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Evaluation of Myocardial Function in Chronic Kidney Disease
A Colour Tissue Velocity Imaging Study

A dissertation submitted to the Royal Institute of Technology in partial fulfilment of the requirements for the degree of Medical Doctor

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To my spiritual father *Jesus Christ*

To my mother *Hatsuko* and my son *Tiago*
If

If you can keep your head when all about you
Are losing theirs and blaming it on you;
If you can trust yourself when all men doubt you,
But make allowance for their doubting too;
If you can wait and not be tired by waiting,
Or being lied about, don't deal in lies,
Or being hated, don't give way to hating,
And yet don't look too good, nor talk too wise:

If you can dream -- and not make dreams your master;
If you can think -- and not make thoughts your aim;
If you can meet with Triumph and Disaster
And treat those two imposters just the same;
If you can bear to hear the truth you've spoken
Twisted by knaves to make a trap for fools,
Or watch the things you gave your life to, broken,
And stoop and build 'em up with worn-out tools;

If you can make one heap of all your winnings
And risk it on one turn of pitch-and-toss,
And lose, and start again at your beginnings
And never breathe a word about your loss;
If you can force your heart and nerve and sinew
To serve your turn long after they are gone,
And so hold on when there is nothing in you
Except the Will which says to them: "Hold on!"

If you can talk with crowds and keep your virtue,
Or walk with kings -- nor lose the common touch,
If neither foes nor loving friends can hurt you,
If all men count with you, but none too much;
If you can fill the unforgiving minute
With sixty seconds' worth of distance run --
Yours is the Earth and everything that's in it,
And -- which is more -- you'll be a Man, my son!

Rudyard Kipling
Abstract

In patients with chronic kidney disease (CKD), overhydration, uremic toxins and left ventricular (LV) dyssynchrony are factors that may lead to LV dysfunction and conduction abnormalities and thus contribute to the high cardiac mortality. Colour tissue velocity imaging (TVI) allows a detailed quantitative analysis of cardiac function in CKD patients, opening new possibilities to evaluate longitudinal myocardial motion, rapid isovolumetric events, LV filling pressure and LV synchronicity. Aims: Using TVI technique: 1. To evaluate myocardial function disturbances and their relations to risk factors in CKD patients. 2. To assess LV synchronicity in HD patients, both at baseline and after HD, and 3. To study acute cardiac effects of HD and i.v. furosemide in HD patients. Methods: 40 predialysis CKD (stages I, II, III, IV and V) (Study II) and 59 HD (Studies I, III, IV and V) patients were studied. In both groups of patients LV function was evaluated using TVI, and in HD patients LV synchronicity was also assessed using tissue synchronization imaging (TSI). In HD patients the evaluations were performed before and after HD (Studies III and V) and i.v. furosemide infusion (Study IV). Results: 1. TVI detected: a) LV contraction disturbances in CKD patients with LVH and normal ejection fraction. b) An increase of LV contractility after HD. c) No changes in cardiac function induced by furosemide. 2. TSI detected the presence of LV dyssynchrony and its improvement after HD. 3. In CKD, cardiac dysfunction seemed to be related to high levels of PTH, phosphate and blood pressure. Conclusions: TVI is a sensitive tool for studies on cardiac function in CKD, allowing a detailed and accurate evaluation of disturbances in LV function. TVI also provides the possibility to follow the changes in LV function and synchronicity induced by different therapeutical interventions. The obtained information may contribute to a better management of CKD patients.
The present thesis is based on the following studies which are referred to by Roman numerals:


III. Hayashi SY.; Brodin LÅ.; Alvestrand A.; Britta Lind B.; Stenvinkel P.; Nascimento MM.; Qureshi AR.; Saha S.; Lindholm B.; Seeberger A. : **Improvement of cardiac function after haemodialysis. Quantitative evaluation by colour tissue velocity imaging.** Nephrol Dial Transplant (2004) 19: 1497–1506.

IV. Hayashi SY.; Seeberger A.; Lind B.; Gunnes S.; Alvestrand A.; Nascimento MM.; Lindholm B.; Brodin LÅ: **Acute effects of low and high intravenous doses of furosemide on myocardial function in anuric hemodialysis patients: A Tissue Doppler study.** Nephrol Dial Transplant. (2008) 23: 1355-1361

V. Hayashi SY.; Seeberger A.; Lind B.; Nowak J.; Nascimento MM.; Lindholm B.; Brodin LÅ: **A single session of hemodialysis improves left ventricular synchronicity in patients with end-stage renal disease: A pilot tissue synchronization imaging study.** Accepted for publication. Nephrol Dial Transplant, may 2008
Preface

The present thesis is submitted to the Royal Institute of Technology (KTH) in partial fulfilment of the requirements for the degree of Medical Doctor. The studies were performed in the Division of Renal Medicine, Department of Clinical Science, Intervention and Technology and Department of Clinical Physiology. Karolinska University Hospital Huddinge. The dissertation will be presented at 09:00, 28th May, 2008.

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and just wants to talk with him and be around him. The first time I was at his office at the Physiology Department I felt at home, because it was as ‘‘organised’’ as my office at home. After that, I saw his picture on his door and, during all these years that I have been his student, he has been that angel looking over me, giving me new projects, supporting and helping me in all possible ways.

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1. Abbreviations

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<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Standard Doppler late diastolic filling wave (atrial contraction)</td>
</tr>
<tr>
<td>A’</td>
<td>Late diastolic myocardial velocity (atrial contraction)</td>
</tr>
<tr>
<td>ch</td>
<td>Chamber</td>
</tr>
<tr>
<td>cm/s</td>
<td>Centimetres/second</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>2D</td>
<td>Two-dimensional image</td>
</tr>
<tr>
<td>2D-SI</td>
<td>Two-dimensional strain imaging</td>
</tr>
<tr>
<td>E</td>
<td>Standard Doppler early diastolic filling wave</td>
</tr>
<tr>
<td>E’</td>
<td>TVI early diastolic myocardial velocity</td>
</tr>
<tr>
<td>E/E’</td>
<td>Estimated LV filling pressure</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>EF</td>
<td>Ejection fraction</td>
</tr>
<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
</tr>
<tr>
<td>FS%</td>
<td>Fractional shortening</td>
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<tr>
<td>HD</td>
<td>Haemodialysis</td>
</tr>
<tr>
<td>IVCV</td>
<td>Isovolumetric contraction velocity</td>
</tr>
<tr>
<td>IVRT</td>
<td>Isovolumetric relaxation time</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>LVH</td>
<td>Left ventricle hypertrophy</td>
</tr>
<tr>
<td>LVMI</td>
<td>Left ventricular mass index</td>
</tr>
<tr>
<td>MAM</td>
<td>Mitral annulus motion</td>
</tr>
<tr>
<td>MD</td>
<td>Maximum delay</td>
</tr>
<tr>
<td>ms</td>
<td>Milliseconds</td>
</tr>
<tr>
<td>PSV</td>
<td>Peak systolic velocity</td>
</tr>
<tr>
<td>RWT</td>
<td>Relative wall thickness</td>
</tr>
<tr>
<td>SR</td>
<td>Strain rate</td>
</tr>
<tr>
<td>TDE</td>
<td>Tissue Doppler echocardiography</td>
</tr>
<tr>
<td>TDD</td>
<td>Tissue Doppler displacement (integrated myocardial tissue velocity)</td>
</tr>
<tr>
<td>TSI</td>
<td>Tissue synchronization imaging</td>
</tr>
<tr>
<td>TVI</td>
<td>Colour tissue velocity imaging</td>
</tr>
<tr>
<td>V</td>
<td>Velocity</td>
</tr>
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Part I

Overview
Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in chronic kidney disease (CKD) (1) and despite the improvements in dialytic procedures and drug therapy, the incidence of cardiovascular mortality is still very high. Modern echocardiography techniques can open new possibilities to evaluate cardiovascular disease in CKD patients and may help in the development of strategies aimed to prevent, or at least delay, the progress of cardiac disturbances in these patients.

Conventional echocardiography is well established as a safe, non-invasive, and versatile diagnostic modality in cardiology. However, there are two important problems when the method is used to perform observational and interventional studies assessing cardiac function in CKD patients. The first is the technical limitation of the method to evaluate cardiac function in hypertrophic myocardium (2), and the second is the lack of reliability to study the acute changes in cardiac function induced by dialysis and drug intervention, when a subjective, semi-quantitative and load dependent method is used.
Recently, the introduction of new techniques in echocardiography helped to partly overcome these limitations and added new possibilities to evaluate some parameters that were previously only possible to be obtained by invasive methods. Most importantly, by using these new techniques, recent studies have underlined the significant role of some aspects of cardiac physiology such as longitudinal motion and synchronism on cardiac mechanics and performance.

Colour tissue velocity imaging (TVI) is a modern echocardiographic technique that is based on the detection of myocardial tissue motion during the cardiac cycle. TVI is an important tool in the clinical investigation of longitudinal myocardial motion, regional and global cardiac function and synchronism, and as TVI measurements are objective, reproducible and quantitative, it is also a valuable tool for clinical interventional studies.

The main purpose of the present thesis was to study longitudinal myocardial motion and LV dyssynchrony in CKD patients, both at baseline and after interventions such as hemodialysis and furosemide treatment, using colour tissue velocity imaging.

1. Physiological background

The heart has an impressive pumping capacity which demands a delicate balance of electrical, mechanical and metabolic systems to function efficiently.

1.1. The biological basis of cardiac contraction

The functioning of the heart depends on action potential generation and propagation, and the sliding of interdigitating thick and thin filaments. Myocardial action potentials reflect the sequential activation and inactivation of inward (Na$^+$ and Ca$^{2+}$) and outward (K$^+$) current carrying ion channels (3). Once the impulse has been generated in the sinus node, it spreads very rapidly throughout the atrium and reaches the atrioventricular node and the ventricles.
The increased concentration of Ca$^{2+}$ ions in the myocardial cytosol that occurs in response to the wave of depolarization, increases the interaction of Ca$^{2+}$ ions with troponin C, resulting in triggering of the contractile proteins. At the end of systole, as the cytosolic Ca$^{2+}$ ion concentration starts to decline, calcium stops interacting with troponin C. The contractile elements are deactivated, the myofibrils return to their original length before contraction and the diastolic phase of the cardiac cycle sets in (4).

1.2. The heart as a physiological pump

Myocardial architecture and motion. Millions of myocytes, situated in the matrix of connective tissue, constitute the myocardium. Myocardial muscle fibres have mainly oblique orientation resulting in helical arrangement, with longitudinal fibre direction in the subendocardial region, transitioning into a circumferential direction in the mid-wall and becoming again longitudinal within the subepicardial surface (5,6).

Because of the intrinsic spiral geometry of the myofibers, shortening and lengthening of the myocardial walls results in rotatory movements. The electrical activation within the subendocardium initiates the contraction sequence. During IVC, the predominant shortening of the subendocardial fibers and stretching of the subepicardial fibers result in a brief clockwise rotation of the apex. However, during ejection, myofibers shorten across the entire myocardial wall, with the direction of rotation governed by the subepicardial fibres, which results in counter-clockwise rotation of the LV apex with respect to the base that is referred to as LV twist or torsion (5,7). A concomitant longitudinal shortening occurs as a consequence of this dominant torsional force of subepicardial fibres and an increase in wall thickness occurs, in part as a consequence of internal rearrangement of the disposition of the fibres composing the LV wall (8,9).
The relaxation starts in the apical subendocardium during the period of isovolumetric relaxation, with subepicardial relaxation evolving in the opposite direction, beginning at the base and starting after the aortic valve closure. The temporal and spatial differences in subendocardium and subepicardium relaxation are critical for the creation of forces required for diastolic suction. During early diastolic filling, there occurs pressure gradient between the LV apex and LV base resulting in suction of blood into the left ventricle (10).

1.3. Left ventricular synchronism

Synchronicity of LV is a prerequisite for effective and energetically efficient LV performance (11-13). Normally, LV contracts synchronously with only 40±29 ms intra-ventricular difference in the timing of peak mechanical systole (14). LV dyssynchrony causes a non-uniform contraction and relaxation that produces an imbalance of forces which, in turn, reduces the mechanical efficiency of ventricular ejection (15) and slows the rate of LV isovolumic pressure fall, impairing diastolic filling (16).

