diastolic function can be evaluated using echocardiographic myocardial strain methods (111). LV diastolic function is often further assessed by Doppler measurement of mitral inflow patterns, reflecting the transmitral pressure gradients and thus the complex interplay between diastolic myocardial recoil (suction), relaxation, compliance and LA contraction. In the transmitral velocity patterns, the inflow velocity during early diastole is represented by the E-wave (E) and the late atrial filling by the A-wave (A) (71, 112). Classification of diastolic function is based on the interrelation of the E- and A-waves, commonly expressed as the E/A-ratio. Further, assessment of myocardial relaxation can be performed by using tissue Doppler measurements of the myocardial velocities in the basal septal and lateral LV wall and mitral annulus velocity (e') can be calculated. By relating the early mitral inflow velocity (E) to e' , the **E/ e' -ratio** is achieved. The E/ e' -ratio has proven to be a rather robust method for estimation of ventricular filling pressures and was used in **Paper II** to characterize the included patients (112).

Assessment of RV function using echocardiography still remains challenging, much due to its complex retrosternal position and anatomy (113). In **Paper II**, several conventional echocardiographic measures of RV systolic and diastolic function were used. In standard echocardiography, estimation of the right heart sizes and volumes is generally performed using surrogate measures, such as **RV basal diameter** and **RA area**. Increased values may indicate RV volume or pressure overload and have proven useful in the assessment of HF patients (114).

RV systolic function can be assessed using a multitude of parameters. **Right ventricular fractional area change (RV FAC)**, expressing the percentage change between RV end-diastolic and end-systolic area, provides a fairly good estimate of RV systolic function. The **tricuspid annular plane systolic excursion (TAPSE)**, measured from the lateral annulus of the tricuspid valve, gives an

estimate of the longitudinal systolic function and was found to correlate well with RV systolic function and to be prognostic in HF (113, 115). The RV free wall contraction velocity during systole measured using tissue Doppler (s') is also a parameter of RV systolic function (115).

RV diastolic function, similarly to the LV, may be assessed using tissue Doppler information from the RV free wall. The measurements are used to generate the early and late diastolic myocardial velocities (e' and a' , respectively) and their ratio, **e'/a' -ratio** can be calculated (116). A decreased e'/a' -ratio is suggestive of diastolic dysfunction.

Cardiovascular magnetic resonance imaging

Cardiovascular magnetic resonance (CMR) has emerged as a versatile and powerful tool for non-invasive and detailed assessment of cardiac morphology and function in patients with cardiovascular disease, fast becoming a gold standard method (117) in e.g. assessment of congenital heart disease. CMR enables analysis of myocardial tissue composition in terms of fibrosis or edema, myocardial strain and wall motion, myocardial perfusion as well as intracardiac flow, in most cases without the need of contrast agents.

General principles of MRI

Magnetic resonance imaging (MRI) is a modality based on the intrinsic properties of the body and the molecules it is composed of. Clinical MRI primarily relies on the signal coming from hydrogen nuclei within the body (118). Other elements also exhibit magnetic resonance properties; however, hydrogen is favorable due to its abundance in tissues with high water or fat content.

The hydrogen nucleus is composed of a single positively charged **proton** that spins around its own axis. The spinning positive charge generates a small **magnetic moment** around each proton, that can be viewed as a tiny spinning bar magnet with a “north” and a “south” pole. The protons are therefore commonly referred to as “**spins**”. The magnetic moments constantly shift and are normally randomly oriented. When a person is placed inside a MRI-scanner, the proton spins in the body are exposed to the powerful external magnetic field created by the main magnet coil, B_0 . The spins will then either align with (**parallel**) or against (**antiparallel**) the axis of this external field. The parallel alignment requires the lowest amount of energy, why there will be slightly more spins aligned with the magnetic field than against in any given volume of spins (**Figure 7B**).

Spins will further begin to **precess** around the axis of B_0 , the frequency of precession being proportional to the strength of the magnetic field according to the Larmor equation:

$$\omega_0 = \gamma \times B_0$$

Where ω_0 is the precession frequency, or **Larmor frequency** (number of precessions per second, in MHz), and γ is the gyromagnetic constant for protons (42,6 MHz/T). In a magnetic field with a

Figure 7. General principles of MR. (A) Precessing spins can be viewed as spinning tops, spinning around the axis of the B_0 magnetic field. (B) Simplified view of precessing spins. (C) As parallel and anti-parallel spins cancel out, the slightly larger number of parallel spins create a resultant magnetic vector M_0 . (D) The rf-pulse flips the magnetic vector at an angle α . (E). (F) The resulting magnetic vector has two components, the longitudinal M_z and the transverse M_{xy} .

strength of 1.5 T, the clinically most common for cardiac applications, the Larmor frequency for protons will be 63.9 MHz.

The precessing spins can be visualized as spinning tops (**Figure 7A**), wobbling around a central axis and simultaneously spinning around their own axis. The central axis is parallel to the B_0 -field and is termed the **z-axis** in an imaginary three-dimensional coordinate system. Simplified, the whole model can be visualized as a cone of spins wobbling around the z-axis with the antiparallel spins as their mirror image (**Figure 7B**). The difference between the number of parallel and antiparallel spins is very small, but enough to provide a resultant **net magnetization vector** along the z-axis when all other spins are cancelled out by each other (**Figure 7C**). This magnetization vector is called M_0 . M_0 is proportional to the strength of the magnetic field and essential in the creation of the MRI signal, explaining the pursuit and usage of higher field strength.

As the spins precess randomly, the magnetization vector will not possess any xy-component. In order to obtain a magnetic resonance signal from this net magnetization M_0 , a radiofrequency (rf) field, matching the Larmor frequency, is applied by the radiofrequency transmitter coil in a short burst, a **rf-pulse**. When the rf-pulse is applied, the spins will resonate and the net magnetization M_0 starts to move away from the z-axis, spinning around it at an angle (**Figure 7D and E**).

By altering the amplitude and duration of the rf-pulse, the amount of energy transferred to the net magnetization M_0 can be altered. The angle of the precession is proportional to this energy and is termed the **flip angle** (α) (**Figure 7E and F**). The flip angle can be set to a specific value.

As long as M_0 is in equilibrium and aligned to B_0 , it is not measurable. When rotation occurs along the xy-planes, the net magnetic vector M_0 can be divided into two components, a longitudinal component parallel to the z-axis (M_z) and a transverse component in the xy-planes (M_{xy}) (**Figure 7F**). As the M_{xy} rotates, it generates its own small oscillating magnetic field and is thus able to induce a current in a receiving coil, producing a MR signal that can be read. A flip angle of 90° naturally produces the strongest signal.

Figure 8. Relaxation of the spin system. (A) T1-relaxation and (B) T2-relaxation.

When the rf-pulse is discontinued, **relaxation** of the spin system will begin, returning it to the equilibrium state (118). This occurs by two simultaneous but separate relaxation processes, relating to the two magnetization components M_z and M_{xy} : the longitudinal **T1 relaxation** and the transverse **T2 relaxation**, respectively. T1- and T2-times represent the time it takes for the respective relaxation to complete. The T1 relaxation is responsible for the recovery of the longitudinal magnetization component along the z-axis from its inclination (flip angle) to the equilibrium, resulting from the release of energy from the spins to the surrounding molecules as heat (spin-lattice interaction) (**Figure 8A**).

The net magnetization M_0 is the sum of all the magnetic moments (spins) in a volume of hydrogen nuclei, and the coherence of the magnetic fields keep the moments spinning together, constantly pointing in the same direction (**Figure 7F** and **8B**). The spins are said to be “**in phase**”. When the rf-pulse is terminated, this coherence breaks up leading to **dephasing**. As dephasing occurs, the transverse oscillating magnetization vector M_{xy} decays, and the T2 relaxation is responsible for this decay (**Figure 8B**). The signal received by the receiver coil is thus oscillating and gradually decaying, a pattern known as **free induction decay** (FID). This occurs due to spin-spin interactions, the magnetic moment of one proton modifying the total magnetic field experienced by a neighboring proton and thus changing its Larmor frequency. The Larmor frequency of individual protons will therefore change constantly, and without the focusing effect of the rf-pulse, loss of coherence leads to a decay in the M_{xy} . Dephasing however occurs faster than the T2 time due to inherent and static inhomogeneities in the main magnetic field B_0 . The combined effect of this and the T2 relaxation represents the actual rate of M_{xy} decay observed when sampling the FID-signal, which is faster than T2, termed T2*. Generally, the longitudinal relaxation is much faster than the transverse. Further, different tissue types have different T1 and T2 relaxation times, enabling weighting of the image in order to generate contrast between the different tissue types.

Figure 9. Gradient fields. (A) Slice selection by regulating the field strength along the z-axis by application of the gradient G_z , (B) frequency encoding by application of the gradient G_x and (C) phase encoding by application of the gradient G_y inducing a phase change in spins.

MR signal to MR image

Producing an image from the received MR signals requires some kind of information of the localization of the measured MR signal. **Spatial encoding** is achieved by applying magnetic field gradients varying the strength of the magnetic field, and thereby the Larmor frequency of the spins, along the direction of the gradient (**Figure 9**) (118, 119).

