Linköping University Medical Dissertations No.1201

Between the Probe and the Pump

An experimental study on cardiac performance analysis based on Echocardiography, tissue and laser Doppler.

LAILA HÜBBERT



Linköping University FACULTY OF HEALTH SCIENCES

Division of Cardiovascular Medicine Department of Medical and Health Sciences Linköping University, Sweden

LINKÖPING 2010

Between the probe and the pump

An experimental study on cardiac performance analysis based on echocardiography, tissue and laser Doppler

Linköping University medical dissertations, No. 1201

Cover picture: Munters Stina, Dala-Järna

© Laila Hübbert, 2010 unless otherwise stated.

Published articles have been reprinted with the permission of the copyright holder.

Printed in Sweden by LiU-Tryck Linköping, Sweden 2010

ISBN: 978-91-7393-327-8 ISSN: 0345-0082

Till Elias, mitt hjärtas melodi



Everything passes Everything changes Just do what you think you should do

B.Dylan

Contents

Abstract		3
Samman	fattning på svenska	4
List of pa	apers	7
Abbrevia	itions	8
Introduc	tion	11
	The Heart	11
	Heart Failure	13
	Diagnostics in Heart Failure	15
	Laser Doppler	18
	Treatment of Heart Failure	19
	The animal model for cardiovascular research	22
Aims		23
Material	and Methods	25
	The animal model	25
	Echocardiography Ultrasound and Doppler	27
	Tissue Doppler Imaging	29
	The laser Doppler perfusion monitoring system	33
	Left ventricular assist device	34
	Statistical analyses	34
Summar	y of results	35
	Echocardiography and myocardial Doppler indices in the anesthetised calf	35
	Laser Doppler perfusion monitoring and tissue Doppler imaging	36
	HeartMate II TM treatment during myocardial depression	38
	Second Harmonic Imaging and Spontaneous Contrast	39
Discussio)n	41
	The animal model	41
	Echocardiography, with tissue Doppler Imaging	43
	Laser Doppler perfusion monitoring, clinical applications	46
	Reproducibility	46
	Limitation	47
Conclusi	ons	48
Acknowl	edgements	49
Referenc	es	5(
Papers I-	IV	57

Abstract

Echocardiography is an ultrasound-based bedside, non-invasive and easily available cardiac diagnostic technique visualising the heart's morphology and function. Quantification of cardiac wall motion can be measured with the tissue Doppler Imaging (TDI) modality which provides in humans a high diagnostic capacity to differentiate healthy from diseased myocardium with reduced function.

Heart failure, as a consequence of, for example, myocardial or ischaemic heart disease, demands both bedside and intraoperative diagnostic procedures for myocardial functional and perfusion assessment. In the late stages of heart failure cardiac left ventricular assist devices (LVAD) may be the treatment of choice. Such new technologies are commonly evaluated in large animals before application in humans is accepted.

With the aim of evaluating TDI's applicability and feasibility in a large animal model 21 calves (aged 3 months and weight around 70 kg), were studied with colour TDI (*Paper I*). Analysis was performed either during coronary artery occlusion when the laser Doppler perfusion imaging technique (LDMP) was refined (*Paper II*), or after implantation of the LVAD, Heart Mate II® (*Papers III, IV*). All animals were haemodynamically monitored (pressures, flows, heart rate) and ECG was continuously recorded. Transthoracic and epicardial echocardiography (TTE) were performed before and after sternotomy and intraoperatively during experimental progressive heart failure. Heart chamber dimensions, native stroke volume, systolic and diastolic regional basal myocardial peak velocities (cm/s; systolic S', early diastolic E', and atrial A', strain (%), strain rate (s⁻¹) and displacement (mm) were determined. Second harmonic imaging (SHI) was applied in order to better visualise air bubbles (*Paper IV*).

In *Paper I* compiled baseline values were established before and after sternotomy for central haemodynamic and echocardiographic parameters, including the TDI myocardial motion variables velocity, strain rate, strain and displacement. Blood pressure and heart rate changed significantly after sternotomy, but the TDI derived data did not change significantly. In *Paper II* we report that movement artefacts of the laser Doppler myocardial perfusion measurements can be reduced, both when myocardium is normally perfused and during coronary occlusion, by using the TDI velocity registrations showing wall motion to be minimal. The optimum interval depends on the application but late systoles as well as late diastole are preferred.

After LVAD implantation in *Paper III* the flow characteristics and myocardial motion during variations in afterload TDI show that myocardial velocities decrease concomitantly with myocardial depression and are significantly correlated to native stroke volume, heart rate, systemic arterial resistance and cardiac output, but not with left ventricular size, fractional shortening or pump speed. Echocardiography together with TDI thereby offers additional means for monitoring and quantifying residual myocardial function during LVAD treatment. SHI is superior in the early detection of single air-bubbles in the ascending aorta prior to significant air embolism during manipulation of the LVAD pump speed, as shown in *Paper IV*. A prompt decrease in size of the left atrium during speed adjustment may be a warning that massive air embolism is imminent whereas the commonly used left atrial pressure not provide the same warning.

Sammanfattning på svenska.

Ekokardiografi (eko) är en icke-invasiv och mobil ultraljudsundersökning av hjärtat. Med denna lättillgängliga teknik kan man visualisera hjärtats anatomi och funktion. Kvantifiering av hjärtmsuskelväggens hastigheter kan utföras med hjälp av vävnads Doppler (TDI) som erbjuder en teknik att identifiera regionala väggrörelsestörningar i hjärtat. Hjärtsvikt som kan vara en följd av hjärtmuskelsjukdom eller t.ex. hjärtinfarkt, kräver en undersökningsmetod som är mobil och patientnära och kan användas på kateter-lab eller i operations sal. I ett sent skede av hjärtsvikt sjukdomen kan behandling med mekanisk hjärtpump (LVAD) vara aktuell.

Ny teknik som t.ex. LVAD bör utprovas i djurförsök innan de kan användas som behandlingsalternativ för patienter. Kalvar och andra större djur används oftast i medicinsk kardiovaskulär forskning eftersom djurens hjärta och kranskärl liksom de stora kärlen liknar människans. Detta gör kalvmodellen mycket användbar vid försök med bl.a. LVAD, mekaniska klaffar och pacemakers.

Totalt har i *arbetena I-IV* studerats sammanlagt 21 kalvar (3 mån gamla med en vikt av ca 70kg), djuren sövdes med barbiturater i en väl utprovad djurmodell.

I studierna har vi använt oss av olika tekniker som eko med vävnads Doppler (TDI), strain rate imaging (SRI) och second harmonic imaging (SHI). I samtliga försök utfördes ekot med Vingmed GE System 5 alt 7 med tillhörande 2.5 MHz prob och eko mätningarna utfördes enl. standard vyer tidigare framtagna för undersökning av människa.

Ny Laser Doppler teknik (LDMP) utvärderades och en då ny mekanisk hjärtpump, HeartMate II[™] (HMII) utprovades. Ekg registrerades för att övervaka hjärtfrekvens och rytm. Invasiva hemodynamiska mätningar utfördes i samtliga försök och med katetrar i carotis artären och lungartären mättes tryck samt on-line-övervakning av hjärtminutvolym (CO). Trycken i vänster förmak (LA) och vänster kammare (LV) mättes invasivt med katetrar. Systemisk vaskulär resistans (SVR) och pulmonell vaskulär resistans (PVR) beräknades utifrån registrerade tryck och flöden. Med blodflödes Doppler mättes hastighetsintegralen under aortaklaffen och med hjälp av den räknades den egna slagvolymen (SV) fram. Med vävnads Doppler bestämdes LV och högerkammarens(RV) basala väggrörlighet (hastighet, strain, strain rate (SR) och displacement (DI))

Arbete I: Alla 21 djuren studerades i syfte att utvärdera TDIs tillämplighet vid kirurgi och för att identifiera ev. förändringar i TDI parametrar när bröstkorgen öppnats. Samtidigt med eko utfördes hemodynamiska mätningar. Peak systolisk (S[^]), tidigdiastolisk (E[^]) och förmakskontraktionens (A[^]) vägghastighet analyserades samt s även strain, SR och DI. Studien visar att ekokardiografi inklusive TDI är tillämpligt och möjligt att använda i djurmodell. Basala kammar TDI värden påverkas inte signifikant av att bröstkorgen öppnas.

Arbete II. TDI användes för att identifiera de intervall i Ekg som är lämpliga att använda vid mätning av hjärtmuskelns genomblödning med ny LDPM teknik. Syftet var att utvärdera om man med hjälp av synkroniserade LDPM, Ekg och TDI kunde få fram en LDPM signal med minskat brus, och i och med det en signal som bättre representerade hjärtmuskelns genomblödning. Vi visade att rörelse artefakter vid mätning med LDPM kan

minskas genom att använda TDI och Ekg i metodutvecklingen. Det optimala intervallet i Ekg cykeln beror på applikationen men intervall sent i systole och sent i diastole kan användas.

Arbete III: Eko med TDI användes här för att studera kammarfunktion och hemodynamik efter implantation av en då ny hjärtpump, HMII och studier gjordes när pumphastighet och de hemodynamiska förutsättningarna varierades: SVR justerades med kärlaktiva mediciner och en tilltagande hjärtmuskel-depression utlöstes av ökande dos β -blockad. Under variation av SVR noterades att TDI hastigheter minskar med ökad hjärtmuskeldepression och är signifikant korrelerade med SV, hjärtfrekvens, SVR och CO men inte med vänster kammarens storlek, fraktions förkortning eller med HMII varvtal. Eko med TDI föreslås därför som ytterligare ett verktyg för övervakning och kvantifiering av hjärtfunktionen under HMII kirurgi.

Arbete IV: Förekomst av luftbubblor i blodbanan (luft-embolier) kan inträffa om blodfyllnaden av vänsterkammaren blir otillräcklig under varvtalsinställning efter LVAD implantation och luftbubblorna kan vara ett resultat av luft läckage i anslutningarna mellan pump och hjärta. Tidig misstanke och upptäckt av enstaka luftbubblor kan leda till justeringar som gör att negativa cirkulatoriska effekter eller organpåverkan av luftembolier undviks. Vi visar att man med SHI tidigt kan identifiera luftbubblor i samband med implantationen av HMII och att en hastig minskning av LA storlek kan vara en tidig varning om att massiv luftemboli är nära förestående.

Studierna visar att tillägg med eko inklusive TDI i djurmodellen kan bidra till den perioperativa bedömningen av hjärtat vid utvärdering av ny medicinsk teknik.

List of papers

Paper I. Echocardiography and myocardial Doppler indices in the anesthetized calf. A closed and open chest study. Hübbert L, Peterzén B, Ahn H, Lönn U, Janerot-Sjöberg B. Manuscript.