1.4. Phases of the cardiac cycle

The cardiac cycle is a manifestation of the periodic contraction and relaxation of cardiac muscle fibres. It is traditionally divided in systolic (isovolumetric contraction and ejection) and diastolic (isovolumetric relaxation and filling) phases.

Ventricular systole. Once the electrical activation reaches the ventricles, onset of ventricular contraction sequence occurs. When the ventricular pressure increases to above the left and right atrial pressure the mitral and tricuspid valves close. This is followed by a short period of time in which the mechanical myocardial events are not followed by changes in intraventricular blood volume, called the isovolumetric contraction (IVC) period. When intra-
cavital pressure rises further to exceed the aortic and pulmonary artery pressure, the aortic and pulmonary valve opens, the ejection phase begins and the blood leaves the ventricles.

**Ventricular diastole.** With the decrease in intra-ventricular pressure, the aortic and pulmonary valves close and another period with no blood volume changes occurs, known as the isovolumetric relaxation time. When the ventricular pressure falls below the atrial pressure, the mitral and tricuspid valves open and ventricular filling from the atrium begins. This period is divided in an early phase \(E\), with a high filling rate, followed by a quiescent phase with slow transvalvular flow, called diastasis, and finishes with the atrium contraction \(A\) \(17\).

### 1.5. Determinants of cardiac performance

**Pre-load.** Pre-load refers to the resting force of the myocardium muscle and is determined by the volume present before the start of contraction, at the end of diastole. According to the Frank-Starling law of the heart, the more the heart is filled during diastole, and to certain extent the longer the muscle, the stronger is the work that can be performed by the muscle \(18\). When pre-load increases, the left ventricle \(LV\) distends during diastole and the stroke volume rises \(19\).

**After-load.** After-load is the force that the ventricle must overcome in order to contract and eject the blood. Increased after-load means that an increased intra-ventricular pressure has to be generated in order to open the aortic valve and then to maintain the blood flow during the ejection phase \(20\).

**Heart rate.** The inherent ability of the ventricular myocardium to increase its strength of contraction independently of neuro-hormonal control, in response to an increase in contraction frequency is known as force-frequency relation or Bowditch treppe. In healthy heart as the contraction frequency is increased from 60 to about 180 bpm the force of the contraction increases and, after a further increase in frequency, the force will decline \(4\).
Contractility. Contractility is the inherent ability of the myocardium to generate force and to shorten independently of changes in the preload or after-load at a fixed heart rate (19). Increased contractility is reflected in higher myocardial fibre shortening velocity, with a more highly developed tension peak and a steeper pressure rise, when pre-load, after-load and heart rate are constant. Decreased contractility is reflected in a lower myocardial fibre shortening velocity, with lower tension peak and a blunted pressure rise, when pre-load, after-load and heart rate are constant (4).

2. Methods for monitoring cardiac function

2.1. General considerations.

There are many ways in which LV function can be determined. Unfortunately, however, there is no measurable quantity that corresponds to an integrated functional assessment, and therefore surrogates that estimate one or another aspect of cardiac function are used instead. It is worth mentioning that it is not possible to completely separate the cellular mechanism of contractility changes from those of load or heart-rate because of the clear overlap between them (21). Therefore, each method has variable dependence on loading conditions and heart rate that limits their accuracy. However, the knowledge of the limitations of each measure permits a more judicious use of them and increases the understanding of the true status of the heart and the changes within it.

2.2. Invasive methods.

Isolated myocardial fibre. Contractility expressed in the isolated myocardial fibre is the maximal velocity of contraction of unloaded muscle fibre (Vmax). This strictly pre-load and after-load independent index fulfils the theoretical requirements for contractility
quantification and greatly contributes to this research field. Nevertheless this model is not
usable under in vivo conditions (4).

*Left ventricular pressure and volume measurements.* The pressure-volume relationship is the
most reliable index for assessing myocardial contractility at rest in the intact circulation and is
virtually insensitive to changes in load. This method is widely used in animal studies and
occasionally also in clinical settings. However, it is invasive, complex, and technically
demanding (22,23).

### 2.3. Echocardiographic measurements of cardiac function

#### 2.3.1. History of echocardiography

Since the introduction of the technique in 1953 by Inge Edler and C Hellmuth Hertz (24),
echocardiography has rapidly advanced from a method that merely traced the blood-tissue
border to a high technological tool that can characterise the myocardium itself. In the
beginning, in the era of M-mode, echocardiography had limited diagnostic value. However,
after the introduction of two dimensional (2D) echocardiography, the technique became the
most preferred non-invasive method to evaluate chamber size and function, valve morphology
and motion, intracardiac masses, and diseases of the pericardium and great vessels. The
subsequent introduction of Doppler echocardiography (25) represents another important
advance and forms the cornerstone in the non-invasive assessment of intra-cardiac pressures
and haemodynamics. The further adaptation of pulse Doppler signal to measurements of low
velocities of myocardial tissue movements laid the ground for tissue Doppler
echocardiography (26). Tissue Doppler echocardiography changed the assessment of cardiac
function from subjective and semi-quantitative to accurate, objective and quantitative.
Additionally, the method has the potential to track changes in cardiac function serially, to
detect sub-clinical disease and to evaluate non-invasively myocardial deformation, isovolumetric contraction, and LV filling pressure. Finally, the introduction of two-dimensional strain imaging \((2D-SI)\) and tissue synchronization imaging \((TSI)\) took echocardiography a step further. 2D-SI allowed for angle-independent measurements of myocardial velocities and TSI improved the evaluation of LV synchronization, an area of growing interest in cardiology \((27)\).

### 2.3.2. Methods for echocardiography assessment of left ventricular function

One of the most frequent indications for obtaining a Doppler echocardiography study is to ascertain LV function. There has been a remarkable advance in the technique to measure cardiac function since the first non-invasive determination of cardiac output in 1971 by Ganz et al \((28)\).

#### 2.3.2.1. Conventional echocardiography measurements of systolic and diastolic function

**Systolic function**

*Percentage of fractional shortening \((%FS)\) - One of the first echocardiographic measurements of LV function was the \( \%FS \) measured using M-mode. Although \( \%FS \) is a quick, simple and reproducible method, it is load dependent and does not provide reliable results in patients with cardiac pathologies of the type that cause regional asymmetries \((26)\).*

*Ejection fraction \((EF)\). EF is the percentage of LV volume ejected in systole. The most common way to estimate EF is by using the modified Simpson’s rule method \((29)\), which divides LV into a series of stacked disks \((30)\). The determination of EF using LV volumes makes possible to evaluate LV function taking into account regional abnormalities. The method is generally performed in clinical practice, has prognostic value \((31)\), guides therapeutic decision making and correlates better than M-mode fractional shortening with*
angiographic studies (32). However, the method is significantly limited by the strong dependency on the skill of the sonographer, the difficulties associated with obtaining technically adequate images in all three required echocardiographic projections, and the load dependence (21).

*Mitral annulus motion (MAM).* An alternative approach for the assessment of systolic LV function is the evaluation of LV longitudinal motion, obtained by measurements of mitral annulus motion (MAM) towards the apex. In 1986, Lundbäck (33) showed the importance of the movement of the mitral annulus for the pump function and in 1988, Höglund (34) described the technique to measure MAM. The technique is to measure the amplitude of the atrio-ventricular plane displacement toward the apex during systole in four different locations around the mitral annulus, using M-mode in apical 2 and 4 ch views. It is a simple technique since mitral annulus can be visualised in almost all patients even if the endocardial borders are difficult to trace. The technique has been shown to correlate with global LV function in a healthy as well as diseased myocardium (35) and, in recent years, has become a well established index of global systolic function in clinical practice (35,36) and also to have prognostic value (37,38).

**Diastolic function.** Analysis of the velocity profile of the flow across the mitral valve during LV filling using Doppler echocardiography is considered to be the cornerstone of diastolic function assessment. However, the analysis is not an unequivocal method to determine intrinsic LV diastolic function since transmitral flow profiles are greatly dependent on intracavity gradients which are not only determined by LV relaxation and compliance but also preload, end-systolic volume, atrial and ventricular stiffness, left atrial pressure, heart rate and age (39,40). *Figure 14* shows the influence of load on mitral inflow.
2.3.2.2. **Myocardial velocity and its derivatives for evaluation of systolic and diastolic function**

2.3.2.2.1. **Colour tissue velocity imaging (TVI)**

*General considerations.* Doppler ultrasound has traditionally been applied in the measurement of blood flow velocities across cardiac valves, arteries and veins. Moving red blood cells reflect low amplitude Doppler signals, but at a relatively high velocity. The low frequency echoes generated by myocardial movements with the velocity usually not exceeding 0.2 m/s are filtered out by built-in high-pass filters. The replacement of high-pass filters with low-pass filters enables myocardial tissue velocities to be displayed (41).

Tissue Doppler echocardiography was described initially by Yoshitoshi (41) and Isaaz (42) and is based on the detection of myocardial tissue motion during the cardiac cycle. In 1992, with the development of colour flow algorithms to visualise myocardial motion, the technique began to gain clinical acceptance. Subsequent software development has led to improved temporal and spatial resolution as well as the post-processing of digitally stored cardiac images enabling quantification of multiple segments of the myocardium in seconds (43). TVI allows an objective and reproducible quantitative estimation of both systole and diastole, and time intervals regionally in the myocardium during the same cardiac cycle (44,45). Since the original validation of the technique (46,47), the modality has been used in a variety of clinical situations, from investigations of the athlete’s heart to implantation of ventricular pacing in the pre-clinical diagnosis of genetic diseases such as hypertrophic cardiomyopathy (48) and even to assess changes in LV function during dobutamine or exercise stress echocardiography (49).
**TVI in the evaluation of systolic function.** TVI opened entirely new possibilities for the quantification of LV function by providing detailed information about myocardial longitudinal motion velocities, displacement and strain rate.

*Peak systolic velocity (PSV).* The velocity of myocardial contraction has been established as a measure of contractility in isolated muscle preparations and in animal studies using sonomicrometry as a reference method (50).

PSV is the positive wave in the myocardial velocity curve that corresponds to the ejection phase (see Figures 1 and 13). PSV corresponds to the LV ejection fraction measured by radionuclide angiography (51), as well as to the peak positive dP/dt in patients with dilated cardiomyopathy and hypertensive heart disease (52,53). The limitation of PSV measurements to evaluate contractility lies in the fact that regional TVI myocardial velocities are affected by heart translation, tethering effect of the adjacent myocardial segments (53), load conditions (54) and ventricular length (55).

*Isovolumetric contraction velocity (IVCV).* Until recently, the most serious disadvantage in the evaluation of isovolumetric contraction was that it could only be measured using an invasive technique. TVI, providing superior temporal resolution, allows the evaluation of myocardial kinetics also during isovolumetric phases (56). IVCV is the positive wave in the velocity curve corresponding to the isovolumetric contraction phase (see Figures 1 and 13). IVCV together with isovolumetric acceleration has been shown to be a good marker of myocardial function (57), as well as a less load-dependent measurement of myocardial contractility (58,59).

*Strain rate imaging (SR).* Strain is defined as relative deformation, whereas strain rate describes the rate of deformation (60). Strain is a strong non-invasive index of LV contractility and is sensitive to the early stages of ischaemia (21). Myocardial strain can be measured non-invasively using regional myocardial velocities acquired with a TVI technique.
The underlying principle is that instantaneous differences in tissue velocities between two adjacent regions imply either compression or lengthening of the tissue in between. The limitation of SR, however, is the significant “noise” in the signal that affects its ability to separate normal from abnormal values (26) and its dependency on heart-rate (62) as well as pre-load (63).

Figure 1. The myocardial velocity curves recorded using TVI have five main components. These include isovolumetric contraction velocity (IVCV) during isovolumetric contraction time (IVCT), peak systolic velocity (PSV), isovolumetric relaxation velocity during isovolumetric relaxation time (IVRT), early diastolic (E’), and late diastolic (A’) myocardial velocities.