Firstly, a certain slice of tissue must be selected. By applying a gradient magnetic field, usually in the direction of the z-axis (head-feet direction of the patient), the strength of the B_0 field and consequently the precession frequency of the spins will vary along this axis. While the gradient is active, a rf-pulse with a frequency corresponding to the Larmor frequency at a chosen point along the gradient is emitted. Only the spins in a plane perpendicular to the gradient at the chosen location will resonate, thereby defining a slice of tissue. This process is called the **slice selection** and the gradient field is termed G_z or G_S .

By further applying a second gradient field (G_x or G_F) in the direction of the x-axis during the read-

out of the MR signal, altering the field strength in this direction, the Larmor frequency of spins will vary along this axis. The resulting MR-signal will then be composed by many different frequencies corresponding to the frequencies of the spins at different locations along the gradient. The complex signal can be decoded using the Fourier transform in order to identify signals from spins at different locations along the x-axis. This process is called **frequency encoding** and provides the spatial resolution of the image.

The slice is now selected along the z-axis and the spin location is obtained by the frequency encoding along the x-axis, however the localization of spins along the y-axis is needed in order to produce the MR image. A third gradient (G_y or G_P) in the y-axis direction is applied, causing the spins to precess at different frequencies and thereby also constantly changing their relative phase along the y-axis (some spins accelerating and some spins slowing their M_{xy} precessing speed along the z-axis). When the gradient is switched off, the precessing frequency of the spins will revert back to original, but the accumulated phase difference will persist. The MR signal received will then be composed of signals with different phases interfering to create one summarized signal. It will be impossible to discern the different signals from this single summarized signal, however by altering the strength of G_y multiple times, the resulting phase shift can be used to mathematically calculate the contribution of individual spins to the signal. This process is termed **phase encoding**. The signal thus contains both frequency information and phase information, pertaining to the x- and y-axes. The data obtained is stored in a two-dimensional matrix, called k-space, with the x-axis representing the frequency encoding and the y-axis representing the phase-encoding. Through a two-dimensional Fourier transform a two-dimensional image of the slice can be created.

The three gradient fields can be combined in order to obtain slices in any spatial orientation. This ability makes MRI a powerful tool, not the least for cardiac applications where oblique slices are needed to produce the standard views of the cardiac chambers.

Field strength

The strength of the main magnetic field B_0 is expressed in units **Tesla (T)**. The typical MRI scanner used for clinical imaging produces 1.5T, however scanners with higher field strengths are increasingly introduced, with 3T scanners presently in clinical use (117) and ultra-high-field 7T scanners being used in research. In the present thesis, both 1.5T and 3T scanners were used. Increasing the field strength is associated with both advantages and challenges. With higher field strengths, the **signal-to-noise ratio (SNR)** increases, allowing for higher image quality and/or reduced scan times with higher spatial and temporal resolution (119). Even if decreased scan times may help reduce artifacts related to patient breathing and movement, some artifacts are inherently more prominent at 3T.

Cardiac and respiratory gating

Application of MRI in cardiovascular imaging poses several challenges, mainly related to the constant beating movement of the heart as well as the breathing motion of the diaphragm blurring the acquired image. In order to eliminate these blurring effects, image gating is performed (118, 119).

Cardiac gating is performed by synchronizing the scan acquisition to the R-wave of the ECG. In

this way, data segments making up the MRI image can be obtained at the same point in time over multiple consecutive heart beats, building a blur free image. Cardiac gating can be prospective, meaning that the acquisition of data is triggered by the registration of the R-wave, or retrospective, meaning that data is continuously acquired and then sorted according to its position in the cardiac cycle. **Prospective cardiac gating** gives better temporal resolution, but implies that a portion at the end of each cardiac cycle is lost, as the scanner interrupts the acquisition in order to wait for the next R-wave. Further, additional data may be lost at the beginning of the next heart cycle due to the delay between the trigger of the R-wave and the actual start of data acquisition. **Retrospective cardiac gating** generally leads to more blurring as each cardiac cycle is interpolated onto a set number of equally spaced imaging phases (**timeframes**) for image reconstruction in each cardiac cycle. As the duration of systole changes much less than the duration of diastole, differences in R–R intervals are generally solved by interpolation of the diastolic phases. Retrospective gating is preferred for acquisition of cine images (moving images) as the whole cardiac cycle is covered and transition between cycles is smooth (119). Cardiac synchronization throughout **Papers I to IV** was achieved using retrospective ECG gating.

For short imaging sequences, respiratory motion does not create problems, since acquisition may well be performed during breath-holds, however, for the more time consuming sequences such as 4D flow acquisition, effects of respiratory motion pose a significant issue. This problem can be overcome by monitoring the respiratory motion during data acquisition. **Respiratory gating** can be achieved by strapping an air bellows to the patients' abdomen or, more commonly, by placing a respiratory navigator window at the level of the diaphragm in a frontal image plane. In the case of continuous data acquisition, such as during 4D flow acquisition, data obtained outside this predefined respiratory window is rejected in the post-processing (119). Respiratory gating may lead to longer acquisition times, as data is discarded depending on patient breathing patterns. This free-breathing technique however offers minimal patient cooperation and was used in the 4D flow acquisitions throughout **Papers I to IV**.

Balanced steady-state free precession imaging

The most widely used technique for assessment of cardiac morphology and function using CMR involves acquisition of moving images of the heart throughout the cardiac cycle in standard imaging planes (similar to echocardiography), termed **cine imaging** or **cine loops**. Retrospectively ECG-gated **balanced steady-state free precession (bSSFP)** is the backbone of any cardiac CMR acquisition, producing bright blood images with good contrast between the myocardium and the intracardiac blood pool at high temporal and spatial resolution, due to high SNR.

The bSSFP acquisition used in **Papers I through IV** include the standard two-chamber (2ch), three-chamber (3ch) and four-chamber (4ch) long-axis views as well as a stack of short-axis views (**Figure 10**). For imaging of the RV inflow and outflow tracts, a RV specific 3ch long-axis view can be used.

Late gadolinium enhancement

In some CMR applications, contrast agents containing **gadolinium (Gd)** are used (71). Gd is a paramagnetic element that reduces the T1 time of tissues containing it in high concentrations. After

Figure 10. Standard cardiac CMR views. (A) Two-chamber (2ch) long-axis view. (B) Three-chamber (3ch) long-axis view. (C) Four-chamber (4ch) long-axis view and (D) Short-axis view. Ao, aorta; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

intravenous injection, the Gd-containing contrast medium distributes in the extracellular space without entering the intact myocytes (120). In areas where the myocyte membranes are disrupted (myocyte necrosis in myocardial infarction) or there is an increase in extracellular space (fibrosis or scarring after myocardial infarction), the gadolinium distribution will be increased and persist for a longer time period than in normal myocardium (usually 10-30 min). By acquiring T1 weighted images according to specific sequences, the resulting high signal from the accumulated Gd produces images where the pathologic areas appear bright white. The tissue will thus present **late gadolinium enhancement (LGE)**. Different myocardial diseases will present different LGE patterns (120). LGE has proven to be an accurate and effective method for assessment of myocardial fibrosis and a strong prognostic predictor of adverse outcome in heart disease (120). LGE was used in **Paper III** to characterize and stratify the included patients.

Flow measurement with MRI – phase contrast

Flow measurement using MRI is performed using a technique called **phase contrast**. This technique exploits almost the same principle as the phase encoding. When a magnetic gradient along a certain direction is applied, spins will alter their frequency of precession in proportion to the strength of the sum of magnetic fields. Consequently, their relative phase will constantly change as long as the gradient is applied. Spins moving along the increasing gradient will successively accelerate and spins moving in the opposite direction will successively decelerate, acquiring shifts in their phase compared to stationary spins. The spins will accumulate this phase shift proportional

to the gradient field strength and the time they spent in the field.

By first applying a gradient in a certain direction for a certain time period, moving spins accumulate a phase shift, but so will the stationary spins. By reversing the gradient for the same amount of time, the stationary spins revert to their original phase, however the moving spins will have accumulated phase differently (**Figure 11**). This phase difference can then be used for encoding velocity information along the direction of the gradient. As B_0 often is not completely homogenous, phase offsets will occur during measurement. By acquiring a reference image without the gradients and subtracting the reference image from the velocity map, these background offsets can be removed (121).

As the spins rotate, thus forming a circle, the phase shifts are measured in degrees within the range of $\pm 180^\circ$. Because of this, the maximal phase shift measurable corresponds to a phase shift of 180° and the velocity encoding thresholds must be set in order to make the peak amplitude correspond to these phase angles. The **velocity encoding (VENC)** is given in cm/s, meaning that a VENC of 100 cm/s describes a measurable range of flow velocities between +100 and -100 cm/s. If velocities exceed this level (VENC is too low), aliasing occurs and conversely, if VENC is set too high, noise and lowered **velocity-to-noise ratio** (the ability to discern real velocity from background noise, **VNR**) occurs (121).