Paper II. Correlation between laser Doppler perfusion monitoring and myocardial tissue Doppler echocardiography in the beating heart. Karlsson M.G.D, Hübbert L, Casimir-Ahn H, Lönn U, Janerot-Sjöberg B, Wårdell K. Med.Biol.Eng.Comput. 2004, 42,770-776

Paper III. Axial flow pump treatment during myocardial depression in calf. An invasive hemodynamic and echocardiographic tissue Doppler study Hubbert L, Peterzén B, Traff S, Janerot-Sjoberg B, Ahn H. ASAIO J. 2008 Jul-Aug;54(4):367-71

Paper IV. Second Harmonic Echocardiography and Spontaneous Contrast during Implantation of a Left Ventricular Assist Device. Hubbert L, Peterzén B, Ahn H, Janerot-Sjoberg B. ASAIO J 2010 Sep;56(5):417-21.

Abbreviations

1D	One-dimensional
2D	Two-dimensional
A´	Tissue Doppler peak late (atrial) diastolic velocity
BNP	Brain natriuretic peptides
BP	Blood pressure
BSA	Body surface area
CABG	Coronary artery bypass grafting
CI	Cardiac index
CO	Cardiac output
CRT	Cardiac resynchronization therapy
СТ	Computed tomography
CVP	Central venous pressure
DI	Displacement imaging
E´	Tissue Doppler peak early diastolic velocity
ECG	Electrocardiogram
FAC%	Fractional area change
HMII	HeartMate II TM
ICD	Implantable cardioverter defibrillator
HR	Heart rate
IHD	Ischaemic heart disease
IVSd	Interventricular septal diastolic thickness
LA	Left atrium
LAD	Left anterior descending coronary artery
LADs	Left atrial dimension in systole
LAP	Left atrial pressure
LDPM	Laser Doppler perfusion monitoring
LV	Left ventricle
LVAD	Left ventricular assist device
LVDd	Left ventricular end-diastolic diameter
LVDs	Left ventricular end-systolic diameter
LVED area	Left ventricular end-diastolic area
LVEF	Left ventricular ejection fraction
LVES area	Left ventricular end-systolic area
LVFS	Left ventricular fractional shortening;
LVPd	Left ventricular posterior wall diastolic thickness
MAP	Mean arterial pressure
MRI	Magnetic resonance imaging,
PAPm,	Mean pulmonary artery pressure
PCI	Percutanous coronary intervention
PCWP	Pulmonary capillary wedge pressure
PVR	Pulmonary vascular resistance, calculated
PWd	Left ventricular posterior wall diastolic thickness
RV	Right ventricle

RVDd	Right ventricular end-diastolic diameter
S´	Tissue Doppler peak systolic velocity
SHI	Second harmonic imaging
SR	Strain rate
SRI	Strain rate imaging
SV	Native stroke volume
SVR	Systemic vascular resistance, calculated
TD	Tissue Doppler
TDI	Tissue Doppler Imaging
TEE	Transoesophageal echocardiography
TTE	Transthoracic echocardiography
TVI	Tissue velocity imaging
VAD	Ventricular assist device
VO_2	Oxygen uptake, V: volume per unit time, O ₂ : oxygen
VTI	Velocity time integral

Introduction.

The Heart

The human heart is a hard-working muscle that even at rest beats about 3600 times and delivers about 300 litres of blood every hour. For the heart's own oxygen and nutritional needs, about 18 litres of blood per hour (5%) pass through the coronary arteries and the heart muscle. The heart has a fantastic adaptability to different conditions with a large reserve capacity of sudden or gradually increasing demands. Together with the blood vessels throughout the body it forms the cardiovascular system (Fig 1).

One of the most striking features of the cardiovascular system is it's dynamism and thereby it's ability to mediate extremes.



Figure 1. The cardiovascular system. In blue; deoxygenated blood from the body through the right side of the heart and into the lung circuit. In red; oxygenated blood from the lungs through the left side of the heart and into the systemic circuit.

The heart consists of two adjacent pumping systems: the right and the left side. Each half of the heart has an inflow part, the atrium and an expulsion part, the ventricle. Between the atria and ventricles and between the ventricles and the great vessels are directional valves. The left and right cardiac chambers are separated by septa and normally there is no blood flow between the left and right side. The heart acts as a pump when the heart muscle contracts around its cavities, the heart's electrical system producing synchronous contraction of the right and left sides of the heart. Even though contraction are synchronous the right and left sides work under different conditions, the right side supplying the low pressure lung circulation system and the left side the high pressure system throughout the rest of the body.

The heart's own blood supply is maintained via the coronary arteries which have the origins in the aortic root. The left and right coronary arteries branch into arteriole and capillaries, and blood from the coronary arteries which has passed the myocardium returns to the venous system via the myocardial veins, and the large venous sinus coronarius finally empties into the right atrium.

The myocardium is mostly composed of three layers; the endocardium which is the inner surface and the epicardium, the outer surface of the myocardium and a mid myocardial layer.¹ In the surface layers the muscle fibres run mostly longitudinal in opposite directions; right handed in the subendocardium (Fig 2; 1, subendocardial fibres) and left handed near the epicardium (Fig 2; 5, subepicardial fibres). In the mid myocardial layer between the endocardium and the epicardium the muscle fibres run in a more circumferential manner (Fig 2; 4, circumferential fibres). The dominant motion of the healthy myocardium during contraction is longitudinal and since the myocardium is incompressible the volume of the myocardium remains constant i.e. when the ventricle shortens the wall thickens.²



Figure 2. The helical structure of the heart. 1, subendocardial fibres.; 2, papillary muscle; 3, vortex cordis; 4, circumferential fibres; 5, subepicardial fibres. From Craig Holdrege, The dynamic heart and circulation.¹ with permission

Heart Failure

Heart failure is a common condition that results in shortness of breath at rest or during exertion, fatigue and signs of fluid retention. These symptoms of heart failure are presented in combination with objective evidence of a structural or functional abnormality of the heart. Various epidemiological studies have shown that about 2-3% of the population have heart failure and the incidence rises rapidly after about the age of 70 with a prevalence of approximately 10% of the population at the age of 70-80. In the elderly the prevalence is equal between the sexes and the overall prevalence of heart failure is increasing because of the ageing of the population.³ The heart sometimes fails even at a younger age, most commonly in men who generally experience an earlier onset of ischaemic heart disease (IHD) compared to women.

Heart failure may be due to a disease of the heart muscle itself, cardiomyopathy. The most prominent cause is inadequate blood supply to the heart muscle which is the case in chronic or acute IHD. In a large group of heart failure patients tachyarrhythmia such as atrial fibrillation is present. Heart failure may also occur in patients with valvular heart disease or defects, and congenital heart disease.

In systemic hypertension the resistance in the vascular system is high for the left side and heart failure may occur as a consequence of or due to improper, extreme or failed myocardial hypertrophy. Right heart failure may develop because of a lung or pulmonary vascular disease such as pulmonary artery hypertension, which increases the pulmonary vascular resistance and right heart workload with consequences similar to systemic hypertension. Heart failure may also result from cardio-toxic effects, as in alcohol-induced cardiomyopathy, or as a result of chemotherapy. Cardiac function may also deteriorate due to endocrine pathology such as thyroid disease or diabetes.³

Whatever the major cause of heart failure is, a remodelling process is initiated. The process is mediated by activation of a neurohormonal mechanism, the renin-angiotensin-aldosterone system (RAAS). Antidiuretic hormone, endothelin, atrial natriuretic hormone, brain natriuretic peptide (BNP) and nitric oxide are also involved. The degree of activation of the RAAS system has been shown to be related to the severity of left ventricular dysfunction. Pathological cardiac remodelling with increased fibrosis in the myocardium develops, heart size increases and geometry and volumes change. This leads to a vicious cycle and causes a progressive decline in cardiac function.⁴

Heart function is also influenced by various haemodynamic variables such as heart rate and contractility, filling of the heart (preload) and the workload the two ventricles have to perform to generate enough pressure and flow when ejecting blood (afterload).

Acute Heart Failure.

Acute heart failure is defined as a rapid onset or change in the signs and symptoms of heart failure. Acute heart failure can either be new heart failure or worsening of a pre-existing chronic heart failure (fig 3). The clinical presentation of acute heart failure shows a variety of symptoms but pulmonary oedema with respiratory distress and low O_2 saturation is commonplace. Cardiogenic chock is a severe form of acute heart failure defined as evidence of tissue hypoperfusion induced by heart failure, where hypoperfusion and pulmonary congestion develop rapidly.³

Cardiogenic chock is typically characterised by reduced systolic blood pressure and absent or low urine output resulting from hypoperfusion of the kidneys. Hypoperfusion of other vital organs may also be present.



- 1. Initial symptoms of heart failure.
- 2. On treatment, stable period of weeks or years.
- 3. Acute on chronic heart failure, number and frequency of attacks individual.
- 4. Time for transplantation or ventricular assist device.
- 5. Palliation

Figure 3. The natural course of heart failure. From Funktion och Livskvaliet, Wickström och Wallström, with permission

Diagnostics in Heart Failure.

Diagnosis of heart failure requires a thorough medical history that may provide guidance as to the underlying cause and simplify the selection of inquiries. At the physical examination auscultation of the heart is important: e.g. a valvular disease may become apparent, an audible third heart sound indicates high filling pressures and an arrhythmia with irregular heart sounds may be disclosed. When lung auscultation is performed rales from congestion, pneumonia or other lung diseases may be heard. Laboratory findings may contribute to the differential diagnosis of diseases with symptoms similar to heart failure and various biomarkers such as troponins and BNP may turn focus towards more specific heart disease. An electrocardiogram (ECG) is mandatory and may show heart diseases such as IHD or arrhythmias.

The ergonometric exercise test combined with ECG and blood pressure recordings is a commonly applied test to determine the reserve capacity cardiac or non-cardiac functions, and is a useful diagnostic test in most cardiac diseases, especially IHD. By the addition of peak oxygen consumption (VO₂) to a maximal cardiopulmonary exercise test, deterioration of heart failure may be monitored, thereby serving as a major decision-making tool for when declining, for example, the optimal timing of planned heart transplantation.⁵

Non-bedside Diagnostic Imaging Techniques in Heart Failure.

Myocardial scintigraphy, i.e. myocardial perfusion imaging accomplished by intravenous isotopes and a gamma camera, is primarily used as a diagnostic tool for IHD when exercise ECG is inconclusive, for evaluation of suspected coronary re-stenosis after intervention and may help in the differential diagnoses of infarction or stunned myocardium, as both perfusion and functional parameters can be displayed. It's high negative predictive value in IHD makes the test unique.

Chest X-Ray examination may reveal congestion of the lung or heart enlargement indicating heart failure.

A computed tomography (CT) scan anatomically maps the cardiovascular system in 3 dimensions if used together with an intravenously contrast agent. The technique, however, is rapidly evolving with decline in radiation exposure, and increases in time and spatial resolution. A CT may disclose, for example thromboembolic disease and can be used in ischaemia diagnosis with recognition of calcification of the coronary arteries. Quantification of coronary artery stenosis and perfusion tools still under evaluation.

Magnetic resonance imaging (MRI) is an imaging modality which can provide data about anatomy, flow and motion in the cardiovascular system. Even here intravenous contrast agents are potentially dangerous but are mandatory and time-resolution is still low. The high magnetic forces applied contraindicates it's use in patients with mechanical devices.