*Tissue Doppler derived displacement (TDD).* Mitral annulus motion (MAM) measurements can also be obtained by the integration of myocardial velocities curves (TDD) from four different locations around mitral annulus (apical 2 and 4 chamber) (51,64). TDD has been shown to improve the accuracy of wall motion analysis (65), but is limited by the angle dependency of the measurements.
**TVI in the evaluation of diastolic function.** The evaluation of diastolic function with TVI includes the measurements of diastolic myocardial velocities (E’, A’), isovolumetric relaxation time (IVRT), and LV filling pressure (E’/E) (Figure 1). TVI has been shown to be a more reliable method for the identification of patients with impaired LV relaxation, regardless of mitral inflow velocity pattern measured using the Doppler technique (66-68). It also allows the non-invasive estimation of LV filling pressure through the ratio of transmitral E flow velocity to E’ myocardial velocity (E/E’) (69-71), improving the global evaluation of diastolic function. Finally, studies have also shown that E’ measurements have a prognostic value in congestive heart failure patients (72).

However, the limitation of myocardial diastolic velocities is the possible load dependency. Many reports have been published regarding the load dependency of diastolic myocardial velocities but the results are conflicting, some showing TVI diastolic velocities as load independent measurements (73), and others demonstrating the opposite (74).

### 2.3.2.3. Two-dimensional strain imaging (2D-SI).

Two-dimensional strain imaging (2D-SI) is a recently introduced echocardiography imaging technique. Ultrasound tissue images contain many small reflectors, naturally-occurring acoustic markers, which move together with the tissue and do not change their pattern significantly between adjacent frames. 2D-SI identifies these characteristic reflectors within the myocardium and tracks them frame-by-frame to yield tissue deformation in two dimensions, thus measuring the true movement of the myocardium. 2D-SI is independent of transducer orientation and allows for the accurate display of tissue velocity and strain rate in two orthogonal dimensions, thus avoiding the angle dependence of all Doppler techniques. Studies evaluating the correlation between 2D-SI and tissue Doppler velocities, strain and
strain rate (75), as well as studies evaluating myocardial torsion (76), have shown good accuracy of the method suggesting the possible use of 2D-SI in LV evaluation.

3. Evaluation of LV dyssynchrony using tissue synchronisation imaging (TSI).

TSI is a new echocardiographic technique that provides an advanced analysis of the synchronicity of myocardial motion, opening entirely new possibilities for imaging and quantification of LV dyssynchrony (27). The TSI software processes acquired TVI data and provide automatic detection of the time to PSV at any discrete point along the myocardial wall with reference to the QRS signal. The obtained temporal data are translated into colour-coded maps of LV contraction synchronicity with colour coding ranges from green (earliest), yellow, orange, to red (latest) giving a detailed quantitative information about the regions of dyssynchronously contracting LV myocardium. The method has proved to be reliable and reproducible and its capacity to identify significant systolic dyssynchrony, and thereby predicting a positive response to CRT, has been found to be superior to that of QRS duration criteria (14,77,78).

4. Renal disease and cardiac function

4.1. General considerations

Chronic kidney disease (CKD) is a worldwide public health problem and despite the advances in dialysis and transplantation, the prognosis of CKD remains poor. Data from the third National Health Nutrition Examination Survey, estimated that the size of the United States CKD population was 11% of the adult population (79). Among the CKD patients, an estimated 5.9 million individuals (3.3%) had Stage 1 CKD (defined as persistent albuminuria with a normal GFR), 5.3 million (3.0%) had Stage 2 CKD (defined as persistent albuminuria
with a GFR of 60 to 89 mL/min/1.73 m, 7.6 million (4.3%) had Stage 3 CKD (defined as GFR, 30 to 59 mL/min/1.73 m), 400,000 individuals (0.2%) had Stage 4 CKD (defined as GFR, 15 to 29 mL/min/1.73 m), and 300,000 individuals (0.2%) had Stage 5 CKD (defined as GFR below 15 mL/min/1.73 m or need for renal replacement therapy with dialysis or transplantation) (80). Additionally, the incidence of Stage 5 CKD patients with end-stage renal disease (ESRD) requiring start of dialysis treatment has increased by 57% from 1991 to 2000 (80); and according to the United States Renal Data System 2001 Annual Report, approximately 64% of the prevalent ESRD patients in US received haemodialysis (81).

CKD patients, particularly those with ESRD, are at much higher risk of CVD in comparison with the age- and sex-matched general population, with increased prevalence of coronary artery disease, small vessel disease, silent myocardial ischaemia, complex arrhythmias (82), LVH, valvar calcifications (83) and arteriosclerosis (84). CVD is a major cause of morbidity and mortality in dialysis patients, accounting for almost 40% of hospitalisations and 79-80% reduction in life expectancy (85,86).

The pathogenesis of cardiovascular damage in CKD is far more complex than in the general population, since the risk factors include traditional risk factors, non-traditional or novel risk factors (87) and also uraemia associated risk factors (85,88-90) (see in Figure 2). CVD in CKD includes disorders of the heart (cardiomyopathy), and disorders of the vascular system (atherosclerosis, arteriolosclerosis and arterial calcifications). These disorders share several common pathophysiological mechanisms and are usually associated and interrelated (91). (See in Figure 3)

4.2. Vascular disease

Atherosclerosis. Atherosclerosis is characterised by the presence of arterial plaques that are primarily intimal, focal, intermittent in distribution, occlusive in nature, and with a
predilection for medium sized arteries and arterial bifurcations. The mechanisms of atherosclerosis are complex and include smoking, lipid disturbances, thrombogenesis, production of vasoactive substances, growth factors, mediators of inflammation, tensile stress and shear stress (92).

Atherosclerosis alters the conduit function of the arteries causing the narrowing or occlusion of arteries resulting in ischaemia or infarction, which is a frequent cause of cardiovascular mortality in ESRD patients (92). Ischemic heart disease in ESRD patients, however, has some particularities. A high burden of coronary disease was found in many asymptomatic patients (93), nevertheless no association between known coronary artery disease and subsequent mortality has yet been found. On the other hand, in 27% of HD patients the symptoms of myocardial ischemia are not caused by atherosclerotic disease (94,95) since ischaemic heart disease in CKD is also associated with underlying cardiomyopathy, arteriosclerosis (96), small vessel disease (caused by hypertension, diabetes mellitus, and calcium phosphate deposition), cardiomyocyte-capillary-mismatch (reduced capillary density, reduced coronary reserve), myocardial fibrosis and impaired myocardial metabolism (97).
Arteriosclerosis. In ESRD patients, arterial stiffness is increased and is associated with acceleration of the arterial aging process. Arteriosclerosis is characterised by thickened, dilated, and non-compliant arteries. The development of arterial stiffness occurs as GFR decreases, with the rate of progression increasing after the initiation of renal replacement therapy (98).

Arteriosclerosis predisposes the patient to LV hypertrophy by diminishing arterial distensibility and by increasing pulse wave velocity resulting in an early return of wave reflections, thus increasing the pulsatile work of the heart. Arteriosclerosis also predisposes to ischaemic heart disease by decreasing subendothelial coronary perfusion (98,99).

Arterial calcification. Arterial calcification is common in CKD patients (100), and is an independent predictor of CV mortality (101). There is accumulating evidence indicating that arterial calcification is an active and regulated process, leading to differentiation of vascular smooth muscle cells into phenotypically distinct osteoblast-like cells with subsequent ossification of the arterial wall (102). Arterial calcifications may occur in two sites: the tunica intima and tunica media. In CKD patients, intimal calcification is associated with older age, a high risk of atherosclerotic disease and arterial stenotic lesions. Medial calcifications promote arterial stiffening and are associated with younger age, a lower risk of atherosclerosis, longer duration of HD, and abnormalities in calcium phosphate metabolism (101,103).

4.3. Cardiomyopathy

Changes in cardiac structure are already present in earlier phases of CKD and tend to progress in parallel with the decline of renal function (104-106). Cardiomyopathy in uraemia is multifactorial in origin and is an ominous prognostic sign. The principal haemodynamic factors responsible for the progression of LVH and left ventricular dilatation in patients with CKD are pressure overload (106-108) and fluid overload (109). LVH is at least initially, a beneficial
compensatory process allowing the LV to produce additional force to increase the cardiac work and to maintain a constant wall tension. In physiological LVH, the increase in LV mass is characterised by a normal organisation of cardiac structure and no collagen increase. The pathological LVH in CKD is characterised by structural changes, collagen accumulation, myocyte hypertrophy, myocyte death, decrease in capillary density resulting in capillary cardiomyocyte mismatch, increased myocardial fibrosis and calcification (110,111) that may progressively lead to systolic and diastolic dysfunction and conduction abnormalities (112-114). (See Figure 4)

**Figure 3.** Pathophysiology of CVD in CKD. The combined effects of chronic hemodynamic overload and non/hemodynamic biochemical and neurohumoral factors characteristic of uremia causes LVH and arterial disease (arteriosclerosis, atherosclerosis). The principal consequences of LVH and arterial alterations are heart failure and ischemic heart disease, which are frequent causes of cardiac death in CKD (92).
4.4. Echocardiographic studies in CKD

Echocardiography has been a valuable tool in the evaluation of CKD patients (115). Previous studies have used conventional echocardiography to evaluate CKD patients with the aims to diagnose cardiac abnormalities (116), to verify their prevalence (116-118), to find out their prognostic value (104,119-121), to determine the possible associated factors and also to evaluate the effects of treatment (116,122-124).

Abnormalities in echocardiographic variables are the rule in CKD patients. Abnormalities of LV size, shape and function are present in between 70 and 80% of dialysis patients (104). An echocardiographic analysis of 334 CKD patients enrolled in the CREATE (Cardiovascular Reduction Early Anaemia Treatment with Epoetin beta) study (118), and with creatinine clearance levels of 15–35 ml/min, showed that 21% had concentric and 29% eccentric LVH, 22% had left ventricular dilation, and only 29% had a normal echocardiogram. Parfrey et al. (120) studied 432 patients starting dialysis, and the echocardiography at baseline revealed LV hypertrophy in 65%, and systolic dysfunction in 16%. Only 16% had a normal echocardiogram. In ESRD, studies have shown a prevalence of LVH from 78% to 90% (116,117), LV dilatation 38% and systolic dysfunction 20% (91).

There is no doubt about the significance of baseline and serial echocardiography evaluation for diagnosis and prognosis in CKD patients (115), as LVH, systolic dysfunction and LV dilatation are strongly associated with the development of cardiac failure and lower survival rates (104,119-121).

5. Dialysis and cardiac function

When haemodialysis is performed, blood flows on one side of a semipermeable membrane (haemofilter), and an osmotically balanced solution of electrolytes and buffer on the other
side. The pores of the semipermeable membrane allow small molecular weight molecules such as water to pass through into the dialysate whereas larger solutes such as blood cells and proteins remain in the blood. Solute transport across the filter membrane occur by two processes: diffusion processes: diffusion which is the main mechanism in haemodialysis, and by ultrafiltration-based convection.

If a dialysable uraemic toxin does indeed cause acute myocardial dysfunction, then it would be possible to confirm this by measurement of cardiac function before and after HD. In fact there are numerous reports of the echocardiographic evaluation of the acute effects of HD on systolic function, but the results are confusing. Some studies show that systolic function improved while others show no changes after HD (122-124). Thus despite numerous studies that have been performed hitherto, the effect of HD on cardiac function remains poorly understood.

6. Hemodynamic effects of furosemide

Furosemide is a diuretic drug that inhibits the \( \text{Na}^+ \), \( \text{K}^+ \), \( 2\text{Cl}^- \) co-transporter localised in cells from the ascending limb of the loop of Henle with a peak of diuretic effect within 15-20 min after intravenous administration. Furosemide has been the standard treatment for heart failure for several decades (125). Diuraesis with consequent decrease of the volume overload is the main effect for symptom relief in patients with preserved renal function. Some studies have suggested that furosemide also has extra renal effects, probably vascular effects (126,127) that may justify its use also in anuric patients. The vascular effect of furosemide, however, is still a matter of debate. Previous studies evaluating the vascular effects of furosemide have shown conflicting results, some demonstrating a decrease in left ventricular filling pressure accompanied by an increase in mean calf venous capacitance (127), others not
succeeding in showing the venodilatory effect (128,129), and others showing a wholly different response such as arterial vasoconstriction, increased LV end-diastolic pressure, and increased systemic vascular resistance (130). The disparity of the results can be related to differences in the vascular bed studied, the species studied, the timing (acute versus chronic effects), systemic versus local effects, direct versus indirect effects, as well as differences in disease states (131).