By altering the gradient fields, velocity information can be obtained in any direction. Measurement of flow is most precise if the imaging plane is positioned orthogonal to the main direction of flow, allowing for calculation of blood volumes passing the plane. The most common clinically used phase contrast flow imaging is thus unidirectional and through plane (2D cine phase contrast) (122).

Figure 11. Principles of phase contrast imaging. (Top) Stationary spins and moving spins in e.g. a blood vessel, in phase. (Bottom) After application of the gradient $G+$ and the reversed gradient $G-$, the moving spins inside the blood vessel will have accumulated a phase difference compared to the stationary spins returned to their initial phase. The phase difference is used for velocity information encoding.

4D flow CMR

By combining the flow velocity encoding information in all three spatial directions inside a chosen three-dimensional sample volume throughout the cardiac cycle, the flow velocity and flow directions can be calculated anywhere inside this volume. Since the measurement combines three-directional, three-dimensional velocity information and time ($3D + \text{time} = 4D$), the method is referred to as **four-dimensional phase contrast imaging** or **4D flow CMR/MRI**. 4D flow CMR does not require administration of intravenous contrast agents, however, in many cases contrast agent dependent sequences are run during the same examination. The administration of contrast will then improve the SNR of the 4D flow acquisition as well.

The 4D flow CMR method thus allows for non-invasive visualization and quantification of complex blood flow patterns inside vessels as well as the beating heart (123), and the number of studies utilizing this method has grown exponentially in the last decades (122, 124).

By superimposing visualizations of 4D flow data on morphologic bSSFP images, comprehensive representations of cardiovascular function and flow physiology are achieved (**Figure on cover**). Analysis of blood flow using 4D flow CMR in both healthy subjects and in patients with various heart diseases and conditions have previously been performed for the ventricles (1, 24, 125-129), atria (130), cardiac valves (131, 132) and valve prosthetics (133, 134) as well as vessels (135-137).

Quantification of blood flow using volumetrics and velocity information have led to formulation of advanced hemodynamic markers of cardiovascular function, such as kinetic energy (24, 126, 130, 138-142), turbulent kinetic energy (95, 131, 134, 143, 144), calculation of pressure gradients (145, 146), wall shear stresses (147) and hemodynamic forces (148).

7. Methods

Study samples

Healthy study subjects (Papers I – IV)

In **Paper I-IV**, a total of 34 healthy subjects were examined as control groups (**Table 1**). In **Paper I**, 10 healthy subjects were included. In **Papers II and III**, 11 and 10 healthy subjects were included, respectively. Data from 7 of these subjects were used in both papers, thus 14 individual subjects were examined. In **Paper IV**, 10 healthy subjects were examined. All subjects had normal electrocardiographic and echocardiographic examinations as well as no history of prior or current heart disease, or use of cardiac medication.

Patients with acquired heart disease (Papers II-III)

In **Paper II and III**, 22 and 26 patients with acquired heart disease were included, respectively, however there was an overlap and data from 10 patients were used in both studies. In total, 38 individual patients were examined (**Table 1**). All patients were outpatients at the Department of Cardiology, Linköping University Hospital, diagnosed with or under clinical suspicion of chronic ischemic heart disease based on pathological stress test results (myocardial perfusion scintigraphy or ECG exercise testing) with typical ischemic appearance and/or a presentation with clinical symptoms of ischemic heart disease corresponding to NYHA I and II, together with the fulfillment of the high risk score of cardiovascular disease of the European Society of Cardiology. Almost all patients were clinically compensated and thus presented NYHA I–II, however in **Paper II** three patients presented NYHA class III.

In **Paper III**, as the assessment of LV function was the main goal, further description of the patient group was needed: 24 patients had objective signs of myocardial ischemia in terms of pathological stress test results (myocardial perfusion scintigraphy, ECG exercise testing or stress echocardiography). The remaining 2 patients were followed at the post infarct clinic due to earlier myocardial infarction, but had no current objective signs of myocardial ischemia in terms of pathological stress test results.

The patient groups in **Papers II and III** were for comparative purposes further divided into strata based on their LV end-diastolic volume index (LVEDVI) value as a measure of LV remodeling, using a threshold of 74 ml/m² (149). In **Paper III**, the patients were also stratified according to presence of LGE.

Patients with congenital heart disease (Paper IV)

In **Paper IV**, 17 patients with diagnosis of ToF that had undergone surgical repair were enrolled from outpatients at the Department of Cardiology, Linköping University Hospital (**Table 1**). The patient group was divided into two strata based on their PR fraction computed from conventional

2D flow CMR data.

Table 1. Overview of the included study subjects and patients.

Paper	Study sample
I	10 healthy subjects (4 male, 6 female), ages 22 to 62 years (mean 46)
II	11 healthy subjects (2 male, 9 female), ages 61 to 71 years (mean 67) 22 patients with chronic ischemic heart disease (11 male, 11 female), ages 62 to 76 years (mean 70), outpatients at the Department of Cardiology in Linköping
III	10 healthy subjects (3 male, 7 female), ages 32 to 71 years (mean 62) 26 patients with chronic ischemic heart disease (16 male, 10 female), ages 54 to 93 years (mean 69), outpatients at the Department of Cardiology in Linköping
IV	10 healthy subjects (6 male, 4 female), ages 22 to 54 years (mean 31) 17 patients with repaired Tetralogy of Fallot (9 male, 8 female) ages 21 to 65 years (mean 33), outpatients at the Department of Cardiology in Linköping

Acquisition and analysis of 4D flow data

4D flow data acquisition

The 4D flow CMR data in all four papers were acquired on either a 1.5 T (**Papers I and IV**) or a 3T (**Papers II and III**) clinical MRI scanner. The 4D flow data were acquired during free breathing using a navigator gating with a slowly adapting acceptance window to suppress respiratory effects, imaging data being accepted at end respiration. Cardiac synchronization was achieved using retrospective ECG gating. The field of view was adjusted to create an acquisition box covering the whole heart for each subject. In **Paper IV**, additional conventional 2D flow data were acquired in planes perpendicular to the proximal ascending aorta and pulmonary trunk.

Further, cine bSSFP imaging was used to acquire 2ch, 3ch, and 4ch morphological long-axis images and a stack of short-axis morphological images during end-expiratory breath holds. The number of slices varied according to the size of each patient's heart. In **Paper III**, LGE images were acquired approximately 20 min after administration of gadopentetate dimeglumine (Gd-DTPA).

4D flow data analysis

In order to comprehensively and accessibly display the vast amounts of acquired 4D flow velocity information, analysis in combination with morphological data is required. In this thesis, visualization and quantification of intraventricular flow was performed according to the method

Figure 12. The semiautomatic 4D flow analysis method (126). The ventricular volumes in end-diastole (EDV) and end-systole (ESV) are segmented from morphological short-axis bSSFP images with guidance by 2ch, 3ch and 4ch long-axis views. The segmented volumes are resampled to fit the acquired 4D flow data and particle traces, each representing a volume of blood, are released forwards and backwards in time, thus encompassing the entire cardiac cycle.

described below, however, flow analysis of any structure included in the data field of view is possible.

Visualization and quantification

A recently developed and validated semiautomatic flow analysis method was used for analysis and quantification of RV and LV flow in **Papers I through III** (127). The RV and/or LV end-diastolic (EDV) and end-systolic (ESV) volumes were segmented in the bSSFP short-axis morphological image stacks, guided by long-axis images (**Figure 12**). The segmentations followed the endocardial contour of the compact ventricular myocardium and the ED and ES times were defined by visual observation of the mitral and tricuspid valves in the 4ch view. ED was defined as the first timeframe in which the atrioventricular valves were completely closed and ES as the last timeframe in which the atrioventricular valves were completely closed. The EDV and ESV were then resampled to match the 4D flow data spatial resolution. **Pathlines**, representing the paths of virtual blood particles, were emitted from the center of each voxel inside the EDV and traced forwards and backwards in time to the time of ES, thus encompassing the whole cardiac cycle (**Figure 12**).

The emitted pathlines, considered to represent all the blood flowing through the ventricle in one cardiac cycle, can be visualized using special software in order to comprehensively display flow events superimposed on the cardiac morphology (**Figure on cover**).

In **Paper I through III**, the emitted pathlines were further divided into four functional **blood flow components** based on their behavior. The blood flow components, previously defined by Bolger et al. (125), are as follows:

- **Direct Flow** : blood that enters the ventricle during diastole and leaves the ventricle during the systole of the analyzed heartbeat.
- **Retained Inflow** : blood that enters the ventricle during diastole but does not leave during the systole of the analyzed heartbeat.
- **Delayed Ejection Flow** : blood that starts and resides inside the ventricle during diastole and leaves during the systole of the analyzed heartbeat.
- **Residual Volume** : blood that resides within the ventricle for at least two cardiac cycles.

Each blood flow component volume was calculated by knowing the volume of each voxel represented by the pathlines of the component. In **Paper III**, the *Retained inflow* and the *Residual volume* was combined to create the **Non-ejecting volume**.