Of these above mentioned techniques only flat-screen X-ray is available at the bedside. They are therefore inconvenient in critically ill patients.⁵

Heart Catheterisation

Heart catheterisation is a partly bedside invasive technique for the estimation of cardiac filling pressures, systemic and pulmonary blood pressure and resistances as well as for flow conditions in the cardiovascular system. With catheterisation it is possible to obtain local cardiac and vascular blood gas values and from these to evaluate cardiac and pulmonary performance or detect cardiac shunting.

It is used as a diagnostic tool for certain cardiac diseases but is also a valuable monitoring tool in the severely ill heart failure patient.

Heart catheterisation is commonly used together with X-ray for catheter localisation and positioning. Together with a locally distributed contrast agent e.g. shunts, regurgitation and stenosis as in the coronary arteries can be visualised.⁵

Echocardiography

In 1954 the cardiologist Inge Edler and physicist Hellmuth Hertz at Lund University, introduced cardiac ultrasonography.^{6,7} Since then the technique has developed at a furious pace and many leading scientists and clinicians have been involved in this successful development.

The continuous development of echocardiography and improvement in performance has made a large impact in the treatment and care of patients with heart failure and other cardiac conditions.



Figure 4. Left panel: Inge Edler and Hellmuth Hertz, 1954. Right panel; The very first recording of ultrasound echo from the heart, 1953. Pictures from Håkan Westling, Lund University, with permission.

Echocardiography is a bedside technique for examination of the heart based on the combination of imaging of the heart and vessels (ultrasound) together with the possibility of measuring velocities and directions of blood flow and tissue movements in the cardiovascular system (Doppler).

Echocardiography is a convenient and bedside examination with no known adverse biological effects.

Despite the fact that it is demanding technique with a significant learning curve it has become the most common examination of the cardiovascular system and is the most frequently used method for assessing cardiac size and function. The procedure is highly standardised and described in established guidelines.⁸⁻¹⁰



Figure 5. Echocardiographs used in the studies; left panel, Vingmed GE Vivid 5, right panel Vingmed GE Vivid 7.

For the echocardiographer it is important to be familiar with the structure-functional relationship of the cardiac contraction to understand the data provided. In order to understand the haemodynamic effects of disease or interventions it is crucial to determine the effects on global or regional myocardial function and echocardiography can be used for decision-making with a high degree of accuracy in a variety of clinical settings.

The time needed to perform an examination depends on the specific situation; from a few minutes in a critically ill patient to hours when mapping valvular disease or congenital heart disease.

Echocardiography can be used during a variety of cardiac surgery procedures since the machine is convenient to use also in the operating room.

More about echocardiography is presented in the Methods section.

Laser Doppler

The ultrasound Doppler technique is easy to work with if there are many red blood cells moving in the same direction as in a large vessel or even when filtering the signal to analyse movements of the hearts tissue. At the capillary level, however, were red blood cells are very few and moving in many directions, the signal is difficult to analyze. In these conditions laser Doppler is superior to ultrasound Doppler and analysis is based on the alteration of the signal presented representing a change in perfusion and not a change in velocity or direction.

Laser Doppler perfusion monitoring (LDPM) is a technique were a fibre-optic probe is placed in contact with the measurement site for estimating blood perfusion in tissue, based on the detection of backscattered Doppler shifted laser light, where the Doppler shifts are generated by the movement of red blood cells.¹¹ However, LDPM is also sensitive to other movements. If the tissue is moving relative to the probe, movement artefacts may arise in the perfusion estimate thus overestimating the perfusion.

Treatment of Heart failure

In order to improve the performance of a failing heart, drug therapy and sometimes surgery are used. In selected cases with advanced disease, a temporary or permanent ventricular assist device (VAD) and finally heart transplantation may be the therapies of choice.

Pharmacologic therapy.

Large international studies has been performed on the medical treatment of heart failure and these form the basis of excellent guidelines supporting decision-making so that patients receive adequate and safe treatment for their heart failure.³ The major targets for drug therapy are the neurohormonal systems mentioned above.

Treatment of acute heart failure or cardiogenic chock:

Therapy number one in acute heart failure is to treat the underlying heart disease. The main goal of treatment of acute severe heart failure is to:

- Reduce the symptoms and restore oxygenation
- Increase cardiac output and organ perfusion, and reduce the filling pressure
- Limit cardiac, renal and other vital organ damage
- Stabilise and improve the haemodynamic state

To achieve these goals, acute heart failure has to be considered a condition with "volume, flow, pressure and resistance" problems. Flow is cardiac output (CO = stroke volume (SV) x heart rate (HR), L/min), the systemic (SVR) and pulmonary vascular resistance (PVR) are governed by dilation or contraction of vessels, and blood pressure (BP) may be regarded as a combination of the effects of flow and resistance (heart and vessels), i.e. BP = Flow x Resistance.

Vasodilators are used for adjustment of CO, SVR and BP through venous and/or arterial vasodilatation in order to achieve reductions in preload and afterload or balance between the two. In cardiogenic chock inotropic agents are used to increase CO and reduce hypoperfusion and congestion. This might stabilise patient at risk for haemodynamic collapse, or serve as a life-saving bridge to more efficient circulatory support with a VAD. A short time VAD can be used to win time as a bridge to decision-making, to recovery or to long-term left ventricular assist device (LVAD) treatment, with or without intended heart transplantation.

A pulmonary artery catheter used in the diagnosis of acute heart failure is not always necessary but may be useful for more extensive monitoring of haemodynamically unstable patients who are not responding to treatment.³

Revascularisation and surgery:

In acute or chronic IHD, specific medication as well as revascularisation improving the blood supply to the heart muscle are essential to reduce symptoms and to avoid or reduce the risk for heart failure. Heart failure in myocardial infarction is usually due to myocardial damage but can also be the consequence of arrhythmias or mechanical damage to the heart such as mitral regurgitation or ventricular septum defect.¹² Revascularisation can be achieved with percutaneous angioplasty (PCI) or coronary artery bypass surgery (CABG), the preferred approach depending on the patient's condition and co-morbidity as well as on the extent and severity of the coronary disease identified by coronary angiography.¹³ Cardiac surgery can also be the treatment of choice for patients with heart failure and cardiac valve disease¹⁴ or patients with congenital heart diseases.

Cardiac resynchronisation therapy

For patients with drug-refractory heart failure and who fulfil certain criteria, a cardiac resynchronisation therapy (CRT) and/or an implantable cardioverter defibrillator (ICD) has been proven beneficial. CRT is a pacemaker that influences myocardial timing and mechanical function of the heart chambers, where optimal timing of atrial systole is linked to an increase in CO.¹⁵

Left ventricular assist devices

LVAD are used as a bridge to heart transplantation or in narrowly selected cases as a longterm palliative device as an alternative to heart transplantation.¹⁶⁻¹⁹ Worldwide over 4000 patients with heart failure have been treated with the most common LVAD, HeartMate II^{TM} (HMII) (fig.6), the longest treatment period being more than 5 years.

LVAD technology is continually improving but there is still a paucity of randomised clinical trials in this patient population due to the nature of the disease, and there is no consensus concerning LVAD indications or selection of patients

Improved technology results in a decrease of adverse events such as infection, sepsis and right heart failure, as well as shorter hospital stays with favourable impact both on patient quality-of-life and treatment costs.²⁰

As a bridge to transplantation, LVAD is today recommended when heart failure deteriorates and the patient is deemed not to last the time to cardiac transplantation. A patient receiving an LVAD pending heart transplantation has lower creatinine and total bilirubin levels after two to four weeks of mechanical support, indicating improved organ perfusion. The lowest risk exists between 1 and 3 months after implantation.

One-year survival among patients supported with a LVAD for more than 30 days before transplantation is high (91.4%).²¹ Patients with the new continuous-flow device had superior survival rates at 2 years 58% vs. 24% for the older pulsative long-term LVAD.²⁰

Perioperative echocardiography for evaluating the native heart and LVAD function is often applied during implantation.^{22, 23}



Figure 6. *The HeartMate II™, LVAD. From Thoratec Corporation, with permission.*

Heart transplantation

Throughout the world about five thousand heart transplantations are performed every year. For selected patients with terminal heart disease and without adequate response to conventional medical and surgical treatment, it is the consensus that heart transplantation significantly increases survival, exercises capacity and quality of life.²⁴

The animal model for cardiovascular research.

Animal studies preferably precede the use, in human, of drugs or surgical interventions in the cardiovascular field. Elaboration of the animal model is not only important for the well-being of future patients, but also ethically correct. Animals used for this purpose must, therefore, be used in the most optimal way possible.

Larger animals are used for research in the field of myocardial ischaemia and device-testing before human implantation, and calves have been used in medical laboratories for at least 50 years.^{25, 26} The haemodynamic conditions and the rapid calcium turnover rate that occurs in calves makes this an ideal model in which to simulate the worst-case scenario for device testing.²⁷ The anatomy of the animals bovine heart and blood vessels is similar to humans which makes the animal suitable for the assessment of various cardiac devices such as ventricular assist devices, novel prosthetic heart valves as well as of other cardiovascular surgical interventions.^{28, 29} The coronary artery anatomy and coronary artery collaterals in the calf are similar to the human heart which is why the animal model has also been used in studies on coronary artery and capillary flow.^{30, 31}

Aims

The overall purpose of this thesis was to evaluate if echocardiography with TDI is applicable and feasible in the animal model during applications of new technology.

The specific aims of the four animal studies were:

- to establish normal baseline values before and after sternotomy for haemodynamic and echocardiographic parameters, including the myocardial motion variables velocity, strain rate, strain and displacement. (*Paper I*)
- to use echocardiography together with the tissue Doppler modality to detect momentary myocardial motion thereby enabling elimination of motion artefact of the developing laser Doppler perfusion signal. (*Paper II*)
- to evaluate flow characteristics and myocardial motion after implantation of a left ventricular assist device during variations of afterload and progressive myocardial depression. (*Paper III*)
- to investigate if the use of intra-operative echocardiographic second harmonic imaging to detect single air bubbles in the ascending aorta, and if this can be used as a warning to diminish the risk of air embolism during implantation of a left ventricular assist device. (*Paper IV*)

Materials and Methods

The animal model.