**Figure 4.** Pathophysiology of cardiomyopathy in CKD. The combined effects of chronic hemodynamic overload and non/hemodynamic biochemical and neurohumoral factors characteristic of uremia results in cardiomyopathy, which results in systolic and diastolic dysfunction. (92)
Aims

The aim of the present thesis was to establish whether:

1. Different echocardiographic methods such as colour tissue velocity imaging, M-mode, anatomical M-mode and two dimensional strain imaging, are comparable and interchangeable for measurements of longitudinal myocardial motion.

2. Systolic and diastolic functions are disturbed in patients with different stages of chronic kidney disease when evaluated using colour tissue velocity imaging and whether there are possible relationships between these cardiac disturbances and cardiac risk factors.

3. Left ventricular synchronism is disturbed in hemodialysis patients with left ventricular hypertrophy when evaluated using tissue synchronization imaging.

4. Cardiac function and synchronicity changes after different types of intervention (hemodialysis and furosemide) when evaluated using colour tissue Doppler velocity imaging and tissue synchronization imaging.
Methods

1. Subjects

In total, 99 patients with different stages of CKD disease were studied. 58 sex and age matched asymptomatic volunteers, with no previous history of cardiac disease, and with no ischaemic heart disease, severe valvular heart disease or congenital heart disease based on rest and stress echocardiographic findings, were used as controls.

All patients and control subjects gave their informed consent and the studies were approved by the local Ethics Committee of Karolinska Institutet at Karolinska University Hospital, Huddinge. The nature and the purpose of the investigations were explained to the subjects before asking for their consent. The investigation conforms with the principles outlined in the Declaration of Helsinki (132)

The age and gender distribution, as well as the classification of renal disease, is presented in Table 1. The information about the causes of CKD in the examined population is provided in Table 2.
Table 1. Study population data for all five studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (N)</th>
<th>Gender women/men</th>
<th>Age (years)</th>
<th>Stage of CKD</th>
<th>Controls (N)</th>
<th>Gender women/men</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>6/9</td>
<td>62±8</td>
<td>ESRD</td>
<td>10</td>
<td>4/6</td>
<td>59±11</td>
</tr>
<tr>
<td>II</td>
<td>40</td>
<td>4/36</td>
<td>60±14</td>
<td>1.2,3,4</td>
<td>27</td>
<td>6/21</td>
<td>58±17</td>
</tr>
<tr>
<td>III</td>
<td>13</td>
<td>7/6</td>
<td>62±10</td>
<td>ESRD</td>
<td>13</td>
<td>7/6</td>
<td>62±10</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>6/12</td>
<td>62±13</td>
<td>ESRD</td>
<td>18</td>
<td>6/12</td>
<td>62±10</td>
</tr>
<tr>
<td>V</td>
<td>13</td>
<td>6/7</td>
<td>64±9</td>
<td>ESRD</td>
<td></td>
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</tbody>
</table>

Inclusion and exclusion criteria. In Studies III, IV and V, the inclusion criterion was that subjects should be ESRD patients in HD three times a week for more than three months. In Study V, only ESRD patients with concomitant LVH were included. The exclusion criteria in Studies III, IV and V were the presence of any clinical evidence of infection, severe valvular heart disease, symptoms of congestive heart failure (New York Heart Association (NYHA) Class III and IV), pericardial disease, clinical and electrocardiographic evidence of myocardial infarction and arrhythmia, or symptoms or signs of myocardial ischaemia and previous renal transplantation. Also excluded from Study V were patients with ECG signs of ventricular conduction abnormalities.

Table 2. Causes of CKD in Studies II, III, IV and V

<table>
<thead>
<tr>
<th>Study</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic nephropathy</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>2</td>
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<tr>
<td>Nephrosclerosis</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td></td>
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<tr>
<td>Polycystic kidney disease</td>
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<td>3</td>
<td>2</td>
<td></td>
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<tr>
<td>Interstitial nephritis</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Vasculitis</td>
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<td>1</td>
<td>2</td>
<td>2</td>
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<td>Glomerulonephrites</td>
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<td>1</td>
<td>4</td>
<td></td>
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<tr>
<td>Amyloidosis</td>
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<tr>
<td>Sarcoidosis</td>
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<td>Mesangioproliferative glomerulopathy</td>
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<tr>
<td>Membranous nephropathy</td>
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<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Obstructive nephropathy</td>
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<td>Chronic pyelonephritis</td>
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<td></td>
</tr>
<tr>
<td>Cortical necrosis</td>
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<td>1</td>
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<tr>
<td>Unknown</td>
<td>14</td>
<td></td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
**Medications.** All medications were suspended 24 hours before the investigation and on the day of the studies, no medication was taken before the last echocardiography examination was finished.

**2. Blood pressure measurements**

Blood pressure was measured before each echocardiographic examination using a standard sphygmomanometer with the subjects in supine position, and after a 10 minute rest. In ESRD patients the measurements were obtained in the arm without arterial-venous fistula.

**3. Hemodialysis parameters**

In *Studies III and V*, all ESRD patients were treated with HD three times per week using polyamide or haemophan dialysers. HD was carried out for 3.0 to 4.5 hours (blood flow rate 230-400 ml/min , dialysate flow rate 500 ml/min) using bicarbonate-buffered dialysate with calcium 1.5 mmol/L, potassium 2±0.2 mmol/L (range 2-3 mmol/L), sodium 139±12 mmol/L (range 136 -140 mmol/L) and bicarbonate 33±2.9 mmol/L (range 25-38 mmol/L). During the HD session, fluid was removed to achieve the patient’s previously clinically determined dry weight. Weight, heart rate and systolic and diastolic blood pressures were measured before and after each HD session.

**4. Biochemical analysis**

In *Studies II, III and V*, blood samples were collected after an overnight fasting from the arterial side of the AV fistula at baseline, and in *Studies III and V* just before the start the HD
session. Plasma samples were prepared and frozen (−70°C) before analysis. Some analyses were also performed on blood samples obtained immediately after the HD session was stopped and the circulating extracorporeal volume of blood had been given back to the patient (see below). Plasma levels of parathyroid hormone (PTH), cholesterol, triglycerides, high-density lipoprotein, high sensitive C-reactive protein (hs-CRP) and cardiac troponin T (cTnT), were measured at baseline. In Study III, endothelin-1 (ET-1) and big-endothelin-1 (Big-ET-1) were also measured. Intact PTH was measured using a commercial electrochemical immunoassay (Elecsys PTH kit, Roche Diagnostics, Mannheim, Germany). Triglycerides and cholesterol were determined using standard enzymatic techniques (Boehringer Mannheim, Mannheim, Germany). HDL-cholesterol levels were analysed after precipitation of apo B-containing proteins with phosphotungstic acid. cTnT was analysed with the third generation troponin T test (Troponin T STAT, Roche Diagnostics). Serum hs-CRP was measured by nephelometry. ET-1 and Big-ET-1 were quantified by enzyme immunoassay (Biomedica, Vienna, Austria). Plasma levels of sodium, potassium, creatinine, urea, albumin, Ca, pH and phosphate were measured before and immediately after HD using routine methods. Albumin corrected albumin was used for further analysis.

5. Echocardiography analysis

5.1. Standard echocardiography

In Study II, all ultrasound examinations were performed on GE System FiVe equipment that is connected to a Macintosh computer loaded with EchoPac 6.3.4 software for post processing of LV digital images. A standard phased array 2.5 MHz multifrequency transducer was used for image acquisition.
In Studies I, III and IV all ultrasound examinations were performed using a Vivid 7 (GE Vingmed Ultrasound AS, Horten, Norway) linked to a PC workstation with pre-installed EchoPacPC 2.1 software and in Study V using a Vivid 7 linked to a PC workstation with pre-installed EchoPacPC BT 05 software. A multifrequency transducer (M3S) was used for image acquisition. The cardiac images during at least three consecutive cardiac cycles were acquired in parasternal long and short axis, and in apical 2-, 3-, and 4-chamber projections with the subject in left lateral position. All 2-dimensional and Doppler variables were acquired according to the guidelines of the American Society of Echocardiography (133) and stored for off-line analysis. Two-dimensional echocardiographic variables included left ventricular end-diastolic and end-systolic dimensions, end-diastolic and systolic inter-ventricular septal wall thickness, and left ventricular posterior wall thickness. All these variables were measured by M-mode in parasternal long-axis views. The ejection fraction was calculated using Simpson’s biplane rule method and complemented by subjective visual estimation, mitral annulus motion (MAM) method (34,134) and through M-mode. The LV mass was calculated according to the Penn convention and indexed for height$^{2.7}$. The LV mass index (LVMI) was calculated by the indexation of mass by height (135) and body surface area. LV hypertrophy was diagnosed when the LVMI > 50 g/m$^{2.7}$ for males and > 47 g/m$^{2.7}$ for females (135). The relative wall thickness (RWT) was calculated according to formula: RWT = (IVS+ PWT)/ LVESd, in order to classify the LV geometric pattern (concentric LVH: RWT ≥ 0.45; eccentric LVH: RWT < 0.45).

Transmitral, aortic and pulmonary venous flow velocities were acquired using pulsed wave Doppler. Cardiac output was determined by the pulsed Doppler technique. Diastolic function was assessed by determining the velocities of early (E) and late (A) diastolic transmitral flow, the ratio E-to-A (E/A) and pulmonary vein flow velocities (PV). In addition, isovolumetric
relaxation time (IVRT), deceleration time of E wave (Edec) and velocity propagation (VP) were also measured.

In Study III, diastolic dysfunction was defined by the presence of E/A < 1 or pulmonary vein systolic/diastolic ratio < 1 or combination of both. In Study II, diastolic dysfunction was defined in three categories, based on the Canadian Consensus on diastolic dysfunction (66):

a) Impaired relaxation (E/A < 1, prolonged Edec > 220 ms, IVRT > 100, PVS/D > 1);

b) Pseudo-normalization pattern (E/A 1-2, Edec 150-200, IVRT 60-100, PVS/PVD < 1)

c) Restrictive pattern (E/A > 2, Edec < 150 ms, IVRT < 60, PVS/PVD < 1).

In Study V, the classification of diastolic function included TVI data and diastolic dysfunction was defined as: a) Impaired relaxation (E/A < 1, prolonged Edec > 220 ms, IVRT > 100, E’< 8 cm/s); b) Pseudo normalization pattern (E/A 1-2, Edec 150-200, IVRT 60-100, E’ < 8 cm/s), and c) Restrictive pattern (E/A > 2, Edec < 150 ms, IVRT < 60, E’ < 8 cm/s) (66).

5.2. Colour Tissue Velocity Imaging (TVI)

After completion of the conventional echocardiography, images from apical 2-, 3-, and 4-chamber views were recorded at the end of expiration with the subject in left lateral position. Cine-loops of three consecutive cardiac cycles were acquired with high temporal resolution (>100 frames/s). The formatted raw data containing both grey scale and TVI information was stored on magneto-optical disk for off-line analysis on a Macintosh computer loaded with EchoPac 6.3.4 software in Study II, on PC workstation with pre-installed EchoPacPC 2.1 software in Studies I, III and IV and PC workstation with pre-installed EchoPacPC BT 05 software in Study V. The software permits real-time digital acquisition of the tissue velocity curve at any point in the myocardial location in the stored cine-loops. The myocardial velocity analysis was performed with 2 mm sampling volume from an optimal measuring position set in the basal segment of inferoseptal, anteroseptal, anterior, anterolateral, inferior, and...
inferolateral LV wall. In Study III myocardial velocities were also measured in the atrio-ventricular plane and mid-wall in the above cited walls. Delineation of the isovolumic contraction and relaxation phases was achieved off-line as previously described by Lind et al. (56). By mathematical processing of the velocity data, the EchoPac PC software also allows the analysis of the myocardial strain rate (SR). Strain rate represents velocity of regional myocardial deformation expressed in s\(^{-1}\) and, when measured at peak systole, reflects myocardial contraction. In order to calculate longitudinal strain rate, the velocity gradients within the area of interest (12 mm) were divided by the distances between the respective measured points and then averaged. The spatial offset was selected as a compromise between acceptable signal-to-noise ratio and longitudinal resolution. Measured variables were myocardial isovolumetric contraction velocity (IVCV), isovolumetric relaxation time (IVRT), peak systolic velocity (PSV), early (E’) and late (A’) diastolic velocities (see Figures 1 and 13), and systolic SR. To estimate the LV filling pressure, the mitral annular velocity at the lateral wall in the longitudinal axis was recorded and used to calculate the E/E’ ratio (71). LV filling pressure was considered to be elevated when E/E’ > 15 and normal when E/E’ < 8 (70). Diastolic dysfunction was defined when E’ < 8 cm/s (66).