Kinetic energy

The KE was calculated for all the four blood flow components in **Papers I** through **III** over the entire cardiac cycle as the sum of KE for all pathlines included the respective component using the volume represented by each pathline (V), the density of blood ($\rho = 1060 \text{ kg/m}^3$), and the velocity of the pathline at every time point (v), according to the equation previously described:

$$KE = \frac{\rho V v^2}{2}$$

Turbulent kinetic energy

As previously described, turbulent flow is inherently ineffective and implies energy losses. When turbulent flow is present, the velocity fluctuates randomly around the mean velocity. The intensity of this velocity fluctuation in any direction can be measured by the standard deviation of the fluctuating velocity component, termed **turbulence intensity** (150).

4D flow CMR allows not only measurements of the mean velocity but also turbulence intensity inside every voxel as velocity fluctuations will decrease the MRI signal magnitude. This signal loss can be related to the standard deviation of the fluctuating velocities, enabling the directionless **turbulent kinetic energy (TKE)** to be calculated (150). The presence of increased levels of turbulence intensity has been associated with detrimental effects on blood cells and endothelium, and high levels of TKE implies higher levels of energy dissipation as heat. Calculation of TKE may thus be utilized as a measure of turbulence related energy loss, and was in **Paper IV** used to measure the hemodynamic effects of pulmonary regurgitation in Fallot patients.

Following previous work on intracardiac TKE (131, 143), in **Paper IV** the TKE in the RV throughout diastole was computed for each diastolic timeframe by integrating the TKE within the RV volume. Accordingly, the RV volume was segmented as described above, but in **Paper IV** all diastolic timeframes between ED and ES were segmented (**Figure 13**). Segmentation was performed to encompass the whole RV outflow tract and a small part of the proximal pulmonary trunk, to ensure that the pulmonary valve plane was well included in the segmentation, not missing any PR. No turbulence was expected to occur in the pulmonary arteries during diastole. After

Figure 13. The analysis method used for calculation of TKE in Paper IV. The right ventricular (RV) volumes were segmented in all diastolic timeframes between end-diastole (ED) and end-systole (ES) from morphological short-axis bSSFP images with guidance by 2ch, 3ch and 4ch long-axis views. The segmented volumes were resampled to fit the acquired 4D flow data and manually registered. TKE was calculated by integrating the TKE within the RV in each diastolic timeframe. Visualization of TKE (red-yellow) together with flow data (white lines) can be superimposed on anatomic images.

resampling of the segmentation to match the 4D flow data, it was in **Paper IV** manually registered to the 4D flow data in order to eliminate effects of patient movement during the scan. This final segmentation was used in the subsequent analysis of TKE, resulting in both visualizations of TKE together with morphological and flow data (**Figure 13** and **21**) as well as quantification of TKE-specific markers.

Several TKE-specific markers were formulated in order to describe the hemodynamic effects of the PR. For every timeframe, the TKE inside the RV volume was integrated spatially to obtain the total amount of RV TKE in the respective timeframe. The RV TKE in the diastolic timeframe with highest TKE was defined as the *Peak Total RV TKE*. The total amount of RV TKE over diastole was divided by the number of diastolic timeframes in order to determine the average TKE inside the segmented RV volume over diastole, *Average RV TKE*. Further, the maximum local TKE was plotted over diastole and the highest value was measured, *Peak RV TKE*. Anatomic localization and the time of occurrence of the Peak RV TKE was assessed (**Figure 21**).

Statistical analysis

Continuous values are given as group means $\pm$ standard deviation (SD). The statistical significance was set to $P < 0.05$ in all the studies.

In **Paper I**, for the comparison of the four blood components (percentage of EDV, KE), EDV and EF between the RV and LV, a Student's t-test for paired observations was used. For the comparison of different flow components in the RV (volume in percentage of EDV and KE), a two-way analysis of variance (ANOVA) with a Tukey post-hoc test was used.

In **Paper II** and **III**, the patient group was stratified into sub-groups. For the comparison of parameters between these study groups and the healthy controls in **Paper II**, a one-way ANOVA with a Fisher's Least Significant Difference post-hoc test was used.

Correlations between LV volume parameters and LV 4D flow measures in **Paper III** were performed using Pearson's parametric correlation analysis. Clinical parameters were compared using t-test, however, in cases where data had a non-Gaussian distribution, the Mann-Whitney U test was used. In the sub-group analyses, one-way ANOVA with a Tukey's post-hoc test was used to compare the patient subgroups based on LVEDVI or LGE and the healthy controls.

In **Paper IV** the results were before statistical analysis assessed for normality by computation of parameter residuals and creation of normality dot plots. All parameters presented satisfactory normality, rendering parametric statistical tests adequate. For the sub-group comparisons, a one-way ANOVA with a Bonferroni post-hoc test was used. Unpaired t-test analyses were performed for parameters present for only two of the sub-groups (QRS-duration and time from ToF repair). Correlations were performed using Pearson's parametric correlation analysis. Simple regression and stepwise multiple regression models were used to predict markers of remodeling. Repeated simple regression analyses were initially performed to determine which parameters should be included in the multiple models as predictors. The most important statistical measures for finding the best regression model are R^2 -value, R^2_{adj} -value (adjusted R^2) and the P-values for the predictors. R^2 is a statistical measure of how close the data is to the regression line, i.e. how much of the variation in the response variable can be explained by the tested predictor. The R^2 -value will always increase with increasing number of predictors included in a multiple regression model, giving the false impression that the model improves. The R^2_{adj} -value is the R^2 -value adjusted for the number of predictors included in the model, and it can only increase if the newly added predictor improves the model more than would be expected by chance. It decreases if the predictor improves the model less than chance.

The strong correlation between the 2D flow CMR parameters and the 4D flow CMR TKE parameters introduced a statistical multicollinearity issue in the regression analyses. Multicollinearity occurs when two or more of the predictor variables in a multiple regression are correlated to each other and not fully independent predictors. This phenomenon is nearly always present when performing multiple regressions on observational data, however it does not affect the predictions made by the whole multiple regression model. Multicollinearity may however not always be a bad thing in multiple regression, and in the performed analyses in **Paper IV**, the issue was found to be self-limiting. A multiple regression model was constructed, and based upon the

simple regressions, the predictor parameters chosen were PR-volume and PR-fraction, *Average RV TKE*, *Peak Total RV TKE* and *Peak RV TKE*. A stepwise multiple regression analysis was performed, and for each step, the least significant (highest P-value) predictor was excluded. By simultaneously assessing the significance of the included predictors as well as the shifts in R^2 -value and R^2_{adj} -value, the model improved when predictors were removed, and a single predictor was left, thus eliminating the multicollinearity issue.

Inter- and intraobserver variability was assessed in **Paper IV** as this method was new. Intraclass correlation coefficient (ICC) for the TKE markers *Average RV TKE*, *Peak Total RV TKE*, and *Peak RV TKE* were used. ICC estimates and their 95% confidence intervals (CI) were based on a single-measure absolute agreement 2-way random-effects model (ICC (2,1)) for the interobserver variability tests and on a single-measure absolute agreement 2-way mixed-effects model (ICC (3,1)) for the intraobserver variability test.

8. Results

As the papers included in this thesis comprise results from studies of both cardiac ventricles, their function in different settings and not least their interaction, the results will be integrated into topical sections with reference to the respective paper.

Blood flow in the normal right and left ventricle (Paper I)

In **Paper I**, blood flow components and their respective KE were assessed in the healthy RV using 4D flow CMR and compared to LV blood flow components and their respective KE.

The overall flow pattern through the RV was dominated by the smooth path of the *Direct flow* component from inflow to the outflow tract, passing through the most basal portion of the ventricle (**Figure 15**). During filling, an asymmetric ring vortex surrounded the tricuspid inlet, the *Direct flow* component contributing to the superior aspect of this ring vortex that extended into the RV outflow tract (**Figure 17A**). The *Retained inflow* entered alongside the *Direct Flow* but followed a more inferior and lateral path, contributing to the inferior portion of the ring vortex and flowing into the central RV, replacing the *Residual volume* as it moved apically. The *Delayed ejection flow* was located along the mid and basal portions of the septum, extending into the left and superior areas of the outflow tract. The *Residual volume* filled the peripheral regions closest to the walls and comprised the volume of the apical third of the RV (**Figure 15**). The apical third of the RV appeared to be rather “flow poor” (**Figure 17A**).

The flow patterns inside the RV and LV presented both differences related to their inherently different configurations, but also similarities. In both ventricles, the route of *Direct flow* was

Figure 14. Pie charts illustrating the four blood flow components as percentage of EDV (percent ± SD) for the RV, LV and one patient with dilated RV. Green = Direct flow, yellow = Retained inflow, blue = Delayed ejection flow and red = Residual volume.

predominantly through the basal and mid regions, however the narrower RV apex was more excluded from flow events than the apical region of the LV. The *Direct flow* in the LV presented a more acute direction change from inflow to outflow than through the gradual curvature of the RV.