Twenty-one calves of the Swedish native breed age 3 months, mean body weight 72 (\pm 9) kg and mean body surface area calculated according to DuBois & DuBois³² of 1.8 (\pm 0.2) m² were studied. All the calves were delivered from the same local breeder. The Local Committee for Animal Research approved the studies, and the university veterinarian was involved in the animal settings and supervised all sessions. The studies were performed during five different sessions (weeks) (Table 1) of which two sessions had additional protocols not reported in Papers I-IV.

session	animals	paper I	paper II	paper III	paper IV
1	3	Х			
2	5	Х			
3	3	Х	Х		
4	5	Х		Х	Х
5	5	Х		Х	Х

Table 1. Table of all 5 sessions and the nr of animals studied in Papers I-IV

The calves were pre-medicated with xylazinehydrochloride (0.15mg/kg) and atropinesulphate (0.06 mg/kg). A central venous catheter was inserted into the external jugular vein for administration of fluids and medication. Pentobarbitalsodium (2mg/kg) was used for induction of anaesthesia, and a tracheotomy was carried out to allow mechanical ventilation (Servo ventilator 900, Siemens-Elema, Sweden). Anaesthesia was maintained with intravenous fentanyl (10 ug/kg/h) and pentobarbitalsodium (2mg/kg/h). Prior to sternotomy all animals received a beta-blocker (5mg metoprolol) to prevent tachycardia. The mean time between the measurements at closed and open chest was 1.2 (\pm 0.48) hours. Dextran 70 with potassium was infused continuously to prevent hypokalemia and to replace fluid loss. A three-lead electrocardiographic recording was used for monitoring of the heart rate and rhythm.

The arterial BP was continuously monitored via a catheter in the common carotid artery. A pulmonary artery catheter with a rapid response thermistor (Edwards Life sciences, Irwine, CA) was inserted via the jugular vein for on-line monitoring of CO, pulmonary artery peressure (PAP) and central venous pressure (CVP).

Left atrial (LA) and left ventricular (LV) catheters were inserted in order to allow invasive measurements of LA and LV pressures. SVR and PVR were calculated conventionally, $SVR = [((MAP-CVP)/CO) \times 80]$ dynes x sec x cm⁻⁵ $PVR = [((PAPm-PCV)/CO) \times 80]$ dynes x sec x cm⁻⁵

In order to prepare for left anterior descending coronary artery (LAD) occlusion measurements in the LDPM study (Paper II), the LAD was dissected free and retractor tapes (Quest Medical Inc., Allen, Texas) were positioned proximally around the LAD.

After the final measurement in the protocol, each animal was given an intravenous overdose of pentobarbitalsodium and cardiac arrest was guaranteed by a high dose of potassium.

Echocardiography, Ultrasound and Doppler

Ultrasound

Ultrasound is high frequency sound not perceived by the human ear. The frequencies of audible sound are 20-20000 Hz and the frequencies of ultrasound used in medicine are 1-100 MHz. Ultrasound penetrates the tissues, and the probe which generates sound waves also has a sensor to detect any waves reflected back (echoes) from interfaces between different tissues. Since the speed of sound through human soft tissue (1540 m/s) and time are known, it is possible to calculate the depth at which reflection takes place. While the probe transmits multiple pulse waves in different directions, the receiver listens to backscattered echoes which are then converted into digital signals and further analysed in the computer. An image of the heart is generated and visualised on the screen and saved as images in digital format.³³

Doppler

Spectral blood flow and tissue Doppler are ultrasound beams emitted and reflected back at a different frequency (shift) depending on the movement of the object being observed. This enables measurement of the speed and direction of the myocardium or blood flow in the cardiovascular system. Signals are presented as a time(x)-velocity(y) curve where positions above the zero line represent movement towards the probe, and those below, movement away from the probe. The velocities recorded are the velocities in the ultrasound beam direction (Fig 10).

Using a modified technique, Doppler velocity information can also be presented as colourcoded images with all pixels sampled simultaneously; red representing movement towards the probe and blue, movement away from the probe.³⁴

Intra operative echocardiographic monitoring.

Echocardiographic examinations were all performed using a clinical echocardiograph (Vingmed GE System 5 and 7, Vingmed GE Healthcare, Horten, Norway) with an adjustable 2.5 MHz transthoracic probe.

Transthoracic echocardiography (TTE) was performed with the animal in the supine position and the probe applied to the left of sternum in the 4th intercostal space in order to achieve a view corresponding to the parasternal view in humans and more apically for the apical 4chamber view. After sternotomy the probe was positioned on the pericardium of the heart in positions corresponding to apical and parasternal view in humans (fig 7).

Cine-loops of three cardiac cycles of TTE standard views⁹ of parasternal long and short axis and apical 2- and 4-chamber views and long axis view were recorded and stored in a central database for off-line post processing and analysis (TVI 6.0 and Echopac, GE Vingmed, Horten Norway).

Two-dimensional grey-scale imaging was gathered for parasternal LV end-diastolic (LVDd) and end-systolic diameters (LVDs), and for LV diastolic septal and posterior wall thicknesses. Right ventricular (RV) diastolic diameter (RVDd) was estimated in end-diastole from the apical 4-chamber view or left parasternal long axis view and measured according to guidelines for the human heart.^{9, 10} From the apical long axis view, sub-aortic diameter and spectral blood flow Doppler were recorded.

Figure 7. The TEE probe applied to the myocardium with a gel standoff. LVAD implanted in the LV. (Paper III)



Second harmonic imaging

Second harmonics are the tones that makes an A note from a guitar sounds different to an A note from a piano.

The pulse or tone emitted from the ultrasound probe, the "original tone", has certain amplitude and a narrow frequency band, called the "fundamental frequency". When receiving backscattered signals from the tissues or blood, a broader band high frequency pulse is detected. The frequency of the fundamental tone was, until recently, normally used for imaging, and for this purpose a filter has been used to separate the fundamental tone from the second harmonics regarded as disturbing noise of different amplitudes and frequencies. Second harmonics are multiples of the original frequency, and are present in all non-sinus signals. In Second harmonic imaging (SHI) these "disturbing" signals are analysed for further information. When the filter is adjusted to accommodate the harmonics a clearer and deeper view into the chest is obtained.³⁵

Pre-bubbled saline has been long been used as ultrasound contrast in the right heart for shunt detection and right ventricular delineation. The air bubbles are highly reflective and easily detected by ultrasound imaging techniques. It has recently been shown that small (2-4 microns) gas-filled micro-bubbles produce overtones due to pulse-provoked oscillations with resonant frequencies around those used for cardiac ultrasound (transmission around 2 MHz). Micro-bubbles are small enough to pass the lung circulation and are nowadays used as an ultrasound contrast agent for the left heart and arteries too.³⁶

Visualisation of contrast in the ascending aorta (Paper IV).

Second harmonic visualisation (SHI) of the ascending aorta was performed to detect single air bubbles indicating small air leakage. In order to induce conditions where air embolism may occur, the haemodynamic conditions were varied and the pump setting increased from low- to high-speed until the aortic cusps closed or the left atrium or ventricle collapsed. While pump speed and haemodynamic conditions were being altered, short axis LVDd, LADs were measured from the epicardial parasternal view at the same time as the left atrial pressure (LAP) and left ventricular pressure (LVP) were measured via catheters.

Tissue Doppler Imaging

When using tissue Doppler imaging (TDI), also called tissue velocity imaging (TVI), the velocity information is generated from the myocardium. A software filter extracts the low-velocity, high-amplitude signals from the tissue prior to registering signals from the blood cells. Echocardiographic TDI offers a method to quantitatively study the values of regional longitudinal and circumferential velocities of the myocardium in cm/s as time-velocity curves or as a colour-coded picture of the heart (Figs 9 and 10). The colour Doppler uses an auto correlation system which presents the velocity information in colours where red shows velocity of movement towards the transducer and blue the opposite. The colour-coded measurement is an average of a few ultrasound waves and the result is a mean velocity for a specific area and may not as spectral (pulse wave) Doppler show instantaneous peak velocities recorded in the ventricular base. Some movement in the base is an effect of contraction in the apex and thereby even passive wall segments show movement. TDI has become a useful non-invasive tool in the assessment of systolic and diastolic myocardial function.³⁷⁻⁴¹







Figure 10. A typical myocardial tissue velocity curve presented with an ECG. Systole in the picture gives the S' peak systolic velocity. Relaxation in the picture gives the E' peak early diastolic velocity. Atrial contraction in the picture gives the A' peak atrial diastolic velocity. In the figure, the IVRT; isovolumic relaxation time and the IVCT; isovolumic contraction time (not measured in this thesis) are also shown.

Displacement imaging, DI

Myocardial displacement is derived from the systolic integral (the area under the systolic velocity curve). This shows how far the wall moves during systole.⁴² If presented in real time colour-coded 2D display, it is often called "tissue tracking".

When colour-coded each colour represents a quantitative length interval and the width of each colour bar is therefore an indicator of regional strain.⁴³

Strain.

In echocardiography strain is used to describe deformation so that regional function of the myocardium, or the deformation indices, can also be visualised in real time as strain rate imaging (SRI). Strain describes deformation in % and is the tissue deformation resulting from an applied force. Positive strain represents stretching and negative strain shortening of the myocardium. Strain rate (SR) has the same direction as strain, and is deformation velocity per unit time unit during which strain takes place (s⁻¹).⁴⁴⁻⁴⁹

For a one-dimensional (1D) object the only strain is in length (L) and the degree of deformation can be described by the formula for strain (ϵ).

 $\epsilon = L - L_0 / L_0 = \Delta L / L_0$ were L is instantaneous length at the time of measurement and L_0 is original length.

When the length of the object is known at the time (t) of deformation the strain can be described as ε (t) = L (t) -L₀(t) / L₀(t).

In this way strain is expressed relative to the initial length (Lagrangian strain) i.e. if a segment of the myocardium shortens its length by 15% in systole the peak Lagrangian strain is -15%.

If the strain is expressed relative to the instantaneous length at an instantaneous time it is called natural (Eularian) strain. During Eularian strain the reference value changes during deformation and Eulerian strain can be assessed even if the original length is unknown. Myocardial segments stretch, shear and slide in many directions as the myocardial muscle movement is complex, but the TDI strain allows only 1D measurement.

A new technique, "speckle-tracking" allows deformation detection in more than one dimension though it analyses natural acoustic markers (speckles) in the myocardium identified in the ultrasonic image. Due to change in orientation of myocardial muscle fibres the ultrasound waves interact with them at differing angulations producing different backscatter intensities.^{48, 49}

If there is a normal gradient in myocardial velocity from the base to the apex of the heart there is a quite uniform distribution of SR within the ventricular myocardial walls. The regional strain curve is derived from the integral of the region under the strain rate (SR) curve.

- Myocardial wall motion: Velocity (cm/s) and Displacement (mm)
- Myocardial wall deformation: Strain Rate (s⁻¹) and Strain (%)

TDI wall motion velocity measurement cannot differentiate between active or passive movement of a myocardial wall segment. Deformation measurements as Strain and SR may allow improved discrimination between active or passive myocardial tissue movement.

Tissue Doppler imaging with strain and displacement imaging (Papers I-III) TDI was performed at 1.7 (3.5) MHz. The typical setting of the colour Doppler when used for velocity measurement was as small sector angle and depth as possible resulting in a mean frame rate of 142 fps (\pm 26). Special attention was paid to avoid aliasing within the image, and if that occurred the pulse repetition frequency was increased. The TDI measurements were performed when the lungs were deflated and without respiratory movements. The basal segment of the anterior, lateral, septal and posterior LV wall and the basal RV wall were analyzed using the default region of interest of around 5 mm. The parameters analysed were: peak S', E' and A' waves of the colour TDI-mode; strain and SR from SRI-mode; and maximal annular displacement from the DI-mode.