5.3. Measurements of mitral annulus motion

5.3.1. Mitral annulus motion measurements with M-Mode

The mitral annulus motion (MAM) measurements towards and away from the cardiac apex were calculated by echocardiography using M-mode from an apical window. The infero-septal and lateral MAM were measured in the four-chamber view. The M-mode cursor was placed at the septal and antero-lateral border of the atrio-ventricular (AV) plane in such a way that the AV plane at septum moved along the M-mode line. By moving the M-mode cursor to
the lateral border of the AV plane, the displacement of the lateral wall was also recorded. The total MAM was measured from the lowest to the highest point of contraction. To avoid shifted velocities due to atrial contraction the systolic excursion of the mitral annulus was assumed to begin 60 milliseconds after the onset of the electrocardiographic QRS complex (136).

5.3.2. Mitral annulus motion measurements with anatomical M-Mode

The anatomical M-mode is a post-processing technique commercially available in the EchoPacPC system (GE Vingmed, Horten, Norway). It generates an M-mode display by reading the 2D pixel samples along a freely positioned cursor line (137). Along a virtual M-mode line, the information of the digitally stored 2D echocardiographic loop is recalculated and then displayed on a temporal axis comparable to the trace obtained by M-mode (138). Angle correction is used to describe the angle between AM-mode line and M-mode line. For AM-mode analysis, after the M-mode measurements, the cursor line was positioned parallel to the longitudinal LV motion and the angle between the M-mode and the AM-mode cursor line was measured on the screen. To avoid a shift of velocities due to atrial contraction, the systolic excursion of the mitral annulus was assumed to begin 60 milliseconds after the onset of the electrocardiographic QRS complex.

5.3.3. Mitral annulus motion measurements with tissue Doppler derived displacement

TVI was performed in the infero-septal and antero-lateral walls with a 2mm sampling volume, in the basal segment using an apical four-chamber view. The software in the EchoPac integrates the velocity curve, allowing for assessment of the systolic longitudinal displacement of the myocardium. The beginning and the end of systole was defined according to the method previously described (139); however, in the present study the total MAM was measured, including the isovolumic phases.
5.3.4. Mitral annulus motion measurements with 2D Strain Imaging

The measurements were performed according to the method described by Leitman et al (75). The algorithm identifies naturally acoustic reflectors and tracks them frame by frame on sequential images. The geometric shift of each element represents local tissue movement. The typical raw data were represented as a temporal sequence of B-mode images. At apical 4 ch view, after manual marking the LV “horseshoe” the endocardial and epicardial LV borders were automatically tracked and delineated. After manual adjustment or approval of the computer-generated myocardial wall delineation, the LV walls were automatically divided in 6 regions of interest and the automated speckle tracking analysis was performed. For the analysis of MAM the sample area automatically placed in the center of the basal region was manually changed to the base of the basal region. Frame-by-frame image tracking extracted from the basal segment of the infero-septal and antero-lateral walls was used to estimate the amplitude of mitral annulus displacement towards the cardiac apex. The systolic excursion of the mitral annulus was assumed to begin 60 milliseconds after the onset of the electrocardiographic QRS complex.
5.4. Tissue Synchronization Imaging (TSI)

The tissue synchronization software processes acquired TVI data and provides automatic detection of the time to PSV at any discrete point within the myocardial wall. The obtained temporal data are translated into a colour-coded image of synchronicity of LV contraction (Figure 7).

The analysed systolic interval was set by default to start 60 ms after the beginning of the electrocardiographic R wave, and to end 200 ms after the closure of the aortic valve, thus including possible a post-systolic contraction. Prior to the analysis, the TSI images were frozen, and scrolled to the end of systole to ensure adequate positioning of regions of interest within the myocardial wall for the whole systole. Subsequently, circular regions of interest (2 mm in diameter) were placed manually on the basal and mid-ventricular myocardium of the opposing LV walls, according to the previously described 12-segment model (27) including inferoseptal, anterolateral, anterior, inferior, anteroseptal and inferolateral walls. Intra-ventricular mechanical dyssynchrony of an LV segment was defined as the delay to PSV >105 ms compared with the shortest time to PSV among all the evaluated 12 segments. This definition of LV dyssynchrony was based on the cut-off value proposed by Perry et al (14) who studied a cohort of 100 volunteers with normal LV systolic function and normal QRS duration. The mean level of dyssynchrony in these individuals was found to be 47±29 ms that gives a mean of +2SD equal to 105 ms as a cut-off for a significant dyssynchrony of LV during systole. The percentage of the dyssynchronous segments, as well as the standard deviation of time to peak systolic velocity between the 12 segments was calculated.
6. Study design

In *Study I*, the infero-septal and antero-lateral MAMs were measured in the four-chamber view using M-mode, anatomical M-mode, TDD and 2D-SI techniques.

In *Studies III and V*, the patients were investigated before and just after finishing HD.

In *Study IV*, the patients (low dose and high dose groups) were investigated immediately before (0 min) furosemide i.v. infusion. In the low dose group, the ultrasound examination was repeated at 10, 20, 30, 40, 50 and 70 minutes after the first dose (dose two - additional 40 mg - was administered at 30 min), and in the high dose group at 10, 20, 30 and 40 minutes after the first dose, as shown in *Figure 6*.

![Figure 6. Study design showing the low (upper panel) and high doses (lower panel) of furosemide infusion and the period where the echocardiography measurements were done.](image_url)
Figure 7. Tissue synchronization imaging before (upper panel) and after (lower panel) a single HD session. Regional longitudinal myocardial velocities (middle panels) obtained at the sampling points placed on the opposing basal infero-septal (yellow) and antero-lateral (green), and midventricular infero-septal (blue) and antero-lateral (red) segments as shown in the left hand panels. Time to systolic peak velocity is color-coded and, before HD, a delay in reaching maximal systolic velocity can be clearly discerned in the lateral segments colored red in 4-chamber view in the left upper panel, but also when inspecting systolic velocity curves in the middle upper panel. The bull’s eye image of this systolic delay is presented in the right hand upper panel. After HD, normalization of time to PSV in previously dyssynchronously contracting LV region is evident in all lower panel images.
7. Statistical analysis

Data were expressed as the mean ± the standard deviation (SD) and median (range). Comparisons between patients and controls were performed using the Mann-Whitney U test (Studies II and III). In HD patients, the paired t-test and the Wilcoxon’s signed rank test were used to assess measurements obtained before and after HD (Studies III and V). For comparisons between MAM motion (Study I) and between before- and after-furosemide (Study IV), analysis of variance (ANOVA), followed by Turkey post hoc test, was performed. For variables with a skewed distribution (Study II), the Wilcoxon rank sum test and the chi-square test of Fisher’s exact test were used. Possible associations were assessed by the Pearson and Spearman coefficients of correlation and linear regression analysis. In Study I, Bland–Altman analysis was used to evaluate the agreement between the methods (140). The measurements of different methods were plotted against their average values. The limits of agreement were defined as ±2SD values from the mean difference. Probability values of greater than 0.05 were considered statistically significant.
Results

1. Interchangeability of different techniques to measure mitral annulus motion (Study I)

Comparison between the methods. There was no significant difference, in average, between MAM measured by M-mode and anatomical M-mode (AM-mode) and between M-mode and 2D strain imaging (2D-SI). In the lateral wall MAM measured by AM-mode was significantly higher than 2D-SI (12.4±2.8 vs. 10.4±3.9, p < 0.05). Tissue Doppler derived displacement (TDD) measurements were significantly lower than M-mode, AM-mode and 2D-SI as demonstrated in Figure 8.

![Figure 8](image)

**Figure 8.** MAM measurements by M-mode, AM-mode, TDD and 2D-SI. The significant different results are represented by different letters, and in the lateral wall also by *
Agreement between the methods. A high variability was observed in MAM measurements obtained using M-mode, AM-mode, TDD and 2D-SI in septal and lateral walls. This variability can be visualized in the Bland-Altman plots presented in Figure 9.

Angle correction. With angle correction up to 8°, the measurements obtained with AM-mode were not significantly different from those obtained by M-mode.

Comments. Despite the good correlation between the methods, they are not interchangeable as demonstrated by a wide range of the limits of agreement between them. TDD measurements were significantly lower in comparison with all other methods.

Figure 9. Bland-Altman plots of MAM, measured by M-mode vs. TDD, M-mode vs. 2D-SI, AM-mode vs. TDD, AM-mode vs. 2D-SI and TDD vs. 2D-SI in septal and lateral walls.
2. Clinical and biochemical characteristics. (Studies II, III and V)

Study II. As expected, creatinine, urea, phosphate and calcium x phosphate levels were higher in group 2 (predialysis CKD, stages IV and V) in comparison with group 1 (CKD, stages I, II and III) as shown in Table 5.

Studies III and V. As expected, weight, potassium, creatinine, urea, calcium and phosphate levels decreased after HD, and haemoglobin and albumin increased after HD.

Table 5. Clinical and biochemical characteristics of Study II, group 1 and group 2 patients, Study III and V, before and after HD

<table>
<thead>
<tr>
<th></th>
<th>STUDY II</th>
<th></th>
<th>STUDY III</th>
<th></th>
<th>STUDY V</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>P</td>
<td>Before HD</td>
<td>After HD</td>
<td>P</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.7±13.8</td>
<td>83.2±12.3</td>
<td>NS</td>
<td>74.8±13</td>
<td>72.4±10</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>73.3±15.8</td>
<td>70.6±15.0</td>
<td>****</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>149±29</td>
<td>144±16</td>
<td>NS</td>
<td>144.2±22.9</td>
<td>137.8±20.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>141.8±19.8</td>
<td>138.3±18.2</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>85±9.5</td>
<td>85±10</td>
<td>NS</td>
<td>81.6±9.5</td>
<td>75.6±11.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79.5±9.0</td>
<td>79.6±12.5</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>130±12</td>
<td>127±15</td>
<td>NS</td>
<td>123±13</td>
<td>129±11</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>117.3±15.1</td>
<td>123.3±13.5</td>
<td>**</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>5.2±0.5</td>
<td>3.5±0.3</td>
<td>***</td>
<td>5.0±0.5</td>
<td>3.5±0.3</td>
<td>****</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>185±41</td>
<td>427±106</td>
<td>***</td>
<td>888±186</td>
<td>350±108</td>
<td>****</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>14.7±3.8</td>
<td>23.8±5.9</td>
<td>***</td>
<td>24.6±5</td>
<td>8.1±3</td>
<td>****</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>24.0±4.6</td>
<td>7.0±2.6</td>
<td>****</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>35.7±4.7</td>
<td>36.7±3.8</td>
<td>NS</td>
<td>36±3.3</td>
<td>39±4.9</td>
<td>*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26.4±10.1</td>
<td>35.9±3.5</td>
<td>***</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.4±0.1</td>
<td>2.4±0.1</td>
<td>NS</td>
<td>2.6±0.15</td>
<td>2.7±0.11</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.7±0.1</td>
<td>2.6±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.1±0.2</td>
<td>1.5±0.4</td>
<td>***</td>
<td>1.7±0.2</td>
<td>0.8±0.2</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.7±0.3</td>
<td>0.8±0.2</td>
<td>****</td>
</tr>
<tr>
<td>Ca²⁺x PO₄²⁻ (mmol²/l²)</td>
<td>3.9±1.0</td>
<td>2.7±0.5</td>
<td>*</td>
<td>4.3±1.4</td>
<td>2.2±0.9</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.4±0.8</td>
<td>2.2±0.6</td>
<td>****</td>
</tr>
<tr>
<td>PTH (ng/L)</td>
<td>108.7±84</td>
<td>120.6±50</td>
<td>NS</td>
<td>225.7±220</td>
<td>307.7±216</td>
<td></td>
</tr>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>7.3±11.4</td>
<td>3.0±1.9</td>
<td>NS</td>
<td>14.4±15.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001

3. Pathological echocardiographic findings at baseline. (Studies I, II, III, IV and V)

Study II. The prevalence of LVH was not significantly different between Group 1 (63%) and Group 2 (66%).
Study V. All participating patients showed signs of concentric LVH, with a mean LVMI after HD indexed by height and body surface area of $73.3\pm18.4$ g/m$^{2.7}$ and $171.5\pm45.1$ g/m$^2$, respectively, and RWT $\geq 0.45$ in all cases.