Previous studies of LV blood flow found that the *Direct flow* component comprised slightly more than one third of the LVEDV and was significantly larger than the three other components (126, 142). These findings were confirmed by the results in **Paper I** ($37 \pm 7\%$, **Figure 14**). Similarly, in the RV the proportion of the *Direct flow* component was substantially larger than the other three flow components ($44 \pm 6\%$, $P < 0.001$), however RV *Direct flow* was also significantly larger than its LV equivalent (44 ± 6 vs $37 \pm 7\%$, $P < 0.01$). The proportion of *Residual volume* was smaller in the RV compared to the LV (23 ± 6 vs $31 \pm 6\%$, $P < 0.05$), whereas *Retained inflow* and *Delayed ejection flow* proportions were equivalent in the two ventricles. The RV *Direct Flow* possessed a significantly larger amount of total KE at the time of ED than the other three flow components (0.41 ± 0.34 mJ, $P < 0.001$), the atrial contraction increasing the KE of the *Direct Flow* more than the other flow components.

Further, one patient with dilated RV due to pulmonary hypertension was examined for comparison of healthy RV blood flow patterns to alterations in primary RV disease. This dataset was never included in **Paper I**, but holds interesting information. In this patient with an EDV of 208 ml, the proportion *Direct flow* of the EDV was merely 12%, while the *Residual volume* was vastly increased (**Figure 14**).

Figure 15. Pathline visualization superimposed on morphological images, illustrating the location of the four RV blood flow components at ED. (A) Anterolateral view, (B) septal view. The Direct flow component (green) resides in the basal part of the ventricle and the Residual volume (red) outlines the functional periphery of the RV, in particular in the apical region. Yellow = Retained inflow, blue = Delayed ejection flow. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle and RVOT, right ventricular outflow tract.

Right ventricular blood flow in acquired heart disease (Paper II)

In **Paper II**, the changes in RV blood flow components and their KE in the presence of subtle LV dysfunction were studied and compared to conventional echocardiographic RV functional parameters. The patient group was divided into two subgroups based on LVEDVI as a measure of remodeling: a lower-LVEDVI and a higher-LVEDVI group.

Similarly to **Paper I**, the overall RV blood flow pattern and blood flow component distribution throughout diastole were visualized. The healthy group presented an organized pattern matching the findings of **Paper I**, however, in the higher-LVEDVI group, the flow component distribution appeared less organized, with higher degrees of component mixing. This pattern was most evident in the patients with the highest LVEDVI values ($>100 \text{ ml/m}^2$). Moreover, in these patients at late diastole the inflowing blood of the superior tricuspid ring vortex was divided into a major counterclockwise flow path up into the RV outflow tract (mainly *Direct flow*) but also into a minor clockwise flow path down towards the apical part of the interventricular septum (viewed from the lateral aspect of the RV) (**Figure 17A and B**).

The higher-LVEDVI group were more remodeled and had lower LVEF than the other groups. Further, the echocardiographic LV E/e' -ratio, describing diastolic function, was significantly higher in the lower-LVEDVI and higher-LVEDVI groups compared to the healthy control group, proposing non-normal LV diastolic function but no elevated filling pressures. There was however

Figure 16. Graphic representation of the findings of Paper II. The right ventricular (RV) proportion of Direct flow of the RV end-diastolic volume (EDV) was significantly lower in the higher-LVEDVI group and possessed a lower kinetic energy (KE) proportion at ED. No significant difference was seen between the RV ejection fraction (RVEF) or RV end-diastolic volume index (RV EDV-index) between the groups.

no statistically significant difference in RVEDVI, RVEF (**Figure 16**) or any of the RV echocardiographic parameters reflecting systolic or diastolic function between the three groups (Table 3 in **Paper II**). Even so, the proportion of RV *Direct flow* of the RVEDV was significantly lower in the higher-LVEDVI group ($38 \pm 5 \%$) compared to both the healthy control ($44 \pm 6 \%$, $P < 0.05$) and the lower-LVEDVI group ($44 \pm 6 \%$, $P < 0.05$) (**Figure 16**) and possessed a significantly lower KE proportion ($52 \pm 6 \%$ vs $65 \pm 7 \%$ and $64 \pm 7 \%$, respectively, $P < 0.001$) of the total RVEDV KE at ED (**Figure 16**), suggesting that these 4D flow specific parameters could detect early impairment of RV function.

Figure 17. Pathline visualization of right ventricular flow components at late diastolic filling. (A) In a 70-year-old healthy subject with LVEDVI of 61 ml/m^2 the inflowing blood is directed towards the outflow tract (RVOT) and the apex is “flow poor”, whilst, (B) in a 72-year-old patient with LVEDVI of 128 ml/m^2 , the inflowing blood is divided into a major counterclock-wise flow path into the RVOT but also into a minor clockwise flow path down towards the apex. Green = Direct flow, yellow = Retained inflow, blue = Delayed ejection flow and red = Residual volume. LA, left atrium; LV, left ventricle; RA, right atrium.

Left ventricular blood flow in acquired heart disease (Paper III)

Previous 4D flow CMR studies have suggested marked abnormalities in 4D flow volume and KE in severely dilated and functionally depressed LVs, the *Direct flow* component diminishing with a concomitant increase in the volume and end diastolic KE of the *Non-ejecting volume* (*Retained inflow* and *Residual volume*) (125). Similar findings were subsequently confirmed in a cohort of well compensated dilated cardiomyopathy patients with moderate remodeling and systolic dysfunction (142). In **Paper III**, alterations in LV blood flow components and their KE in the presence of only subtle LV dysfunction and remodeling were studied under the hypothesis that the potentially adverse changes in flow volumes and KE previously measured would be detectable early in the course of disease.

Similarly to **Paper II**, in **Paper III** sub-group analyses were performed based on LVEDVI, but also on the presence or absence of LGE. Patients in the higher-LVEDVI group had significantly lower proportion of *Direct flow* ($33 \pm 6\%$) of the LVEDV compared to the lower-LVEDVI group ($42 \pm 6\%$, $P < 0.05$) and healthy controls ($42 \pm 8\%$, $P < 0.05$) (**Figure 18**), that possessed a lower proportion of KE ($50 \pm 11\%$ vs $63 \pm 10\%$ and $60 \pm 14\%$, $P < 0.05$, respectively) of the LVEDV total KE at ED. In tandem with this, the higher-LVEDVI group had significantly higher proportion of *Non-ejecting volume* than the lower-LVEDVI group ($48 \pm 5\%$ vs $41 \pm 5\%$, $P < 0.05$) (**Figure 18**). No intergroup differences were seen between the groups stratified according to LGE and the healthy control group.

For the whole patient group ($n=26$), correlation analyses between the *Direct flow* and *Non-ejecting volume* proportions and LVEDVI were performed, as well as between *Direct flow* and *Non-ejecting volume* KE proportions and LVEDVI (**Figure 19**). With increasing LVEDVI, the *Direct flow* proportion fell and the *Non-ejecting volume* proportion increased ($r = -0.643$ and $r = 0.669$, $P <$

Figure 18. Pie charts illustrating the four blood flow components as percentage of EDV (percent $\pm$ SD) for the three sub-groups stratified by LVEDVI. Green = Direct flow, yellow = Retained inflow, blue = Delayed ejection flow and red = Residual volume. Non-ejecting volume is comprised by the Retained inflow and the Residual volume flow components (yellow + red = orange).

0.001, respectively). The KE proportion of the total LVEDV KE of the two flow components showed the same pattern with increasing LVEDVI ($r = -0.482$ and $r = 0.532$, $P = 0.013$ and $P = 0.005$, respectively) (**Figure 19**). Further, the *Direct flow* and *Direct flow* KE proportions correlated positively to LVEF ($r = 0.68$, $P < 0.001$ and $r = 0.47$, $P < 0.05$, respectively), while the *Non-ejecting volume* proportion and its KE proportion correlated negatively to LVEF ($r = -0.74$, $P < 0.001$ and $r = -0.44$, $P < 0.05$, respectively), all together suggesting a shift both in volume and KE distribution from the *Direct flow* to the *Non-ejecting volume* as remodeling progressed and LVEF diminished.

Figure 19. Correlation plots. (A) Direct flow (DF, green) volume proportion (ratio) of LVEDV vs LVEDVI, (B) DF kinetic energy (KE) ratio at ED vs LVEDVI, (C) Non-ejecting volume (NE, orange) ratio vs LVEDVI and (D) NE KE ratio at ED vs LVEDVI.

Right ventricular turbulent kinetic energy in repaired Tetralogy of Fallot (Paper IV)

In **Paper IV** the hemodynamic effects of PR were investigated in the RV of 17 patients with surgically corrected ToF, with respect to turbulent kinetic energy (TKE) using 4D flow CMR. The patient group was divided into two strata for comparative purposes based on PR fraction computed from conventional 2D flow CMR data using the patient group median of 11% as a cutoff (median 11%, range 1 – 50%): one group with lower PR fraction ($\leq 11\%$, $n = 9$) and one group with higher PR fraction ($>11\%$, $n = 8$).

The group with higher PR fraction presented higher RV remodeling indices (RVEDVI and RVESVI) than both the other two groups and both patient subgroups had lower RVEF than the healthy control group. PR volumes and fractions were computed from conventional 2D flow CMR data, and as expected, the higher PR fraction group also had larger PR volumes than both the lower PR fraction group and the healthy PR-free control group.