In *Paper III* the data were compared and related to invasive haemodynamic parameters. A multimodal approach with echocardiography and haemodynamic measurements was carried out on 13 occasions in each animal: during baseline conditions; during 3 different pump speeds, during normal and low systemic vascular resistance SVR; during increasing doses of the β -blocker esmolol; and finally during maximal pump speed before circulatory collapse.



Figure 8. Protocol for paper III. Measurements were performed on 13 occasions during the experiment with variation of haemodynamic conditions. The systemic vascular resistance, SVR was medically modified, and progressive myocardial depression was induced by increasing doses of β -blocker.

To estimate the native stroke volume (SV native i.e flow through the aortic valve) the subaortic systolic flow velocity time integral (VTI) was multiplied by the subvalvular area, assumed circular and calculated from the measured subvalvular diameter.⁵⁰

As a substitute for volumes and LV ejection fraction (LVEF) the LV end-systolic (LVES) and LV end-diastolic (LVED) areas were obtained by planimetry of the midventricular LV short axis and fractional area change (FAC%) was calculated as [(LVED_{area}-LVES_{area}/LVED_{area}) x 100]

The laser Doppler perfusion monitoring system (paper II)

A technique based on digital signal processing has shown it possible to correlate the output signal from a LDPM system to the cardiac cycle, using the ECG signal. By applying this ECG tracing technique, the LDPM signal can be studied at a specific point in time in the cardiac cycle where the tissue motion is minimal, thus offering the possibility to reduce the influence of movement artefacts.⁵¹ For the perfusion study in 3 calves, a specially designed small and lightweight intramuscular fibre optic probe (LD probe) was used to guide laser light (632.8 nm, 5mW) between the LDPM system and the myocardium. The probe was inserted 3-5 mm into the LV anterior myocardium in the area corresponded to the supply of the LAD. Simultaneous sampling of the ECG signal from the beating heart made it possible to correlate the signals to the cardiac cycle.

LDPM data were sampled and stored during normal conditions and at the end of LAD occlusion (caused by a retractor tape) at the same time as TDI data and ECG were collected and the signals of both Doppler modalities were correlated to the ECG.



Figure 11. The LDPM system for myocardial capillary perfusion measurements. *Picture from Daniel MG Karlsson.*

Measurements of LDPM were performed in several probe positions. The area of choice for TDI measurements were guided visually by the position of the LDPM probe and the probe was also visualised in the myocardium by the echocardiography.

The unprocessed LDPM signal and the total backscattered intensity were sampled and stored on a computer for off-line processing.

LDPM was then studied at a point in time in the cardiac cycle where the myocardial tissue velocity was minimal, identified by TDI with a flat velocity curve < 1cm/s during the cardiac cycle. To determine if ischaemia was induced by LAD occlusion, the peak velocity in systole S', which has been shown to decreases in ischaemic myocardium,⁵²was measured and compared with baseline. The average LDPM signal over a cardiac cycle was calculated from the last 15 heartbeats during LAD occlusion.

Left ventricular assist device (Papers III and IV)

The HeartMate IITM (HMII) is an axial flow pump using electrical power to rotate an impeller. The pump house weight is 375g with dimensions approx. 4 x 6cm. The hermetically sealed pump shell is constructed of titanium and contains a brushless electromagnetic direct-current field motor. The rotor, i.e. impeller is held in position within the centre of the housing by two ceramic bearings. The motor has the capacity to spin the rotor between 6,000 and 15,000 rpm. Inflow and outflow stators are constructed to give laminar blood flow through the LVAD.

The system also includes a sewing cuff, an outflow graft, a power cable for transcutaneous tunnelling, a controller, a system monitor, a power base unit and wearable batteries. The sewing ring mounted apically in the LV holds the inflow graft within the LV. The pump has no valves, and the blood exits the LV through the LVAD and flows through the graft and through an anastomosis into the ascending aorta. There is a bent relief outside the proximal part of the graft. To test the HMII function before implantation it is submerged in saline and is let to run for five minutes. After implantation and pump start, the pump may not be stopped because of risk for emboli. The pump speed can be manually adjusted via the system monitor

Statistical analyses.

All statistical analyses were performed using the commercially available software program, Statistica TM (StatSoft, version 7.0, Tulsa, OK, USA,). p < 0.05 was regarded as significant.

In *Paper I* Wilcoxon Matched Pairs non-parametric test is used for statistical analysis and the values are reported in median (range) and mean (SD).

In *Paper II* a statistical analysis was performed using MATLAB v. 6.1 (MathWorks Inc., USA) and StatisticaTM v. 6.0.

Due to a rather limited number of animals and observations non-parametric test were applied in *Papers III-IV* and median values (range) are reported.

In *Paper III*, after statistical advice, the variance-adjusted sum of squares (type III sum of square similar to R^2) was used to see how the TDI results were affected by the covariates.

Summary of results.

Echocardiography and myocardial Doppler indices in the anesthetised calf. (Paper I)

Both HR and MAP increased after sternotomy. A tendency towards increased CO related to increase in HR was recorded after sternotomy. Stroke volume (SV), mean pulmonary artery pressure (PAPm), CO, Cardiac index (CI), CVP, Pulmonary capillary wedge pressure (PCWP), SVR or PVR did not change significantly.

	Closed (n=13)		Open (n= 21)		Chee et al. ⁵³
	Median (range)	Mean (SD)	Median (range)	Mean (SD)	Closed (n=16) mean (SD)
Weight (kg)	70 (60-90)	72 (9)			106 (12)
BSA (m ²)	1.8 (1.5-1.8)	1.8 (0.2)			2.2 (0.7)
HR (bpm)	87 (55-120)	81 (14)	102 (59-162)*	104 (28)	65 (12)
MAP (mmHg)	97 (80-135)	106 (24)	115 (77-158)*	116 (25)	114 (17)
PAPm (mmHg)	25 (18-37)	27(6)	25 (13-55)	28 (13)	22 (8.3)
CO (L/min)	5.7 (4,2-10)	6.6 (2.1)	7.0 (3,7-11)	7.0 (1.9)	8.0 (1.9)
SV (ml)	82 (54-146)	92 (31)	69 (32-105)	67 (19)	na
CI (L/min/m ²)	3.2 (2,3-6,2)	3.6 (1.1)	3.9 (2.3-6.2)	3.8 (1.1)	3.6 (1.1)
SVR (dynes x sec x cm ⁻⁵)	1220 (972-1845)	1295 (482)	1244 (657-2395)	1355 (522)	na
CVP (mmHg)	12 (8-18)	13 (5.9)	10 (1-19)	12 (7)	9.4 (6.8)
PCWP (mmHg)	14 (11-20)	16 (4.8)	13 (2-24)	13 (6)	13 (3)
PVR (dynes x sec x cm ⁻⁵)	141 (74-266)	157 (67)	146 (66-471)	162 (95)	na
LVDd (cm)	4.4 (3.1-8.1)	4.7 1.5	5.0 (4.0-7.0)	5.2 (0.9)	5.6 (0.8)
LVDs (cm)	3.3 (2.2-4.4)	3.3 0.6	4.3 (2.4-5.4)	4.2 (0.8)	3.5 (0.7)
LVFS (%)	26 (10-50)	21 (14)	20 (4-45)	19 (11)	37 (10)
IVSd (cm)	1.4 (1.1-1.9)	1.5 (0.2)	1.4 (1.1-1.9)	1.4 (0.2)	1.2 (0.2)
PWd (cm)	1.5 (1.0-1.8)	1.5 (0.3)	1.4 (1.0-2.0)	1.4 (0.2)	na
RVDd (cm)	2.5 (1.0-3.3)	2.4 (0.6)	2.2 (0.8-3.0)	2.0 (0.7)	3.2 (0.5)

Table 2. Results from central haemodynamic monitoring and the conventional echocardiographic examination in closed and open chest. *= p-level of < 0.05

When comparing echocardiography before and after sternotomy no statistically significant changes were found, although a tendency towards an increase in LVDs and LVDd can be noted. The LVDd values found in our study were in the normal range of what is reported both for male and female humans.⁹

With the colour-coded tissue Doppler modality the SRI, strain and DI did not show any significant changes and the range was large.

Peak velocity measurement showed no significant difference in basal left or right ventricular S', E' or A' values between closed and open chest. However a tendency towards a decrease of E and a concomitant tendency of increase in A' were observed after sternotomy both in the left and right ventricle.

Laser Doppler perfusion monitoring and tissue Doppler imaging (Paper II)

The LDPM signal was low during the same intervals in the cardiac cycle as a low (< 1 cm/s) TDI was registered. Otherwise the LDPM signal was high throughout the cardiac cycle. The low velocities were found in late systole and late diastole and in this time frame the stable LDPM signal correlated with the low myocardial velocities detected by TDI. The diastolic interval was found between the E' and A' waves close to the ECG P peak. By using this correlation, intervals with minimal artefacts in the LDPM signal were localised thereby giving a more accurate estimate of local myocardial perfusion. During LAD occlusion (35-105 sec) (Fig 12) the systolic S' was significant lower (p< 0002, n=14) compared to baseline, indicating ischaemia.



Figure 12. *TDI signal and corresponding LDPM signal calculated from 15 heartbeats during LAD occlusion. The arrow marks the interval in late systole where low TDI and LDPM signals are noted 0,23 s from the R-peak of the ECG.*

LAD occlusion measurements from 2 animals are shown in fig. 13. The TDI-detected low velocities intervals were found in the late systole. LDPM interval and TDI intervals overlapped in all cases except one. The averaged (n=14) overlap of the TDI and LDPM intervals was 63 (22) % in relation to total interval length and 84 (27) % in relation to TDI interval.



Figure 13. The numbers beside each bar describe the amount of overlap between laser Doppler interval and tissue Doppler interval relative to the total interval length and to tissue Doppler interval length, respectively. TDI-int; Intervals with low velocity (< 1 cm/s) and LDPM-int; low LDPM signal (< 50% of baseline), during LAD occlusion in two animals.

HeartMate II treatment during myocardial depression. (Paper III).

After the HMII had been implanted and started, MAP as well as LVED and LVES areas decreased. CO was maintained, indicating a decrease in workload on the heart due to the LVAD's unloading effect. TDI readings of the posterior basal segment decreased during the experiment, and significant reductions in the longitudinal velocity (p<0.02) were recorded. The fact that CO was maintained indicates that most of the blood flow was generated by the LVAD (Fig 14). The VTI declined together with the TDI at the end of the experiments. The LVED area and LVES area decreased when SVR was lowered, but increased again during the first infusion of β -blocker with induction of myocardial depression. The LVED and LVES areas did not respond in the same way as the TDI but the areas had a tendency to increase when the TDI velocities decreased and vice versa.