Table 6. Prevalence of echocardiographic abnormalities in all studies.

<table>
<thead>
<tr>
<th>CE abnormalities</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
<th>Study V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic dysfunction</td>
<td>4 (15%)</td>
<td>2 (5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic dysfunction</td>
<td>11 (42%)</td>
<td>26 (65%)</td>
<td>10 (77%)</td>
<td>18 (100%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>LVH</td>
<td>13 (50%)</td>
<td>26 (65%)</td>
<td>12 (92%)</td>
<td>14 (66%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Left atrial enlargement</td>
<td>8 (30%)</td>
<td>13 (32%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV enlargement</td>
<td>3 (11%)</td>
<td></td>
<td></td>
<td></td>
<td>3 (17%)</td>
</tr>
</tbody>
</table>

CE – conventional echocardiography


4.1. Systolic function evaluated by conventional echocardiography and colour tissue velocity imaging at baseline. *(Studies II, III)*

Study II. Systolic velocities *(IVCV, PSV, SR)* did not differ between Group 1 and 2 and controls. Analysing only patients with normal EF, TVI demonstrated that patients with LVH had significantly lower IVCV (LVH $2.8\pm1.3$, no LVH $3.9\pm1.5$ and controls $3.8\pm1.5$ cm/s, p<0.05) and PSV (LVH $5.5\pm1.0$, no LVH $6.4\pm1.2$ and controls $6.4\pm1.3$ cm/s, p<0.05) than patients without LVH and controls. These findings were even more pronounced when sub-analyses of patients with different types of LVH were performed. Patients with eccentric LVH had significantly lower IVCV and PSV compared with controls, indicating contractility and contraction disturbances.
Study III. ESRD patients showed significantly lower PSV before HD compared with controls indicating systolic dysfunction. Analysing only patients with normal EF (excluding the two patients with low calculated EF), PSV was significantly lower in patients in comparison with controls (5.2±0.7 vs. 6.0±1.2, p<0.05).

Comments. TVI could detect contractility and contraction disturbances in CKD patients with LVH and normal EF. Before HD, TVI could also detect contraction disturbances in HD patients with normal EF.

Table 7. Conventional echocardiography and TVI indices of systolic function:

- Comparison between controls and group 1 (CKD, stages I, II and III) and group 2 (pre-dialysis CKD, stages IV and V). (Study II)

- Comparison between controls and ESRD patients. (Study III)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study II</th>
<th>Study III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Group 1</td>
</tr>
<tr>
<td>EF%</td>
<td>60.9±9.8</td>
<td>60.8±11</td>
</tr>
<tr>
<td>IVCV (cm/s)</td>
<td>3.8±1.5</td>
<td>2.9±1.4</td>
</tr>
<tr>
<td>PSV (cm/s)</td>
<td>6.4±1.3</td>
<td>5.5±1.2</td>
</tr>
</tbody>
</table>

* p < 0.05 (controls vs. ESRD before HD)

4.2. Correlations between LV systolic function and risk factors in HD patients

Study III – Inverse correlations were found between plasma levels of phosphate and mitral annular, basal, and mid wall PSV (r=-0.58, p<0.05; r=-0.84, p<0.001; r=-0.82, p<0.001, respectively) as well as between phosphate and mitral annulus IVCV (r=- 0.66; p=0.01). The calcium-phosphate product also showed inverse correlations with basal and mid wall PSV (r=-0.68, p<0.01; r=-0.66, p=0.01, respectively) and mitral annulus IVCV (r=-0.67; p<0.05).
4.3. Effects of HD on LV systolic function. (Study III)

Study III. IVCV, PSV and SR increased significantly after HD, indicating improved systolic function as shown in Figure 10.

Table 8. Conventional echocardiography and TVI variables of systolic function before and after HD. (Study III)

<table>
<thead>
<tr>
<th>Variables</th>
<th>STUDY III</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before HD</td>
<td>After HD</td>
</tr>
<tr>
<td>EF (%)</td>
<td>61±15</td>
<td>58±10</td>
</tr>
<tr>
<td>IVCV (cm/s)</td>
<td>4.0±1.7***</td>
<td>5.5±1.9</td>
</tr>
<tr>
<td>PSV (cm/s)</td>
<td>5.0±0.87**</td>
<td>5.7±0.84</td>
</tr>
<tr>
<td>SR (s⁻¹)</td>
<td>0.7±0.2**</td>
<td>0.9±0.2</td>
</tr>
</tbody>
</table>

** P < 0.05, *** P < 0.01

Figure 10. Isovolumetric contraction velocity (IVCV), peak systolic velocity (PSV), and strain rate (SR), before and after HD at basal region (Study III).

4.4. Effects of furosemide on left ventricle and right ventricle systolic function in HD patients

Study IV. No significant changes in cardiac output, IVCV, PSV, SR, LV MAM, were observed, indicating that neither 40 mg (plus additional 40 mg after 30 min) nor 250 mg of furosemide had any measurable effects on LV and RV systolic function.
5. Evaluation of diastolic function

5.1. Diastolic function in predialysis (Stages IV and V) and HD patients evaluated by conventional echocardiography and TVI at baseline. (Studies II, III, IV)

Study II. E’ velocities were significantly lower in predialysis CKD (Stages IV and V – group 2) compared with controls but there was no significant difference between controls and CKD (Stages I, II and III- group 1), indicating more pronounced diastolic dysfunction in the patients with severe CKD. After omitting patients with evidence of ischemic heart disease, the E’ velocities were still lower in Group 2 compared with controls (6.1±1.9 vs. 8.0±2.9 cm/s, p<0.05).

Study III. In ESRD patients the average of E’ velocities were significantly lower compared with controls, indicating altered diastolic filling.

Study IV - All patients presented with high values of E/E’ (>10), indicating a capillary wedge pressure > 12 mmHg.

Table 10. Variables of diastolic function. Study II: Comparison between controls and group 1(CKD - stages I, II and III) and group 2 (CKD - stages IV and V). Studies III and V: Comparison between controls and HD patients.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>STUDY II</th>
<th>STUDY III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Group 1</td>
</tr>
<tr>
<td>Mitral E (cm/s)</td>
<td>72.7±17</td>
<td>64.8±13</td>
</tr>
<tr>
<td>Mitral A (cm/s)</td>
<td>69.9±17</td>
<td>66.6±19</td>
</tr>
<tr>
<td>Mitral E/A</td>
<td>1.0±0.4</td>
<td>0.9±0.4</td>
</tr>
<tr>
<td>Diastolic dysfunction #</td>
<td>14 (63%)</td>
<td>12 (66%)</td>
</tr>
<tr>
<td>E’(cm/s)*</td>
<td>8.0±2.9</td>
<td>6.8±2.2</td>
</tr>
<tr>
<td>A’ (cm/s)*</td>
<td>7.6±2.2</td>
<td>6.6±1.8</td>
</tr>
<tr>
<td>IVRT (ms)**</td>
<td>88±26</td>
<td>121±18</td>
</tr>
</tbody>
</table>

* < 0.05, ** < 0.001, a (controls vs. group 2), b (controls vs. before HD).
# Diastolic dysfunction evaluated with conventional echocardiography
5.2. Correlations between diastolic function parameters and risk factors.

*Study II* - In Group 2 (predialysis CKD, stages IV and V): E’ velocities correlated negatively with plasma PTH ($r^2=-0.50$, $p<0.05$), systolic blood pressure (SBP) ($\text{Rho}=-0.68$, $p<0.01$) and pulse pressure ($r^2=-0.50$, $p<0.05$). A’ velocities correlated positively with PTH ($r^2=0.62$, $p<0.01$). IVRT showed positive correlations with PTH ($\text{Rho}=-0.51$, $p<0.05$) and DBP ($\text{Rho}=0.56$, $p<0.05$). Linear regression analysis demonstrated that E’ velocities are dependent on SBP ($p<0.001$) and also that E’/A’ are dependent on PTH ($p<0.005$).

5.3. Effects of HD on diastolic function. (*Study III*)

*Study III*. There was a significant correlation between change in body weight and change in standard Doppler E ($r=-0.79$, $p<0.01$). The HD session decreased E/E’, indicating a decrease in LV filling pressure as shown in *Table 11*.

*Table 11*. Conventional echocardiography and TVI parameters of diastolic function before and after HD in *Study III*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before HD</th>
<th>After HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral E (cm/s)</td>
<td>88±31**</td>
<td>69±17</td>
</tr>
<tr>
<td>Mitral A (cm/s)</td>
<td>89±31</td>
<td>86±28</td>
</tr>
<tr>
<td>Mitral E/A</td>
<td>1±0.3</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>E’ (cm/s)</td>
<td>5.7±1.7</td>
<td>5.2±1.7</td>
</tr>
<tr>
<td>A’ (cm/s)</td>
<td>6.6±1.7*</td>
<td>7.4±1.5</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>101.2±32</td>
<td>93.4±22</td>
</tr>
<tr>
<td>E/E’</td>
<td>16.7±7.7*</td>
<td>12.2±4</td>
</tr>
</tbody>
</table>

* $p<0.05$, ** $p<0.01$
5.4. Effects of Furosemide on LV and RV diastolic function in HD patients

Study IV. No significant changes in right ventricle and left ventricle E’, A’, E’/A’ and left ventricle IVRT and LV filling pressure were observed, indicating that neither 40 mg (plus additional 40 mg after 30 min) nor 250 mg of furosemide had any measurable effects on LV filling pressure and LV and RV diastolic function.

6. LV dyssynchrony in HD patients (Study V)

6.1 Evaluation of LV dyssynchrony in HD patients.

Before HD, all 13 patients presented with LV dyssynchronous areas characterized by a LV mechanical maximum delay >105 ms (300±89 ms), standard deviation >34.4 (27) and at least one delayed segment at basal or midwall level.

6.2. Effects of HD on LV dyssynchrony

HD resulted in normalization of LV synchronicity in 3 (23%) patients. Furthermore, HD caused a significant decrease in the maximum systolic LV mechanical delay, 327 (range: 119 to 303) vs. 265 (range: 50 to 410), paralleled by a significant reduction in the percentage of segments with >105 ms delay, 33 (range: 8.3 to 83) vs. 16 (range 0 to 50) as shown in Figure 11.

Table 13. Maximum delay and % of delayed segments before and after HD in Study V.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before HD</th>
<th>After HD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max delay (ms)</td>
<td>300.8±89.2</td>
<td>225.5±116</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>% delayed segments</td>
<td>36.5±14</td>
<td>19.2±14</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
7. Main findings

1. The evaluation of LV longitudinal motion can be performed by 4 different methods: M-mode, anatomical M-mode, tissue Doppler derived displacement and 2 dimensional strain imaging, however the methods are not interchangeable.

2. TVI improved the evaluation of systolic function in CKD patients with LVH and ESRD patients. In CKD patients with LVH and normal EF and ESRD patients with normal EF, TVI was able to detect impaired systolic function.

3. Phosphate levels seem to be related with systolic dysfunction in ESRD before HD, indicating a possible role of hyperphosphatemia on systolic dysfunction observed before HD.

4. Diastolic dysfunction was found to be more pronounced in patients with severe CKD in comparison with patients with mild/moderate CKD when evaluated using TVI.

5. PTH seems to be associated with diastolic dysfunction, in patients with severe CKD

6. LV dyssynchrony was present in ESRD patients with LVH before HD.

7. A single session of HD improved LV myocardial contraction, contractility and dyssynchrony in ESRD patients without signs of heart failure or ischemic heart disease.

8. Low and high doses of intravenous furosemide did not induce any significant changes in cardiac hemodynamics in anuric hemodialysis patients.
Discussion

1. General discussion

Cardiovascular disease is well documented as the leading cause of mortality in patients with chronic kidney disease, a fact that certainly challenges the scientific community and calls for increased efforts to search for more effective ways to prevent, identify and treat cardiac complications in this patient category.

In recent years, new developments in echocardiographic techniques have created a basis for much more detailed and accurate evaluation of cardiac pathologies in clinical practice and research. Colour tissue velocity imaging (TVI), two dimensional strain imaging (2D-SI) and tissue synchronization imaging (TSI) are some of these newly developed techniques and were applied in the present thesis.