The higher PR fraction group had significantly higher diastolic RV TKE levels than both the lower PR fraction group and the healthy control group, reflected by higher values of *Average RV TKE*, *Peak Total RV TKE* (**Figure 20**) and *Peak RV TKE* (Table 2 in **Paper IV**). The visualizations of TKE showed that the substantially larger TKE development in the higher PR fraction group was located in the RVOT during early diastolic filling, reaching centrally and apically inside the RV chamber as a plume around the regurgitant jet (**Figure 21**). *Peak RV TKE* was in this group consistently situated in the RVOT. Contrary to the higher PR fraction patients, the much lower *Peak RV TKE* values were in the majority of healthy subjects situated in the RV inflow region during diastolic filling. This TKE pattern was also seen in the lower PR fraction group, however the three patients presenting the highest PR fractions in this group (4 – 11%) also had TKE development in the RVOT. *Peak RV TKE* values in these three patients were indeed higher (176 – 246 mJ/m^3) than those presented by the healthy control group (Table 2 in **Paper IV**).

Figure 20. Turbulent kinetic energy (TKE) in the right ventricle (RV) throughout diastole. (A) Healthy control group, (B) Fallot group with lower PR fraction ($\leq 11\%$), (C) Fallot group with higher PR fraction ($>11\%$). No significant difference was found between the healthy control group and the lower PR fraction group, reflected by the similar appearance of the (A) and (B) graphs. In graph (C), the higher TKE-levels during early diastolic filling are clearly visible: (a) PR fraction of 50%, (b) 39%, (c) 40%, (d) 30% and (e) 28%, respectively.

PR fractions were related to the TKE-parameters and *Peak RV TKE* was found to correlate the strongest ($r = 0.87$), followed by *Peak Total RV TKE* ($r = 0.85$) and *Average RV TKE* ($r = 0.80$), $P < 0.05$ for all. The initial simple regression analyses found all conventional 2D flow as well as the 4D flow parameters moderately predictive of RV remodeling (RVEDVI), with the *Peak Total RV TKE* as the strongest predictor of RVEDVI ($R^2 = 0.47$), followed by *Average RV TKE* ($R^2 = 0.44$) and PR volume ($R^2 = 0.37$). The significant 2D and 4D flow parameters were included in the subsequent backward stepwise multiple regression analysis in order to create a prediction model for RVEDVI (Table 3 in **Paper IV**). In this multiple regression analysis however only one predictor remained: *Peak Total RV TKE* was found to be the strongest independent predictor of RVEDVI at $R^2 = 0.47$ with an adjusted $R^2 = 0.44$ for the whole model. Throughout the analysis, the adjusted R^2 -values increased.

Figure 21. Visualization of turbulent kinetic energy (TKE) in the right ventricle (RV) of a repaired Tetralogy of Fallot patient with PR fraction 30% in three progressive timeframes. (A, B) At early diastolic filling, inflow of blood from the right atrium (RA) passes the tricuspid valve (TV). (B, C) The regurgitant blood (white arrow) from the pulmonary trunk (Pulm) enters through the incompetent pulmonary valve (PV). (A-C) Adjacent to the regurgitant flow, TKE development is seen as a yellow-red plume (higher TKE = yellow, lower TKE = red). (Graphs A-C) *Peak RV TKE* is shown graphically over diastole, a line marking the respective diastolic timeframe. The anatomic position of the *Peak RV TKE* value (green dot) is present centrally in the TKE plume. The RV is visualized as a semitransparent mask and a 4ch image provides anatomic orientation.

9. Discussion

The studies included in this thesis have provided new physiologic and pathophysiologic insights into the complex multidimensional blood flow within the cardiac ventricles using novel 4D flow CMR techniques and analyses. Further, novel blood flow specific markers of cardiac dysfunction have been formulated and evaluated, which in the future may assist the clinical every-day assessment of patients with cardiovascular disease as well as aid in decision making. Below follows a discussion of the presented results as well as some technical considerations.

Physiological considerations

In **Paper I**, 4D flow CMR was successfully used to investigate the distribution, volume and energetics of different flow components in the normal RV and in **Paper II** the RV of patients with primary left-sided ischemic heart disease, highlighting the importance of interventricular interaction. Overall, the patients in **Paper II** and **III** were well managed and showed low NYHA levels creating possibilities for detection of subtle alterations of ventricular function.

In the healthy RVs in **Paper I**, the portion of RV inflow that passes directly to outflow (*Direct flow*) was located in the basal region of the ventricular cavity and was proportionally larger than the other flow components. The *Direct flow* component in the healthy RV further possessed higher presystolic KE compared with the other RV flow components. The flow path from RV inflow to outflow followed a gradual curvature through the basal and middle parts of the ventricle, reflecting the RV anatomy with separated inflow and outflow tracts. The apical region was found to not participate as much in flow exchange, supporting previous 3D echocardiographic findings observing that the apical region of the RV contributed less to overall RV contraction than the inflow and outflow regions (151). The visualizations in **Paper I** suggest that the *Residual volume* outlines the functional periphery of the RV, in particular the apical region, providing a fluid-fluid interface that influences the routes of the other more exchanging flow components, potentially facilitating the smooth redirection of inflowing blood towards the outflow tract while preserving its KE (152). This flow distribution through the RV enables a substantial proportion of the RV inflow to pass directly to ensuing outflow, a diastolic flow organization that in the normal RV appears to create favorable conditions for effective systolic ejection.

In **Paper II**, the healthy controls presented *Direct flow* proportions similar to those measured in **Paper I**, however in the higher-LVEDVI group a significantly lower proportion of RV *Direct flow* was detectable, suggesting that the RV hemodynamics are altered in these patients compared to the healthy subjects and the less remodeled patients. This lower relative amount of *Direct flow* is due to altered diastolic RV inflow patterns, with blood being retained in the ventricular cavity instead of passing directly to the RVOT and ejection. Further, the relative amount of KE possessed by the *Direct flow* component at ED was lower in the higher-LVEDVI group compared to the other two groups, suggesting that in total a lower proportion of the RV EDV contributes to efficient systolic ejection. Moreover, this suggests that a larger relative amount of the KE of the RV inflowing blood in these patients is transferred into other flow components or other energy forms. This was actually clearly evident for the mildly dysfunctional LVs in **Paper III**, and it is reasonable to believe that

the same shift in blood flow component volumes and energetics occurs in the impaired RV.

Although the absolute values of the KE possessed by the *Direct flow* are much lower than the estimated stroke work of the ventricle and the importance of KE has been discussed, a KE preservation at ED reflects a transit of inflowing blood through the ventricle that may be favorable for an efficient systolic ejection. In both **Paper II** and **III**, impaired ventricular function resulted in lowered KE of the *Direct flow* component. The preservation of presystolic KE may have an impact on pulmonary and aortic valve function. In a large animal study, a presystolic increase in LV outflow tract volume has been suggested to prepare the aortic valve for ejection (153). Furthermore, preservation of the inflow KE may also facilitate the filling process by a smaller inflow-impeding pressure rise between inflow orifice and the endocardial surface of the expanding ventricles (154).

There may be several reasonable mechanisms underlying the RV hemodynamic changes seen in **Paper II**. It is well known that the LV plays a central role in RV flow and pressure generation and that the most common cause of RV failure is primary LV dysfunction, generally considered to be due to indirect interventricular interaction. However, in **Paper II** it is not reasonable to believe that the main explanation of the hemodynamic alterations seen in the higher-LVEDVI group is merely due to increased RV afterload secondary to increased LV filling pressures and pulmonary hypertension. Both patient groups had higher indices of diastolic dysfunction than the control group, but the measured values were still below the level where significant elevated filling pressures and pulmonary congestion are likely to be present (112). Moreover, no difference was found between the groups regarding the RVEF, which has been found to be inversely correlated with RV afterload (48).

The oblique LV muscle fibers contribute to RV function by the shared interventricular septum (41, 155). Ischemic LV disease eventually results in myocardial remodeling and chamber dilation (60), which can lead to increased transversing of septal oblique muscle fibers (43). The observed RV flow alterations could thus be explained by altered properties of the shared interventricular septum in the patients with higher LVEDVI. In the patients with the highest degree of LV remodeling ($>100 \text{ ml/m}^2$), the late inflowing blood in the superior portion of the tricuspid ring vortex divided into a major counterclockwise flow path up into the RVOT as seen in **Paper I**, but also into a minor clockwise flow path. This “abnormal” clockwise flow path was located in the vicinity of the septum and it could be speculated that altered properties of the septum may redirect this portion of the late inflowing blood away from the RVOT, reducing the amount of *Direct flow*.

There was further no statistically significant difference in any echocardiographic parameters of systolic or diastolic RV function between the three groups. As many conventional echocardiographic parameters for assessment of RV function are based on RV free wall measurements, a compromised septal function may be overseen and global RV function may not be fully assessed. An advantage of RV 4D flow measurements, and in particular the *Direct flow* component, is that not only the RV free wall is taken into account, but the configuration and function of the entire ventricular chamber including the interventricular septum, and its interaction with flow.