TDI velocities were not significantly affected by the LVED dimension or the pump speed, and the greatest effect on velocity was the result of individual variations and unknown factors. If the various parameters were analysed individually as the only variable influencing TDI there was a significant (p<0.05) relationship between TDI and native SV (20%), HR (19%), SVR (9%), and CO (8%) but none of these could alone explain more than 20% changes in myocardial velocities.



Figure 14. Diagram showing the relationship between CO and TDI systolic peak measured in LV posterior basal segment during 13 different steps of the study protocol.

Second Harmonic imaging and Spontaneous Contrast. (Paper IV)

Visualisation of air bubbles in the ascending aorta was possible with epicardial SHI, and the bubbles appeared before or simultaneously with a threatening fall in heart chamber size and pressure. There was a significant decrease in LADs (p<0.007), LAP (p<0.008), LVDd (p<0.001) and LVPd (p<0.03) when pump speed was increased from baseline to maximum speed. The most obvious finding was a significant reduction in LADs preceding detection of air bubbles in the ascending aorta (fig 15). The LAP change did not predict the occurrence of air bubbles (fig 16).



Figure 15. Diagram showing the median decrease in left atrial systolic diameter (LADs) and left ventricular end-diastolic diameter (LVDd) from baseline to maximum pump speed when divided into two groups depending on whether or not aortic air bubbles were detected. Only differences in LADs the between groups were significant, with a more pronounced fall in the air bubble group.

In six of ten animals air bubbles occurred when the speed was increased, and in all six bubbles were first visualised in the ascending aorta and later in the LV (Fig 16). In two animal delayed lethal massive air embolism occurred minutes after air bubbles appeared in the ascending aorta.



Figure 16. *Air bubbles visualised in the ascending aorta while increasing pump speed. LV: left ventricle, LA: left atrium, Aorta: ascending aorta.*

Discussion

The animal model

Central haemodynamic and cardiovascular anatomy in calves are known to be similar to humans and this contributed to our choice of model.⁵⁴ The haemodynamic parameters measured in our study were also in concordance with those observed in humans. Several baseline haemodynamic and echocardiographic data have been published from normal awake calves.^{55,57} and a few report data from sedated or anaesthetised calves.^{58, 59}

Chee et al.⁵³published a study on central haemodynamic and echocardiographic indices in anaesthetised calves with closed chest. They used up to 50% larger and older animals than we did and dimensions and flows reported were proportionally larger than ours. However the size-independent haemodynamic were similar to ours.

The BSA was calculated according to the DuBois & DuBois formula (head to tail length) and the result used for indexing. When the LVDd was divided by the BSA in our animals and calculated from data reported from Chee's study, a similar quota occurred (2.5 respectively 2.4 cm/m²). This LVDd index found in this study is in the normal range seen in both male and female humans.¹⁰ The results show that animal size should be considered when applying animal models to cardiovascular research.

Anaesthetic agents.

It is known that induction of and anaesthesia itself induces haemodynamic depression which is why we monitored baseline readings during anaesthesia as well as in the awake state prior to induction.

Hypnotic agents have been used in veterinary medicine since the 1950's. The agents are valuable in calming animals and are most effective when used in animals that are relatively calm at the time of administration. The major advantage of this agent is to make induction of anaesthesia easier. Drug interaction may occur and in cardiovascular research the separation of drug effects and cardiovascular responses may be difficult. The α -adrenergic blocking agents have a direct effect on BP and in some cases HR. Atropine sulphate is a parasympatholytic agent is useful in controlling the salivary and respiratory tract secretion. The usual doses recommended for pre-anaesthesia do not markedly affect BP but do cause tachycardia. Individual animals will have varying degree of tachycardia depending upon the vagal tone and individual large doses of atropine may act as a cardiac depressant.⁶⁰ The effect of atropine sulphate last for hours and to prevent tachycardia, the animals in our studies were given β -blocker prior to sternotomy. Despite the β -blocker an increase in HR was recorded as a consequence of the drugs administered, surgery, or both.

General anaesthesia, as administered in these studies, was maintained with intravenous fentanyl and pentobarbital sodium. Today, Isoflurane or Desflurane inhaled trough intubation are considered the agents of choice for general anaesthesia in calves, as in animal anaesthesia in general.

The advantage of the inhaled anaesthetic agents is a smooth induction and very fast recovery, and cardiopulmonary variables remain within reference ranges throughout anaesthesia.⁶¹

We performed acute experiments, and the animals were terminated in the laboratory, which is why recovery from general anaesthesia was not considered when choosing the anaesthetic agent and by the time of the first sessions, there were no equipment for inhaled anaesthetics available in the laboratory.

Laminar blood flow.

Haemodynamic measurements were dependent on an adequate measurement of BP. The impeller-based LVAD delivers laminar blood flow with a lowering of the pulse width in the vessels. However, the pulsations from the native heart and the vessels properties produce a low pulsation in the vessels even in the presence of the LVAD. Using the IntelliVue (Philips Healthcare, DA Best, The Netherlands) technique, a 'red disconnect' alarm is activated if mean pressure falls below 10 mmHg and loss of pulsation will cause a 'pulsation' alarm. The algorithm (in which the numeric will be determined every second using a 0.6 Hz low path filter) for the calculation of BP works in the absence of an alarm.

In this way a low-pulsatile pressure is determined even at with weak pulsations, but measurements made at the time for an alarm are questionable.

Echocardiography with tissue Doppler imaging

For many years the echocardiographic 2D grey-scale imaging with spectral blood flow Doppler analysis was the standard methods available for quantification of ventricular function. With recently introduced technologies, regional myocardial function and perfusion may be monitored, but these must be applied and evaluated in each specific situation. Together with invasive haemodynamic monitoring and echocardiography, TDI provides further and repeatable cardiovascular function parameters.

Technological advancements have resulted in TDI-derived parameters such as velocity, strain, SR and displacement, as well as speckle tracking and contrast echocardiography, providing more accurate quantification of global and regional ventricular function. These techniques have been extensively studied in transthoracic echocardiography (TTE) but recently using the tansoesophageal (TEE) approach too.⁶² The TEE probe routinely used in the operating room setting and this should also be the technique of choice in calf studies. The anatomy of the calf chest in these studies made it difficult to examine the heart with TEE echocardiography, and at the time of the first experiment sessions, the TDI and SHI function of the TEE probe were not even available for clinical use.

Tissue Doppler imaging

There are several publications from humans on tissue Doppler-derived indices in humans.^{37-41,63} All TDI-derived data on myocardial wall motion and deformation are angle-dependent which may affect TDI velocity data as well as TDI-derived strain data. Aware of the fact that the ultrasound beam should be parallel to the myocardial wall in longitudinal measurements, TDI was performed with default settings (region of interest 5 mm diameter, 3 samples/pixels) and the angle of the sector was as small as possible (15-20 degrees) and the depth was (7-11 cm) though we prioritised the high frame rate advised for accurate TDI measurements.

We observe that the S´ velocities and also the mean E´ and A´ velocities in the present study are in the range of those found for awake humans. No significant changes were recorded after sternotomy but the statistical power was quite low and the range wide. A tendency towards decreased E´ and a concomitant tendency towards an increase in A´ were observed after sternotomy in both the left and right ventricle that might have been an effect of a higher HR as described in humans. The E'/A´- ratio, however is 3.4 in our anaesthetised calves which is higher than that reported in awake humans, and this may be considered even if statistical analysis is not possible from data available. As described earlier in patients without LVAD S´ also fell parallel to myocardial depression in our calves after LVAD implantation which suggests this to be a useful additional tool when evaluating myocardial performance during LVAD treatments.

Strain

The difference between Lagrangian and Eulerian strain rate has practical consequences. The Lagrangian SR is measured as the velocity difference, relative to a fixed initial length. The Eulerian SR in systole, with the muscle fibres decreasing in length, is the change in velocity relative to the instantaneous length. The Eulerian SR will thus be higher than in the Lagrangian, and the difference increases with higher strain.

In diastole, the differences will decrease as long as the Lagrangian strain is calculated from the initial end diastolic length. If the end-systolic length is taken as initial length, the instantaneous length is increasing, and the Eulerian SR will be lower than the Lagrangian. The practical consequences are that peak systolic SR will be higher in magnitude when using Eulerian SR. In TDI a correction is already applied and the scanner measures Eularian SR integrated to Lagrangian strain.

Speckle tracking (2D strain) is a new method for deformation and motion analysis based on the tracking of natural acoustic markers in the myocardium which does not have the angular dependence of TDI. This technique measures Lagrangian strain and the optimal frame rate is lower (55-90 frames/s) than in Eularian SR derived from TDI.

Deformation imaging, SRI, allows 1D measurements based on TDI and 2D measurements based on speckle-tracking imaging. SR in speckle tracking is derived from the strain curve, and if strain is measured directly by methods such as speckle tracking, the inverse correction has to be applied for comparison with standard SR from tissue Doppler.⁶³

The Speckle tracking technique was not available in our laboratory and the data from humans that we used for comparison with our TDI, strain and displacement data were measured using TDI.

Strain measurements in this thesis showed values similar to humans, but the ranges were large. This might be an effect of the poor reproducibility seen in many of today's available Echocardiographic machines (personal communication) which also explains why SRI has not yet been introduced into clinical practice. The disadvantage of strain rate is the signal to noise ratio since the signal is a gradient between two points and noise from these two will be added. This results in a noisy signal which affects the reproducibility of the method. The median and mean values for displacement in our studies were lower than in humans, which might be an explained by size differences. New and more specific modalities applying lower frame rate for strain and displacement imaging may improve the accuracy of measurements,⁴⁷even if speckle tracking has not yet been shown reliable and reproducible enough for clinical practice.

Air bubble detection.

Air embolism is always a potential risk associated with open heart surgery and studies using transcranial Doppler have shown a significant correlation between air emboli during cardiopulmonary bypass and postoperative neuropsychological impairment.⁶⁴⁻⁶⁶ Carbon dioxide insufflations in the open chest has been shown to decrease the number of cerebral air emboli during heart surgery.⁶⁷

Scalia et al.⁶⁸using the first generation of LVADs reported that significant efflux of microbubbles from the graft into the ascending aorta could be seen with standard TEE in 91% of patients. Whether or not these figures are applicable to the new generations of LVADs is unknown.⁶⁹

In clinical practice it is often recommended to carefully monitor LAP and maintain it at 10-15 mmHg in order to avoid a rapid fall in preload. This knowledge is based on clinical experience rather than on studies in humans. In our study we focused on the problem of detecting air bubbles at an early stage. By visualising the LVAD outflow tract in the ascending aorta using SHI we could detect even small numbers of micro-bubbles, but we found no significant association with changes in LAP. On the contrary a decrease in LA or LV size predicted the appearance of air bubbles and we suggest further studies based on these findings to determine if this can be used instead of LAP for monitoring and optimisation of pump speed during the implantation.

Cavitation

When implanting a LVAD there is a risk of air being trapped inside the pump, and air leakage may occur through the device system connections or suture lines due to negative pressures in the left side of the heart if the preload of the LA and LV is inadequate. Air bubbles within the heart look similar regardless of origin.