The conceptual framework for the current series of studies involves certain important factors. First, TVI technique allows the evaluation of new aspects of cardiac function, such as right and left ventricular longitudinal myocardial motion, myocardial deformation,
isovolumetric contraction, LV filling pressure and LV synchronism which can increase the understanding of pathophysiological mechanisms of cardiovascular involvement in CKD. Second, TVI has been shown to improve the echocardiographic evaluation of patients with LVH, a very common pathological entity in CKD patients. Third, TVI provides the possibility of an objective, quantitative and reproducible evaluation of cardiac function, thereby improving the evaluation of changes in cardiac function after different interventions. Fourth, only few studies conducted hitherto evaluated myocardial velocities in CKD patients; and the majority of them focused on diastolic function. In the present thesis, several conventional echocardiography and TVI variables were used in order to obtain a global estimation of cardiac function and dyssynchrony in CKD patients. Finally, since TVI was used to evaluate myocardial longitudinal motion in all studies presented in this thesis, a methodological study evaluating and comparing different methods to measure longitudinal motion was also included.

2. Echocardiographic assessment of left ventricular longitudinal motion. (Study I)

The evaluation of longitudinal myocardial motion is of importance, since it plays an important role in LV systolic performance (141). It is the first myocardial motion to be altered in hypertension (142). It is also the first to be affected by ischaemic myocardial injury (143), since the systolic interstitial pressure and flow resistance in the inner layer of the myocardium is higher, and the sub-endocardial longitudinally orientated fibres are hence more exposed to the deleterious effects of the impaired oxygen supply resulting from coronary atherosclerosis (144,145).

Longitudinal motion can be evaluated by measurements of mitral annulus motion (MAM) toward the apex. In Study I, MAM was assessed by measurements of the longitudinal
myocardial displacement derived from TVI myocardial velocities (Tissue Doppler displacement – TDD) and the results were compared with the results obtained using three other methods. The objective was to determine whether TDD measurements are comparable and interchangeable with an established method (M-mode), M-mode with angle correction (anatomical M-mode), or with a new method that theoretically measures the true motion of the myocardium (2D-SI). The results demonstrated that all 4 techniques are related to each other, but not interchangeable.

The first method that describes measurement of MAM is based on the use of M-mode, which is an established indicator of LV function with prognostic implications (35,141,146).

A possible contribution of M-mode to the non-interchangeability of the methods evaluated in this study may arise by the fact that M-mode can both under or overestimate the true values of MAM. The combination of the longitudinal and radial motion of the ventricular wall may result in a not strictly longitudinal displacement of MAM. Consequently, depending on the relation between the angle of insonation and true movement of the LV wall, MAM measurements obtained by M-mode can be under or overestimate, this effect could be minimized by the use of angle correction applying anatomical M-mode technique. However in the group of individuals analysed in the study, the necessary angle correction was only up to 8° and no significant difference was found among MAM obtained by M-mode and AM-mode.

MAM measurements can also be obtained by integrating the long-axis myocardial tissue velocity curve obtained using TVI. The use of TVI for MAM measurements is particularly useful in the setting of poor 2D image quality. The contribution of TVI to the lack of interchangeability was the significantly lower values of MAM obtained by this technique in comparison with all three other methods. One possible reason for this finding could be related to the fact that TDD is a measure of the mean value of myocardial velocities through the
longitudinal motion (seen from a fixed point), and myocardial velocities decrease from base to apex (147).

Finally, MAM measurements can also be obtained using two dimensional strain imaging (2D-SI). 2D-SI is novel software for real-time quantitative echocardiographic assessment of myocardial motion. This technique measures the motion of the myocardium by following the movement of natural acoustic markers, which move together with the tissue and do not change their pattern significantly between adjacent frames. Therefore, 2D-SI is an angle independent measurement of MAM and hypothetically, can be used as a reference method since it seems to measure the true movement of the myocardium. In view of the fact that both M-mode and TDD are angle dependent, different values can be obtained in comparison with 2D-SI measurements.

In summary, Study I showed that the four evaluated methods to assess MAM are not interchangeable, and the knowledge of their limitations is important in order to obtain and interpret MAM more accurately. This finding is of particular interest in clinical practice, since it is important for the sonographer to know that, when borderline values are obtained using one method, normal reference values from another method cannot be used. Regarding angle correction for MAM measurements, our results do not support the necessity of using AM-mode with angle correction up to 8°. However, further studies are needed to evaluate the possible necessity of using AM-mode in order to correct for higher angles when measuring MAM.

2. Evaluation of cardiac function in predialysis CKD (Stages I, II, III, IV and V) and HD patients using colour tissue velocity imaging. (Studies II and III)

One of the most common echocardiographic findings in CKD is LVH, which has been shown to be an independent factor associated with mortality (119). LVH is already present in
earlier phases of CKD (38.9% (106) to 87% (148)) and tends to progress in parallel with the decline of renal function (104-106,149) reaching 75% of patients at the start of dialysis (107). In Study II, 40 pre-dialysis CKD patients were studied and a high prevalence of LVH was found here also. LVH was present in 63% of the patients with CKD (Stages I, II and III), and in 66% of the patients with predialysis CKD (Stages IV and V). The data from Study III cannot be used to evaluate the prevalence of LVH in hemodialysis patients but for the purpose of this discussion, it is worth to mentioning that LVH was present in 92% of the studied patients.

When a group of patients with such a high prevalence of LVH is studied, it should be kept in mind that the value of the conventional echocardiography for the evaluation of cardiac function in hypertrophic myocardium is limited (150). For example, previous studies have shown that in LVH contractile function may be depressed despite the presence of normal LV fractional shortening (2,151-153). Furthermore, conventional echocardiography does not allow the detection of LVH at its transitional stage from compensated hypertrophy to myocardial dysfunction and heart failure (154,155). These diagnostic shortcomings are, to some extent, related to the high relative LV wall thickness. Thus, as only small changes occur during systole in the outer contour of the heart (156), the contracting and thickening myocardium will decrease LV end systolic diameter in the short axis. Since EF and % FS reflect stroke volume derived from short axis changes in LV internal diameter, the ejection fraction will remain normal, or even increase, despite the presence of disturbed contractility (2) as shown in Figure 12.

With the development of TVI, the echocardiographic evaluation of systolic and diastolic function in LVH has improved (157). This improvement may be related to the fact that TVI measures the changes in the myocardium itself and not in the stroke volume. Additionally, TVI evaluates longitudinal motion and an earlier detection of cardiac dysfunction can thus be
obtained in comparison with the evaluation of radial motion, since longitudinal motion is the first to be altered during myocardial injury (156,158).

TVI provides the possibility to differentiate between physiological and pathological forms of LVH allowing the detection of compensatory hypertrophy at its transition to heart failure (153). Previous studies have demonstrated that peak systolic velocity is a sensitive marker of impaired LV systolic function even in individuals with “normal” LV ejection fraction (159,160). Accordingly, in the present studies, low values of systolic myocardial velocities indicating the presence of systolic dysfunction were observed in CKD patients with LVH (Study II) and HD patients (Study III) (92% with LVH) even in the presence of a normal ejection fraction.

**Figure 12. Effects of left ventricular wall thickness in stroke volume measurements**

As far as the diastolic function is concerned, in study II, in accordance with previous studies in patients with preserved renal function, the employment of the TVI diagnostic criteria of E´<8 cm/s (66) allowed an easier discrimination between patients with normal and
pseudo-normal pattern of diastolic function (68,157). Additionally, the application of TVI resulted in the identification of more accentuated diastolic dysfunction in patients with severe CKD. This finding was possible because different from the changes in diastolic mitral inflow pattern (E, E/A) as shown in Figure 14 and other standard Doppler indexes that changed in a typical parabolic pattern during the progression from normal to severe diastolic dysfunction, early diastolic myocardial velocities (E’) progressively decrease with the progression of the disease (66).

Since impaired ventricular diastolic function is considered to be one of the major causes of cardiac failure and LVH regression induced by the treatment in ESRD patients had a favorable effect on cardiovascular mortality (116), the diagnostic information provided by TVI could be of importance and can be used as therapeutic guidance and prognostic instrument.

4. The evaluation of left ventricular synchronicity in HD patients using tissue synchronization imaging

Previously, a prolonged duration of QRS complex in surface electrocardiogram was considered as a marker of LV dyssynchrony and used as a tool in the selection of patients for cardiac resynchronisation therapy. However, the accuracy of the QRS duration criteria appears to be poor as significant mechanical dyssynchrony is usually absent in 30-58% of patients with QRS duration longer than 120 ms whilst, being at same time present in 65% of individuals with QRS complex shorter than 120 ms (14,161).

The recent introduction of tissue synchronisation imaging (TSI), have improved and opened entirely new possibilities for imaging and quantification of LV dyssynchrony (77,162).
LV dyssynchrony was found to be present in patients with left ventricular hypertrophy and congestive heart failure. In congestive heart failure it has been recognized as a significant contributor to increased morbidity and mortality, independent of reduced EF and
increased QRS-complex width (163,164). In ESRD patients, left ventricular hypertrophy, heart failure and complex arrhythmias are associated with increased cardiovascular mortality as well, but the role of LV dyssynchrony in the pathophysiology of cardiovascular complications in ESRD patients without heart failure and with normal QRS duration, is still not known.

The hypothesis of Study V was that LV dyssynchrony might be present also in ESRD patients with LVH, and that HD might produce transient improvement of LV systolic synchronicity. The results of this pilot study demonstrated that LV dyssynchrony indeed was present in the studied ESRD patients, and in some of them, this abnormality diminished after HD. The possible factors associated with LV dyssynchrony detected in Study V will be discussed in the following sections.

The identification of LV synchronicity disturbances in patients with ESRD is of clinical importance since the uniformity of LV contraction is a prerequisite for effective and energetically efficient LV performance (13). Mechanical LV dyssynchrony has marked deleterious effects on ventricular pump function leading to mitral regurgitation (13) as well as impairments in systolic and diastolic performance, electrophysiology and regional myocardial perfusion and metabolism (13,165). Mechanical LV dyssynchrony impairs systolic performance by creating an imbalance of forces within the myocardium with regionally poorly coordinated stretching and shortening of myocardial fibres and subsequent abnormal stress to myocardial tissue (13). As a consequence, the kinetics of regional LV contractions is not effectively coupled to the systolic pressure build-up, but rather causes an intracavitral shift of intraventricular blood volume. Chronic dyssynchrony leads to ventricular remodeling of both early and late activated segments (166), with increasing ventricular cavity volumes and changes in LV geometry (167). Similar effects can be expected in ESRD patients and the
occurrence of LV dyssynchrony, if confirmed in a larger patient population, ought to be seen as an important risk factor and bad prognostic omen, as it is in patients with heart failure (163).

5. The evaluation of cardiac effects of different interventions in HD patients using colour tissue velocity imaging. (Studies III, IV and V)

5.1. Acute effects of hemodialysis on cardiac function and dyssynchrony

The evaluation of cardiovascular status and its response to acute changes in volume and solutes induced by HD is important for the management of patients with ESRD. This is, however, not an easy task since the majority of the methods to measure cardiac function are load dependent. Consequently, the detected changes may reflect the effects of load changes and not real changes in cardiac function. For this reason, a load independent method would be the ideal to evaluate the true effects of HD on myocardial function.

The most reliable load independent method to evaluate LV contractility is the simultaneous measurement of LV pressure and volume and the analysis of thus obtained pressure-volume curve (22). However, the procedure has the disadvantage of being invasive and is therefore seldom used in clinical studies.

The method that has been commonly used to evaluate the acute effects of HD on cardiac function is conventional echocardiography. However, several factors limit the possibilities to accurately evaluate changes in cardiac function after HD using this method, and the obtained results are conflicting (124,168).

The accuracy of the conventional echocardiography measurements depend on visual and semi quantitative assessment of wall motion and are subjected to a considerable inter-observer variability (169) making this technique inappropriate to evaluate acute, short lasting and subtle changes induced by HD. Most importantly, altered loading conditions may profoundly
influence the most commonly used indices of cardiac function causing a significant misinterpretation of the data (124,170,171). In Study III, for example five out of five patients in whom HD induced an increase in the calculated EF and FS%, had reduced systolic blood pressure following dialysis, whereas systolic blood pressure increased in five out of seven patients in whom calculated EF and %FS decreased following HD, demonstrating the effects of after-load changes on these systolic function parameters. Additionally, HD also elicited significant changes in the early diastolic rapid filling wave ($E$) recorded by pulsed Doppler and these alterations correlated well with changes in weight, thus demonstrating the pronounced preload dependence of Doppler indices of LV diastolic function (170,171). The effects of load conditions on mitral inflow are demonstrated in Figure 14.