As much as the RV and LV are interconnected units of the same heart and in many ways similar, they also present significant differences, not the least of a hemodynamic nature. Even if the LV

Direct flow extended further into the apical region compared with the RV *Direct flow* and comprised a smaller portion of the EDV than the RV *Direct flow*, it still presented a larger proportion of LVEDV and higher KE than the other LV blood flow components. The differences seen in LV and RV blood flow distribution mirror their anatomic and functional differences. Similarly to the findings of **Paper I**, among the four functional components of the LV diastolic volume, the *Direct flow* has the most direct route and fastest transit through the LV. Contrary to the gradual and smooth redirection of flow in the RV, the LV inflow and outflow tracts are nearly opposite in direction, which produces a turning point for the blood in the midventricular and apical regions. The change in direction between ventricular inflow and outflow was less acute on the right side compared to the left side. The movements of the atrioventricular valves are important for normal ventricular function (156), the directions and extent of atrioventricular plane displacement differing between the right and the left heart. On the left side, motion occurs largely in the direction of the ventricular long axis. In contrast, the tricuspid annulus moves as if hinged, with lateral displacement much greater than septal, creating a sweep towards the RV outflow tract. The predominant direction of ventricular inflow seems to follow the atrioventricular plane displacement in both cases.

Despite the larger RV *Direct flow* compared with the LV, the total end-diastolic KE of the *Direct Flow* was not larger on the right side. This finding may in part be explained by a greater LV diastolic atrioventricular pressure gradient, in particular during atrial contraction. This is in agreement with higher diastolic inflow velocities across the atrioventricular valve on the left side detected using Doppler echocardiography (3).

Similarly to the RV flow alterations seen in **Paper II**, previous 4D flow CMR studies have found clear abnormalities in 4D flow volume and energy in moderately to severely dilated and functionally depressed LVs compared to healthy LVs (125, 142). The study of quantitative measures of LV flow component volumes and end-diastolic KE in patients presenting a range of normal to mild LV remodeling and normal to mildly depressed LV systolic function in **Paper III** fills the gap in this spectrum of heart failure patients studied with 4D flow CMR to date. A clearer picture emerges when these results are combined with prior work, both on LV and on RV (126, 127, 138, 140-142): the proportion of the *Direct flow* diminishes with increased LV volumes. At the same time, the amount and proportion of the *Non-ejecting volume* (*Retained inflow* and *Residual volume*) of LVEDV increase.

By pre-systole, the preserved KE of *Direct flow* may, as previously discussed, imply less work required for ejection of the stroke volume. As the *Non-ejecting volume* does not leave the ventricle during the ensuing systole, its KE does not contribute directly to ejection. The results in **Paper III** and also prior studies in patients demonstrate that in remodeled, dysfunctional ventricles, there is a shift whereby the KE of the *Direct flow* diminish, while that of the *Non-ejecting volume* increase (125, 142, 152).

The LV *Direct flow* volume in **Paper III** was lower in the higher LVEDVI group compared to both the lower LVEDVI group and the controls. On the other hand, also LVEF was lower in the higher LVEDVI group compared to the controls whereas no difference was observed between the two patient sub groups. Although the *Direct flow* volume and KE correlated at least moderately to LVEF, these parameters are not interchangeable. The LV ejected volume constitutes both *Direct flow* and *Delayed ejection flow*. *Direct flow* may reflect more aspects of diastolic-systolic coupling

than *Delayed ejection flow* and thus LVEF, particularly at these subtle stages of LV dysfunction. Further, 4D flow based KE measures may better track efficiency or potential for excess diastolic pressure than measures merely based on volume; the shift in KE from *Direct flow* to *Non-ejecting volume* may contribute to higher intraventricular pressure due to deceleration of flow. Future comparison of 4D flow CMR parameters to other metrics of ventricular work and demand would be required to confirm their accuracy in reflecting ventricular efficiency.

In **Paper IV**, TKE in the RV of patients with rToF and a spectrum of PR was investigated using 4D flow CMR, and TKE was found to be increased in the rToF patients with PR compared to healthy controls. The visualization of TKE inside the RV volume of the higher PR fraction patients showed that the development of TKE and the *Peak RV TKE* values were located in the outflow tract at the time of early RV diastole, coinciding with the occurrence of PR. The similarities between the anatomic locale and time of occurrence of the PR and TKE development in the higher PR fraction patients suggest that TKE reflects the severity of the regurgitation.

The correlation between TKE and the severity of regurgitation was previously shown for mitral regurgitation (131). In the mitral regurgitation study, Peak Total TKE levels were however substantially higher (ranging between 13-37 mJ) than the highest levels observed in Paper IV (*Peak Total RV TKE* 5.95 ± 3.15 mJ in the higher PR fraction group). This would naturally be expected, as pressure gradients between the left ventricle and left atrium in the setting of systolic mitral regurgitation widely exceeds those between the pulmonary artery and the RV during diastole. However, the mechanism behind the development of elevated TKE would be similar, namely regurgitating blood causing energy dissipation through turbulence. No statistically significant difference in TKE levels were seen between the healthy controls and the lower PR fraction group and the levels of *Peak Total RV TKE* were tended to be lower than previously observed in healthy LVs (1.91 ± 0.78 mJ vs the corresponding measurement “*Peak Total LV TKE*” of 2.5 ± 1.2 mJ), once again reflecting the higher pressure gradients of the left heart (143).

Further, RV TKE related slightly stronger to indices of RV remodeling than conventional 2D flow-based parameters of PR and in the multiple regression model, the strongest independent predictor variable for RVEDVI was the 4D flow-specific parameter *Peak Total RV TKE*. Even if the correlation between the conventional 2D flow parameters and the 4D flow-specific TKE parameters was high, these parameters might still not be fully interchangeable, as the 4D flow-specific TKE parameters reflect not only the amount of PR but also flow-specific effects of the PR on the RV. It is reasonable to believe that there are other factors besides the RV volume overload due to worsening PR that contribute to RV dilation.

One factor may be the aneurysmal development in the RVOT due to the transannular patching during the initial repair procedure. This local remodeling will contribute to an increase in RVEDVI (157, 158), and it has been discussed that resection of the aneurysm during PVR procedures could partly explain the postoperative reduction of RV volumes (159). One study however found that a significant portion of the included patients presented outflow aneurysms even though transannular patching was not initially performed, raising the question of other factors important for aneurysmal development and RV remodeling.

Another factor may be progressive fibrosis and general dilation of the RV. Evidence of fibrosis occurring in the RV outflow tract in patients with rToF has been presented in multiple studies (160,

161), but also in areas outside the surgical incision (162). Previous studies have demonstrated that disturbed flow is linked to endothelial dysfunction (98, 99, 163), possibly impacting subendocardial structures causing fibrotization and remodeling, in this case presumably in the RVOT and adjacent ventricular segments.

In **Paper IV**, a progressive increase in PR fraction brought about not only progressively higher local *Peak RV TKE* values, but also an increase in *Total Peak RV TKE* and *Average RV TKE*, reflecting a higher energy loss and flow disturbance inside the entire RV throughout diastole. The *Peak Total RV TKE* was in the present cohort the strongest predictor of RVEDVI, suggesting that elevated TKE in the RV could act as a mediator of myocardial fibrosis, aneurysmal development and thus increasing RVEDVI (**Figure 22**). This proposed mechanism of fibrotization and dilation can be further supported by the uniform occurrence of *Peak RV TKE* in the outflow tract during diastole in the patients with larger PR. Visualizations of TKE however showed that the PR and development of TKE also extended further down into the chamber than only the RVOT, which in turn could explain fibrotization of areas outside the region of surgical repair, possibly reflected by the more global TKE measurements *Peak Total RV TKE* and *Average RV TKE* being stronger predictors of higher RVEDVI than was the more local *Peak RV TKE*. The severity of PR has previously been related to an increase of the RV extracellular volume, synonymous with increased levels of diffuse RV myocardial fibrosis (164).

Figure 22. Flow chart of the hypothesized pathophysiologic mechanisms of right ventricular (RV) remodeling as a result of pulmonary regurgitation (PR) in adults with repaired Tetralogy of Fallot (rToF). PR produces disturbed flow, that besides volume overload, through mechanotransduction may cause tissue damage such as endothelial dysfunction and fibrosis even outside the area of operation. This may in turn aggravate RV dilation. 4D flow specific TKE, a possible mediator of fibrotization may serve as a marker for risk stratification of rToF patients.

A recent study showed that RV fibrosis was associated to restrictive physiology, that has been proposed to protect the RV from dilation initially after repair (165). The patient cohort in that study was however substantially younger than in **Paper IV** (10.2 ± 2.6 vs 30 ± 8 and 36 ± 14 years, respectively) and, as the authors themselves discuss, the volume overload from PR is known to be well tolerated during adolescence. With time this initially protective fibrotization may instead lead to ventricular dilation and aneurysmal development. In line with this, Munkhammar et al. also found a positive correlation between PR severity, the degree of fibrosis, as well as larger RV volumes (165).

4D flow-specific TKE parameters could thus reflect different pathophysiologic mechanisms of late RV remodeling in patients with rToF, integrating not only the PR size but also aspects of mechanotransduction. The impact of fibrosis on late complications has been discussed (166), however, understanding of the mechanisms leading to dilation and fibrosis may be important for the early detection of rToF patients in need of PVR. 4D flow specific TKE may serve as a marker for risk stratification of these patients.