It is also known, however, that cavitations can be induced by an impeller and lead to air bubbles.^{70, 71} There is a possibility that cavitations contributed to air bubbles during our experiments, especially when the preload was low and pump suction high.

Our findings might be of value in the clinical situation where air bubbles in the ascending aorta and decreasing LA size could be early sign of insufficient preload. Air embolism is a common occurrence in open heart procedures and this is not species-related. Even if our experimental procedure does not simulate the clinical situation where pump speed is increased in small stages while being carefully monitored. It does, however, indicate the risk of aggressively increasing pump speed during implantation.

We now employ SHI, for LA dimension monitoring and ascending aortic air bubble detection in our patients during implantation of the HMII so as to avoid air embolism when adjusting pump speed.

To our knowledge this has not been reported previously and further clinical studies are required to assess the usefulness of these findings.

Laser Doppler perfusion monitoring, clinical implications

By taking tissue motion into consideration it is possible to reduce movement artefacts from the LDPM signal. Movement artefacts can be reduced if the LDPM signal is correlated to the ECG and measured during minimal wall motion. The intervals of choice have now been identified with the help of TDI as the late systole and late diastole. Data have been reported from phantom studies that a time delay (asynchronous) between the TDI and ECG signals can occur.⁷² Aware of this issue we identified the known ECG-correlated velocities in the image, and a short time delay was not regarded as significant for our results The future of this technique is to monitor regions at risk for low perfusion in the myocardial wall after cardiac surgery. The probe is small and could be kept in place through in the chest wall for a short time after surgery in the same way as a pacemaker cable is handled after surgery in the clinical routine practice.

Fors et al.⁷³recently performed a study on humans undergoing CABG and found that the most appropriate fixed time during the cardiac cycle to measure the perfusion with laser Doppler is in the late systole and late diastole. Late diastole was considered a better choice, since it is longer and the perfusion signal thereby reaches a stable level. A proper ECG signal is essential in order to use the ECG-triggering method. In this study it was possible to record the signal in eight of ten patients the day after surgery, and the probe was removed in all patients without complications.

Reproducibility

We did not evaluate the reproducibility of our measurements but previous studies in humans have shown that the inter-observer reproducibility of TDI indices was highest in the basal parts of the ventricles with coefficients of variation between 9-14% for the different basal segments.⁷⁴ We therefore chose those segments for evaluations in our studies. The variability of diastolic parameters however exceeds those in systole. A fair inter- and intra- observer variability has been shown for strain and SR but the reproducibility is less than for TDI velocity measurements.⁷⁵ A large inconsistent variation in strain and SR measurement, depending on machine and software used, has also been shown recently (personal communication). DI has been shown to be a relatively reproducible and robust method that is easily applied.

Limitations

The number of animals used in our studies was small and available results from closed chest echocardiographic examinations intended for normal values are especially few. These TTE examinations were first performed in the morning after the animal had been anesthetised, and it was agreed to only take measurements when this did not interfere with the basic protocol for the different sessions. There are however already results from closed chest studies in calves²⁷ and in our studies no significant changes were reported in myocardial motion indices after opening the chest were seen.

In our studies we used male calves and to our knowledge there are no data available evaluating the possibility of gender-dependent differences.

The effect of age is also not usually considered in such animal experiments studies and our healthy normal animals aged 3 months are presumed too young to suffer cardiovascular disease.

Conclusions

The studies show that echocardiography including tissue Doppler and harmonic imaging contribute to the perioperative evaluation of the application of new medical devises in the anaesthetised calf.

- Transthoracic echocardiography including TDI is applicable and feasible for the anaesthetised calf model and normal baseline central haemodynamic and echocardiographic values before and after sternotomy are provided. Echocardiographic myocardial velocity, strain and displacement are not significantly affected by sternotomy.
- Movement artefacts in signals from the laser Doppler perfusion monitoring device can be reduced by using the results of myocardial tissue Doppler registration. The optimum interval depends on the application and late systole and late diastole can be used.
- The flow characteristics and myocardial motion of a LVAD during variations in afterload show that myocardial velocity decreases with the myocardial depression and is significantly correlated to native stroke volume, heart rate, systemic arterial resistance and cardiac output, but not to left ventricular size, fractional shortening or pump speed. Echocardiography with TDIs thereby offers an additional means of monitoring and quantifying residual myocardial function during LVAD treatment.
- Second harmonic echocardiography is useful in the early detection of spontaneous ultrasound contrast (air bubbles) during implantation of a LVAD. A prompt decrease in size of the left atrium during pump speed adjustment may be a warning that massive air embolism is imminent.

Acknowledgements

This thesis is a result of a joint contribution from the Clinics of; Cardiology, Cardiovascular Surgery and Anaesthesiology, and Clinical Physiology at former Linköping Heart Centre, all in cooperation with the Department of Biomedical Engineering, Linköping University. Financial support by grants from the Country Council of Östergötland, the Linköping University foundations and the Swedish National Research Council. Acknowledgments to CMIV at Linköping University.

Special thanks to

My supervisor, professor **Birgitta Janerot-Sjöberg** for great tutorial, and that you took your time to navigate my way between the islets, despite all the other more important voyages of yours (not to talk about your daily overtime hours).

I have really appreciated all of our long conversations about research and life.

The chief and informal co-supervisor through this project, **Henrik Ahn.** You do always navigate through your own brilliant charts, and you do indeed make the sailing through the sea of research more interesting.

Bent Peterzén, co-author, without your knowledge and support this thesis had never been finished. I promise you, the following sessions in Gammalnäs by the sea, will not be scientific at all.

Urban Lönn, co-author, your enthusiasm and dynamic personality was the foundation of the whole project. Thank you for opened the door to the lab for me, and it was really a privilege to work with you.

The co-authors, **Daniel Karlsson, Stefan Träff** and especially my friend, **Karin Wårdell**. **Lena Lindström** and **Tomasz Kukulski**, for that you steered me into the Echo-archipelago many years ago and **Ulf Dahlström**, co-supervisor, who kept me very busy out there. The crew in the lab represented by **Dan Linghammar**, for all technical assistance, and for treating the animals so respectfully and well.

Other helping hands; Hans Granfeldt, Bo Carnstam, Ulf Wallgren and Jonas Andersson-Lindholm. Göran Eklund and the helpful staff at Clinical Physiology.

All the **colleagues and friends at the Cardiology clinic** and especially the crew of the **Heart failure division**. You make it all worth it!

Christine Sonnhag and **Hans Rutberg**, the former heads of Linköping Heart Centre, who gave us all the prerequisites to be able to implement a joint research project like this.

A warm thought to our lost colleagues **Bengt Wranne** och **Björn Bergdahl** which both have contributed to planning of this thesis.

And finally, a big and warm thanks to the **Hübbert and Kairento families**. Sis, the long calls with you in Lappland is the anchor of my life. And **Elias**, goa unge, you are the Captain.

Reference List

- 1. Holrege C The Heart: a pulsing and perceptive center in *The Dynamic Heart and Circulation* (Fair Oaks, CA: AWSNA; 2008:8, 2010).
- 2. Lundback, S. Cardiac pumping and function of the ventricular septum. *Acta Physiol Scand. Suppl* **550**, 1-101 (1986).
- 3. Dickstein, K. *et al.* ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur. Heart J.* **29**, 2388-2442 (2008).
- 4. Ma,T.K., Kam,K.K., Yan,B.P., & Lam,Y.Y. Renin-angiotensin-aldosterone system blockade for cardiovascular diseases: current status. *Br. J. Pharmacol.* **160**, 1273-1292 (2010).
- 5. Braunwald E Examination of the patient in *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine* 1-349 2010).
- 6. Edler, I. & Hertz, C.H. The early work of ultrasound in medicine at the University of Lund. *J. Clin. Ultrasound* **5**, 352-356 (1977).
- 7. Edler, I. & Hertz, C.H. The use of ultrasonic reflectoscope for the continuous recording of the movements of heart walls. 1954. *Clin. Physiol Funct. Imaging* **24**, 118-136 (2004).
- 8. Bierig, S.M., Ehler, D., Knoll, M.L., & Waggoner, A.D. American Society of Echocardiography minimum standards for the cardiac sonographer: a position paper. *J. Am. Soc. Echocardiogr.* **19**, 471-474 (2006).
- 9. Lang, R.M. *et al.* Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J. Am. Soc. Echocardiogr.* **18**, 1440-1463 (2005).
- 10. Rudski,L.G. *et al.* Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. *J. Am. Soc. Echocardiogr.* **23**, 685-713 (2010).
- 11. Nilsson,G.E., Tenland,T., & Obert,P.A. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans. Biomed. Eng* **27**, 12-19 (1980).
- 12. Van de,W.F. *et al.* Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. *Eur. Heart J.* **29**, 2909-2945 (2008).

- 13. Bassand, J.P. *et al.* Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. *Eur. Heart J.* **28**, 1598-1660 (2007).
- 14. Vahanian, A. *et al.* Guidelines on the management of valvular heart disease: The Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology. *Eur. Heart J.* **28**, 230-268 (2007).
- 15. Vardas, P.E. *et al.* Guidelines for cardiac pacing and cardiac resynchronization therapy: The Task Force for Cardiac Pacing and Cardiac Resynchronization Therapy of the European Society of Cardiology. Developed in collaboration with the European Heart Rhythm Association. *Eur. Heart J.* **28**, 2256-2295 (2007).
- 16. Maybaum, S. *et al.* Cardiac improvement during mechanical circulatory support: a prospective multicenter study of the LVAD Working Group. *Circulation* **115**, 2497-2505 (2007).
- 17. Miller, L.W. *et al.* Use of a continuous-flow device in patients awaiting heart transplantation. *N. Engl. J. Med.* **357**, 885-896 (2007).
- 18. Park, S.J. *et al.* Left ventricular assist devices as destination therapy: a new look at survival. *J. Thorac. Cardiovasc. Surg.* **129**, 9-17 (2005).
- 19. Rose,E.A. *et al.* The REMATCH trial: rationale, design, and end points. Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure. *Ann. Thorac. Surg.* **67**, 723-730 (1999).
- 20. Slaughter, M.S. *et al.* Advanced heart failure treated with continuous-flow left ventricular assist device. *N. Engl. J. Med.* **361**, 2241-2251 (2009).
- Gammie, J.S., Edwards, L.B., Griffith, B.P., Pierson, R.N., III, & Tsao, L. Optimal timing of cardiac transplantation after ventricular assist device implantation. *J. Thorac. Cardiovasc. Surg.* 127, 1789-1799 (2004).
- Chumnanvej,S., Wood,M.J., MacGillivray,T.E., & Melo,M.F. Perioperative echocardiographic examination for ventricular assist device implantation. *Anesth. Analg.* 105, 583-601 (2007).
- 23. Orihashi, K. *et al.* New applications of two-dimensional transesophageal echocardiography in cardiac surgery. *J. Cardiothorac. Vasc. Anesth.* **5**, 33-39 (1991).
- 24. Mehra, M.R. *et al.* Listing criteria for heart transplantation: International Society for Heart and Lung Transplantation guidelines for the care of cardiac transplant candidates-2006. *J. Heart Lung Transplant.* **25**, 1024-1042 (2006).
- 25. LARSON,R.E. & MCGOON,D.C. Experimental observations on total prosthetic reconstruction of the aortic valve. *Surg. Forum* **12**, 242-243 (1961).
- 26. LARSON,R.E. & Mc Goon,D.C. Total prosthetic replacement of aortic valve in calves. *Arch. Surg.* **88**, 135-144 (1964).
- 27. Chee,H.K. *et al.* Baseline hemodynamic and echocardiographic indices in anesthetized calves. *ASAIO J.* **50**, 267-271 (2004).