In **Paper I** and **II**, the occurrence of a diastolic vortex reaching into the RV outflow tract was discussed from an energy preservation point of view. In the presence of large regurgitant jet and development of turbulence stretching downwards into the central portions of the RV, the potentially energy conserving diastolic filling vortex was seen to be diminished or completely absent due to PR. This may in turn hinder the normal filling of the ventricle, further aggravating a diastolic dysfunction.

Methodological considerations

4D flow CMR is a powerful non-invasive, contrast-free technique that enables comprehensive visualization and quantification of blood flow inside the beating human heart. Blood flow specific markers of ventricular function, such as component volumetrics, KE and TKE were used in this thesis to characterize this intraventricular flow.

As with any other imaging modality, 4D flow CMR is naturally associated with limitations and methodological considerations. The inter- and intraobserver variability has however previously been assessed for blood flow component analysis with very good results (127, 167), as well as in **Paper IV** for the TKE analysis with similarly high levels of reproducibility.

In all papers included in this thesis, the morphologic images were obtained at end-expiration, and similarly, the 4D flow data were obtained using a navigator-gated pulse sequence that during free breathing only accepts data in the end-expiratory phase. Consequently, data are only representative for the end-expiratory volume and pressure situation. Since the RV operates at significantly lower pressures than the LV, the inflow and outflows naturally vary with respiration due to changes in intrathoracic pressures.

The present 4D flow findings relate only to subjects in supine position at rest and in sinus rhythm, without significant valvular disease. For the RV, it is reasonable to believe that the relative contribution from the two caval veins to the atrial inflow is different in the standing position from the supine position, which may influence RV flow patterns and energetics. Different flow patterns

would also be seen with varying heart rates and altered rhythm (such as atrial fibrillation) or valve disease. Ongoing studies on patients with dobutamine infusions (increasing inotropy) as well as on patients with atrial fibrillation are now evaluating the potential flow alterations occurring in these conditions.

Valvular disease was an exclusion criterion from the pathline analyses of **Papers I-III**, mainly due to technical aspects relating to segmentation of the ventricular chamber and the definition of the blood flow components. Since the *Direct flow* component was defined as the blood volume entering and exiting the ventricle during the analyzed heartbeat (based upon the most basal segmentation plane), mitral or tricuspid regurgitation would falsely increase the *Direct flow* component. Further development of the technique aims at defining dual basal planes, thus discerning the regurgitant volume from the real outflow volume.

Further, with atrioventricular valvular stenosis or aortic/pulmonary regurgitation, the increased blood velocities may induce phase wrapping and aliasing artifacts as the VENC generally is set for flow velocities inside the ventricles. Incorrect VENC is thus problematic, however phase unwrapping during post processing may correct these effects to some extent. Novel techniques employing dual-VENC may in the future reduce these issues further. Phase wraps do not affect the signal magnitude used for TKE analysis (150) and the technique can thus be used in patients with valvular regurgitation, as was done in **Paper IV**.

The blood flow of the single cardiac cycle reconstructed in 4D flow CMR represents an average of the main flow events reoccurring in the several hundred cardiac cycles it takes to acquire the data. Lesser beat-to-beat variations will thus be smoothed, reducing the ability of 4D flow CMR to assess very small or fast flow changes.

Patient movement during CMR scan may give rise to motion artifacts or data mismatch, especially problematic in some patients with cardiac disease that may have difficulties to fully comply during the CMR examination. This issue was addressed in **Papers I-III** by acquiring morphological data both before and after the 4D flow data, and in **Paper IV** by manual registration of the segmented volume to the 4D flow. Further, by application of the principle of conservation of mass, the ventricular inflow and outflow were compared in **Papers I-III** as a quality control of acquired data, with no significant differences. Registration algorithms might improve matching of the 4D flow data to the underlying morphological data and reduce in this type of error (168).

The accuracy of 4D flow data may further be degraded by eddy currents or concomitant gradient fields; the effects of these sources of error being minimized by tailored post-processing. Careful attention to the quality of the post-processed data was fundamental and included meticulous observation for evidence of aberrant pathlines that could indicate data imperfections.

Pathlines, representing the paths of virtual blood particles are computed by numerical integration over time, and are thus susceptible to noise, tending to accumulate more noise the longer they are traced. Tracing the pathlines from ED forwards and backwards in time reduces this error, as the tracing time is halved. TKE data may contain artifacts that may influence the measured values of especially *Peak RV TKE*, why median filtering of TKE was performed. For the integrative measurements, *Peak Total RV TKE* and *Average RV TKE*, occurrence of both negative and positive artifacts were considered to cancel each other out and no filtering was performed for the calculation

of these.

Due to the vast amounts of data, 4D flow acquisitions are associated with long scan times. During the years spanned by the papers included in this thesis, significant improvements on the method have been made, mainly reflected in the scan time. This improvement is evident, as scan times were reduced from ~30 min (including navigator efficiency) in **Paper I** to ~15 min in **Paper III**. Scan time is determined by not only the heart rate and respiratory gating efficiency, but also by the magnetic field strength and the acquisition sequences, where the spiral read-out used in **Paper IV** reduced the acquisition time more than two-fold with preserved data quality (169).

Clinical considerations

Assessment of cardiac function is a complex process involving understanding of both physiologic and pathophysiologic aspects of cardiovascular structures as well as the blood flowing through them. Cardiovascular disease is today an enormous health care burden, why early detection and treatment is of paramount importance.

Even so, many aspects of cardiac physiology have not yet been investigated, especially of the somewhat historically overlooked RV function. 4D flow CMR has the potential to significantly improve clinical assessment of cardiovascular disease by comprehensive visualization and quantification of intracardiac blood flow not achievable by conventional echocardiographic Doppler examinations.

The flow-based aspects of RV diastolic-systolic coupling found in **Paper I** and **II**, together with previous work on the LV, can provide novel useful insights into ventricular physiology as well as interventricular interaction in health and disease.

Ventricular remodeling and dysfunction are progressive. Detection at its earliest, asymptomatic stages is a clinical priority in order to reduce patient morbidity and mortality, especially since LV dysfunction can be present long before clinical manifestations. Both in **Paper II** and **III**, 4D flow CMR proved its ability to detect and identify subtle pathological changes early in the course of disease, where other modalities might oversee them. The 4D blood flow-specific measures *Direct flow* and *Direct flow* KE may thus clinically be used as markers of impaired cardiac function, and used in both diagnostics and classification as well as in monitoring of patients, much like echocardiographic EF is used today. The progressive changes in the amounts and proportions of ejecting and non-ejecting volumes and energetics may be able to predict progression or define clinically relevant thresholds along the transition to more severe remodeling and eventual failure, providing possibilities of earlier and more optimized treatment, in turn leading to improved quality of life in these patients.

In **Paper IV**, the 4D flow CMR application was further extended to an important group of patients with congenital heart disease. This growing patient group present clinicians with novel, even more complex challenges associated with late post-surgical complications. The measures of intra-ventricular TKE propose novel hemodynamic aspects of pathophysiological mechanisms of PR after ToF repair, and have the potential to serve as markers for risk stratification of these patients and aid cardiologists in the decision-making before ventricular failure or irreversible remodeling

occurs.

Further, in these patients the unique possibilities for visualization of intracardiac blood flow and turbulence intensity provided by 4D flow CMR may improve pre- and post-surgical assessment. By visualizing the multidimensional blood flow patterns pre-operatively, surgeons will be able to study complex aberrant flow patterns, e.g. shunt flows and better plan procedures. Post-operatively, 4D flow CMR may give the opportunity to study the hemodynamic effects of different surgical approaches.

The semiautomatic analysis of 4D CMR velocity data thus allows fairly user-friendly analysis and visualization of distinct functional flow components as well as TKE inside the beating heart, making the technique feasible for clinical assessment of individual cases. However, with development of fully automated segmentation (170) and even faster acquisition sequences, the analysis can be sped up and the reproducibility improved, facilitating transition of the 4D flow CMR technique into every-day clinical use.

Conclusions

- Semiautomatic analysis of 4D flow CMR data allows visualization and quantification of distinct functional flow components in the ventricle of normal and diseased hearts (**Papers I-III**) as well as of TKE in the RV of patients with rToF and PR (**Paper IV**).
- Diastolic flow through the normal ventricles appears to create favorable conditions for efficient systolic ejection for the inflowing blood passing directly to outflow (*Direct flow*) (**Paper I**).
- Progressive shifts in the proportions of LV and also RV *Direct flow* and *Non-ejecting flow* components occur across the spectrum from normal to mildly remodeled LVs (**Paper III** and **II**).
- 4D flow specific markers could detect both subtle RV and LV impairment even in early stages of LV disease (**Papers II** and **III**).
- 4D flow specific TKE markers related slightly stronger to RV remodeling than conventional PR parameters and propose novel hemodynamic aspects of PR in rToF patients (**Paper IV**).
- 4D flow specific markers have the potential to add to the understanding of cardiac physiology and pathophysiology as well as to optimized and individualized treatment of patients with heart disease.

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