- 28. Tuzun, E. *et al.* Myocardial hemodynamics, physiology, and perfusion with an axial flow left ventricular assist device in the calf. *ASAIO J.* **50**, 47-53 (2004).
- LaPeyre, D.M. *et al.* In vivo evaluation of a trileaflet mechanical heart valve. *ASAIO J.* 40, M707-M713 (1994).
- 30. Karlsson, M.G. *et al.* Myocardial tissue motion influence on laser Doppler perfusion monitoring using tissue Doppler imaging. *Med. Biol. Eng Comput.* **42**, 770-776 (2004).
- 31. Root, C.R. & Tashjian, R.J. Thoracic and abdominal arteriography in calves. *Am. J. Vet. Res.* **32**, 1193-1205 (1971).
- 32. Du,B.D. & Du Bois,E.F. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* **5**, 303-311 (1989).
- 33. Edler, I. & Lindstrom, K. The history of echocardiography. *Ultrasound Med. Biol.* **30**, 1565-1644 (2004).
- 34. YOSHIDA, T. *et al.* Analysis of heart motion with ultrasonic Doppler method and its clinical application. *Am. Heart J.* **61**, 61-75 (1961).
- 35. Thomas, J.D. & Rubin, D.N. Tissue harmonic imaging: why does it work? J. Am. Soc. Echocardiogr. 11, 803-808 (1998).
- 36. Senior, R. *et al.* Contrast echocardiography: evidence-based recommendations by European Association of Echocardiography. *Eur. J. Echocardiogr.* **10**, 194-212 (2009).
- Kukulski, T. *et al.* A comparison of regional myocardial velocity information derived by pulsed and color Doppler techniques: an in vitro and in vivo study. *Echocardiography*. 17, 639-651 (2000).
- 38. Kukulski, T. *et al.* Normal regional right ventricular function and its change with age: a Doppler myocardial imaging study. *J. Am. Soc. Echocardiogr.* **13**, 194-204 (2000).
- 39. Strotmann, J.M., Hatle, L., & Sutherland, G.R. Doppler myocardial imaging in the assessment of normal and ischemic myocardial function--past, present and future. *Int. J. Cardiovasc. Imaging* **17**, 89-98 (2001).
- 40. Waggoner, A.D. & Bierig, S.M. Tissue Doppler imaging: a useful echocardiographic method for the cardiac sonographer to assess systolic and diastolic ventricular function. *J. Am. Soc. Echocardiogr.* **14**, 1143-1152 (2001).
- 41. Wilkenshoff, U.M., Hatle, L., Sovany, A., Wranne, B., & Sutherland, G.R. Age-dependent changes in regional diastolic function evaluated by color Doppler myocardial imaging: a comparison with pulsed Doppler indexes of global function. *J. Am. Soc. Echocardiogr.* **14**, 959-969 (2001).
- 42. Ballo,P., Bocelli,A., Motto,A., & Mondillo,S. Concordance between M-mode, pulsed Tissue Doppler, and colour Tissue Doppler in the assessment of mitral annulus systolic excursion in normal subjects. *Eur. J. Echocardiogr.* **9**, 748-753 (2008).

- 43. Andersen, N.H. & Poulsen, S.H. Evaluation of the longitudinal contraction of the left ventricle in normal subjects by Doppler tissue tracking and strain rate. *J. Am. Soc. Echocardiogr.* **16**, 716-723 (2003).
- 44. Heimdal,A., Stoylen,A., Torp,H., & Skjaerpe,T. Real-time strain rate imaging of the left ventricle by ultrasound. *J. Am. Soc. Echocardiogr.* **11**, 1013-1019 (1998).
- 45. D'hooge, J. *et al.* Regional strain and strain rate measurements by cardiac ultrasound: principles, implementation and limitations. *Eur. J. Echocardiogr.* **1**, 154-170 (2000).
- 46. Voigt, J.U. *et al.* Assessment of regional longitudinal myocardial strain rate derived from doppler myocardial imaging indexes in normal and infarcted myocardium. *J. Am. Soc. Echocardiogr.* **13**, 588-598 (2000).
- 47. Dandel, M., Lehmkuhl, H., Knosalla, C., Suramelashvili, N., & Hetzer, R. Strain and strain rate imaging by echocardiography basic concepts and clinical applicability. *Curr. Cardiol. Rev.* **5**, 133-148 (2009).
- 48. Jamal, F. *et al.* Can changes in systolic longitudinal deformation quantify regional myocardial function after an acute infarction? An ultrasonic strain rate and strain study. *J. Am. Soc. Echocardiogr.* **15**, 723-730 (2002).
- 49. Urheim, S., Edvardsen, T., Torp, H., Angelsen, B., & Smiseth, O.A. Myocardial strain by Doppler echocardiography. Validation of a new method to quantify regional myocardial function. *Circulation* **102**, 1158-1164 (2000).
- 50. Sjoberg,B.J. & Wranne,B. Cardiac output determined by ultrasound-Doppler: clinical applications. *Clin. Physiol* **10**, 463-473 (1990).
- 51. Karlsson,M.G., Casimir-Ahn,H., Lonn,U., & Wardell,K. Analysis and processing of laser Doppler perfusion monitoring signals recorded from the beating heart. *Med. Biol. Eng Comput.* **41**, 255-262 (2003).
- 52. Edvardsen, T. *et al.* Acute regional myocardial ischemia identified by 2-dimensional multiregion tissue Doppler imaging technique. *J. Am. Soc. Echocardiogr.* **13**, 986-994 (2000).
- 53. Chee,H.K. *et al.* Baseline hemodynamic and echocardiographic indices in anesthetized calves. *ASAIO J.* **50**, 267-271 (2004).
- 54. Root, C.R. & Tashjian, R.J. Thoracic and abdominal arteriography in calves. *Am. J. Vet. Res.* **32**, 1193-1205 (1971).
- 55. Amory,H., Jakovljevic,S., & Lekeux,P. Quantitative M-mode and two-dimensional echocardiography in calves. *Vet. Rec.* **128**, 25-31 (1991).
- Amory,H., Kafidi,N., & Lekeux,P. Echocardiographic evaluation of cardiac morphologic and functional variables in double-muscled calves. *Am. J. Vet. Res.* 53, 1540-1547 (1992).
- 57. Amory, H. *et al.* Growth-induced haemodynamic changes in healthy Friesian calves. *Vet. Rec.* **132**, 426-434 (1993).

- Lin,H.C., Thurmon,J.C., Tranquilli,W.J., Benson,G.J., & Olson,W.A. Hemodynamic response of calves to tiletamine-zolazepam-xylazine anesthesia. *Am. J. Vet. Res.* 52, 1606-1610 (1991).
- 59. Stowe, C.M. & Good, A.L. Estimation of cardiac output in calves and sheep by the dye and Fick oxygen techniques. *Am. J. Physiol* **198**, 987-990 (1960).
- 60. D.Gross Preanesthesi, Anesthesia, Chemical Restraint, and the Recognition and Treatment of Pain and Distress in *Animal Models in cardiovascular research* 17-54 2009).
- 61. Keegan, R.D., Greene, S.A., Valdez, R.A., & Knowles, D.K. Cardiovascular effects of desflurane in mechanically ventilated calves. *Am. J. Vet. Res.* **67**, 387-391 (2006).
- 62. Marcucci, C., Lauer, R., & Mahajan, A. New echocardiographic techniques for evaluating left ventricular myocardial function. *Semin. Cardiothorac. Vasc. Anesth.* **12**, 228-247 (2008).
- 63. Sun, J.P. *et al.* Noninvasive quantification of regional myocardial function using Doppler-derived velocity, displacement, strain rate, and strain in healthy volunteers: effects of aging. *J. Am. Soc. Echocardiogr.* **17**, 132-138 (2004).
- 64. Ahlgren, E., Lundqvist, A., Nordlund, A., Aren, C., & Rutberg, H. Neurocognitive impairment and driving performance after coronary artery bypass surgery. *Eur. J. Cardiothorac. Surg.* **23**, 334-340 (2003).
- Borger, M.A. *et al.* Neuropsychologic impairment after coronary bypass surgery: effect of gaseous microemboli during perfusionist interventions. *J. Thorac. Cardiovasc. Surg.* 121, 743-749 (2001).
- 66. Pugsley, W. *et al.* The impact of microemboli during cardiopulmonary bypass on neuropsychological functioning. *Stroke* **25**, 1393-1399 (1994).
- 67. Svenarud,P., Persson,M., & van der,L.J. Effect of CO2 insufflation on the number and behavior of air microemboli in open-heart surgery: a randomized clinical trial. *Circulation* **109**, 1127-1132 (2004).
- 68. Scalia,G.M., McCarthy,P.M., Savage,R.M., Smedira,N.G., & Thomas,J.D. Clinical utility of echocardiography in the management of implantable ventricular assist devices. *J. Am. Soc. Echocardiogr.* **13**, 754-763 (2000).
- 69. Piccione, W., Jr. Left ventricular assist device implantation: short and long-term surgical complications. *J. Heart Lung Transplant.* **19**, S89-S94 (2000).
- 70. Potapov, E.V. *et al.* Transcranial detection of microembolic signals in patients with a novel nonpulsatile implantable LVAD. *ASAIO J.* **47**, 249-253 (2001).
- 71. Yamazaki,K. *et al.* An intraventricular axial flow blood pump integrated with a bearing purge system. *ASAIO J.* **41**, M327-M332 (1995).
- 72. Walker, A., Olsson, E., Wranne, B., Ringqvist, I., & Ask, P. Time delays in ultrasound systems can result in fallacious measurements. *Ultrasound Med. Biol.* **28**, 259-263 (2002).

- C.Fors, H.Ahn, & K.Wårdell. Online Laser Doppler Measurements of Myocardial Perfusion ECIFMBE 2008, IFMBE Proceeding 22. 1718-1721. 2008. Ref Type: Conference Proceeding
- 74. Fraser, A.G. *et al.* Feasibility and reproducibility of off-line tissue Doppler measurement of regional myocardial function during dobutamine stress echocardiography. *Eur. J. Echocardiogr.* **4**, 43-53 (2003).
- 75. Sun,J.P. *et al.* Noninvasive quantification of regional myocardial function using Doppler-derived velocity, displacement, strain rate, and strain in healthy volunteers: effects of aging. *J. Am. Soc. Echocardiogr.* **17**, 132-138 (2004).