In the present thesis, TVI was used to evaluate the acute changes in cardiac function and dyssynchrony induced by HD. One of the most important aspect favoring the inclusion of TVI in the echocardiographic evaluation of CKD patients is the improvement offered by this method to assess changes in cardiac function induced by different interventions (49,65,172). The main reason for this improvement is that all variables are quantitative and can be measured objectively and with high reproducibility (44,173). The second reason is the possibility to detect early changes in cardiac function since longitudinal motion can be evaluated. The third reason is that TVI has been shown to improve the sensitivity of echocardiography to detect subtle changes in cardiac function induced by drug intervention (49,65,172). And finally, following the introduction of TVI, two new valuable variables in the evaluation of changes in cardiac function after HD have been added to the echocardiography arsenal. The first is the isovolumetric contraction velocity and the second is the estimate of LV filling pressure.

Another factor that could be an advantage for using TVI in the evaluation of changes in cardiac function induced by HD is the fact that some of the TVI variables have been described
to be less load dependent. However, the load dependency is also a problem for TVI measurements. Regarding systolic function, PSV (174) and SR (61) have been shown to be load dependent and consequently, the variables do not really precisely define contractility. On the other hand, isovolumetric contraction velocity (IVCV) can provide a less load dependent measurement of myocardial contractility since most of the LV systolic pressure generation occurs when the aortic and mitral valves are closed and no changes in LV blood volume occur (59). In Study III, HD resulted in increased values of PSV and SR, but also IVCV increased, thus indicating a real increase in myocardial contractility.

Regarding diastolic function, the results of some preliminary studies evaluating the effects of pre-load changes on diastolic myocardial velocities suggested that these measurements may be load independent. However, these results were not confirmed by subsequent studies (67, 71, 73). Consequently, it is not possible to determine the real effects of the metabolic changes induced by HD on diastolic function. The real usefulness of TVI is the possibility to estimate LV filling pressure providing more information about volume status and its changes induced by HD. In Study III, it was possible to detect a high pulmonary capillary wedge pressure before HD that significantly decreased after HD.

There are few previous studies evaluating the effects of HD on myocardial velocities. However, there are some important differences between these studies and ours. The first difference is related to the method used to measure myocardial velocities. In the above-mentioned studies pulsed tissue Doppler was used, a technique different from TVI that was applied in our studies. One disadvantage of using pulsed tissue Doppler lies in the risk to overestimate the true velocity value. Additionally, the limitation in the data acquisition to only one myocardial data point at a time, and the fact that the acquired data has to be analyzed online, makes the analysis of changes in global cardiac function very time consuming. The advantage of using TVI as compared to pulsed tissue Doppler lies in the possibility to obtain
myocardial information practically at any discrete point within the imaged myocardial wall during the same cardiac cycle and with better temporal resolution. And finally, it is possible to store the raw data for subsequent off line analysis, decreasing the time of the examination for the patient and also the time for the analysis of changes in global cardiac function, consequently enabling far more efficient use of TVI in the research field.

The second difference between the present results and the results of above-mentioned studies is related to the type of the performed measurements. The majority of the previous studies focused mainly on the effects of pre-load changes on diastolic myocardial velocities (74,175,176). To our knowledge only in two studies, performed by Gilmartin et al. (122) and Bauer et al (73), pulsed tissue Doppler was used for measurements of myocardial velocities in order to evaluate the effects of HD on systolic function, finding the same increase in myocardial velocities as was observed in Study III.

However, in these studies (43,(73)) only regional myocardial velocities were evaluated and it is not possible to make an assessment of global function based on measurements in only 2 LV walls. In our studies, using TVI, it was possible to evaluate not only the effects of HD on diastolic function but also the effect of HD on regional and global left ventricle systolic and diastolic function (51), isovolumetric contraction, myocardial deformation, myocardial displacement and LV filling pressure. Moreover, the possibility offered by the applied TVI software to evaluate simultaneously 12 segments made it easier also to detect possible changes on LV dyssynchrony.

In this way, much more information could be gathered about cardiac function and its changes induced by HD in ESRD patients. And the analysis of TVI variables demonstrated: 1. an elevated LV filling pressure before HD, which decreased significantly after HD, 2. decreased myocardial contraction (PSV) before HD and an improvement in myocardial contractility and
contraction after HD and 3. the presence of LV dyssynchrony prior to, and its partial reduction after HD.

The possible factors related to the improvement of LV function and dyssynchrony after HD is discussed in a following section.

5.2. Acute effects of furosemide on cardiac function evaluated using colour tissue velocity imaging

Congestive heart failure is a common clinical condition in CKD patients and acute pulmonary oedema is one of the leading causes for emergency dialysis. Given the respiratory distress involved, initial stabilisation using non-dialysis procedures is important, mainly in the event of immediate dialysis being impossible. Diuraesis with consequent decrease of the volume overload is the main effect for symptom relief in patients with preserved renal function. Some studies have suggested that furosemide also has extra renal effects, probably vascular effects (126,127) that may justify its use also in anuric patients. However, studies evaluating the vascular effects of furosemide in ESRD patients have shown conflicting results. When five anephric patients were investigated by Johnston et al (129), no changes in venous capacitance and limb blood were observed. In contrast, Mukherjee et al (177), evaluating eleven functionally anephric hypertensive patients, demonstrated a short-lasting rise in forearm blood flow. In contrast to the findings of these authors (129,177), Schmieder et al (178) demonstrated a significant furosemide-induced increase in total peripheral resistance.

In Study IV the acute effects of low and high intravenous doses of furosemide on central cardiac hemodynamics in anuric HD patients were evaluated using conventional echocardiography and TVI. Both techniques allowed a detailed and sensitive analysis of changes in RV and LV hydrodynamics and mechanics (see Figure 13). The results demonstrate that even with the use of a sensitive method we did not note any effects of furosemide on central hemodynamics, which contrasts with the results of previous studies.
showing a vascular effect of furosemide in anuric patients. This discrepancy between the results may be possibly explained by differences between the respective studied populations. In contrast to the present experiments, in the study of Schmieder et al (178), the patients were studied on a non-dialysis day whereas Dikshit et al (127) investigated three anuric patients with acute renal failure.

The absence of changes in central hemodynamics after low and high intravenous doses of furosemide in anuric ESRD patients may have three possible explanations: 1) Furosemide has no vascular effects in the presence of ESRD; 2) Furosemide may cause peripheral vascular effects in the presence of ESRD, but these are so subtle that they do not significantly influence cardiac hemodynamics. 3) Although a recently published study showed that TVI was able to detect small changes in myocardial velocities during afterload reduction in hypertensive patients treated with valsartan (172), TVI may not be sensitive enough to detect very subtle changes in central hemodynamics after furosemide infusion. If this should be the case, a question that arises is whether such very subtle changes are of clinical importance. Taken together, the results of Study IV do not support the use of furosemide in anuric ESRD patients with acute pulmonary edema.

6. Factors influencing systolic and diastolic function in pre-dialysis CKD (Stages IV and V) (Study II) and HD patients (Study III), and LV synchronicity in HD patient (Study V)

The presence of myocardial dysfunction in CKD (Study II) and in ESRD patients before HD and the improvement of myocardial contractility, contraction and synchronism after dialysis observed in Studies III and V indicate that non-transient and transient factors should contribute to cardiac dysfunction and dyssynchrony in CKD patients.
Hypertension and PTH were the factors found to be related to diastolic dysfunction in patients with severe CKD (Study II). Left ventricular hypertrophy was the non-transitory factor found to be related to LV dyssynchrony in ESRD patients (Study V). Accumulation of fluid and solutes were the transitory factors found to be related with systolic dysfunction and dyssynchrony in Studies III and V.

**Figure 14.** Changes in the pattern of mitral flow and velocity ratio (E/A) ratio during transition from normal diastolic function to severe dysfunction and the effect of preload and changes in the pressure difference between left atrium and left ventricle. In the presence of relaxation disturbances, volume overload increasing the transmitral pressure gradient can shift the pattern from relaxation disturbance to pseudonormal or restrictive pattern. When this augmented pre-load decreases, a shift in the opposite direction occurs, going back to a relaxation pattern.

It must be clarified, however, that despite the found correlations, the number of patients in Studies III and V is limited and it is therefore difficult to draw any definite conclusions. Since these correlations are in accordance with what was observed in previous studies, we discussed these possible associations.
6.1. Factors influencing cardiac function in predialysis CKD patients (Stages IV and V)

Hypertension. In Study II, a negative association between systolic blood pressure and diastolic function was observed. This finding is in accordance with previous studies in hypertensive patients in whom impaired ventricular diastolic function was a common finding. The vast majority of patients with significant CKD present high blood pressure (179) and a clear relationship between higher blood pressure values and a worse cardiovascular outcome has been shown, emphasizing the importance of an adequate blood pressure control in the management of patients with CKD (180,181).

Parathyroid hormone (PTH). In Study II, a negative association between PTH and early diastolic myocardial velocities (E') was observed in predialysis CKD patients (Stages IV and V). Secondary hyperparathyroidism starts at earlier stages of CKD and will occur in the majority of patients as glomerular filtration rate (GFR) approaches CKD stage V (96). High levels of PTH have been shown to play a role in the development of uremic cardiomyopathy (182). PTH seems to damage cardiac myocytes (182), to decrease cardiac contractility (183), to have a permissive role in interstitial myocardial fibrosis (184), BP independent wall thickening of intramyocardial arterioles and impaired vasodilation (185).

6.2. Factors influencing LV synchronicity in HD patients (Study V)

Left ventricular hypertrophy. The LV dyssynchrony observed before HD can be, at least partly, caused by the presence of LVH. LVH has been found to be associated with LV asynchrony in patients with pressure overload and preserved renal function (186). In ESRD patients, the maladaptive LVH with its abundant fibrosis and structural heterogeneity (121), would provide even better substrate for the occurrence of dyssynchronous systolic LV motion.
6.3. *Transient factors influencing cardiac function and synchronicity*

*Fluid retention.* In ESRD patients, the fluid state is in a continuous disequilibrium characterized by a rapid reduction during dialysis and a slow increase between dialysis sessions. Both extremes of fluid state have to be compensated for by the cardiovascular system, and cardiovascular instability is the most common complication during HD. The imbalance between the amount of fluid removed and the refilling capacity of the intravascular compartment can occur originating from erroneous dialysis target weight, the mode of the dialysis technique, and the altered response of a pathological cardiovascular system to the stress of fluid fluctuation.

In *Study III*, there was a significant increase in SR, PSV and IVCV after HD showing an increase of myocardial contraction and contractility. The significant decrease in weight, LV filling pressure and LV end diastolic diameter and the absence of significant changes in blood pressure after HD, indicates a decrease in pre-load with no significant changes in afterload. The expected response to an acute decrease in pre-load caused by HD in patients with normal cardiac function (in *Study III*, none of the patients had clinical signs or symptoms of heart failure or had severe heart dilatation - mean LVEDd 46.9±5.2 mm) would be, according to Frank Starling law, a decrease of myocardial contractility, i.e. the reaction opposite to that observed in *Study III*. This suggests, that volume overload or uremic toxins accumulated before HD, may cause a transitory impairment of myocardial contractility, or that ESRD patients with normal systolic function are able to compensate the acute effects of changes in volume by increasing myocardial contractility. An improvement of myocardial perfusion can be also a possibility since a decrease in LV end diastolic volume improves the balance between cardiac oxygen demand and supply (187). Finally, another factor that could contribute to the improvement of systolic function observed in *Study III* is the improvement of myocardial synchronism observed in *Study V*. 
Study V. In study V, an improvement of myocardial synchronism was observed after HD. The effect of increased afterload on LV dyssynchrony was previously demonstrated by Villari et al (186) in patients with aortic stenosis in whom LV nonuniformity normalized after valve replacement. In the present study, the improvement of LV dyssynchrony after HD as well as the positive association between the percentage of delayed segments and LV end diastolic diameter before HD, suggests that increased pre-load may also influence LV synchronicity. The possible interaction between load and dyssynchrony can be suggested by the results of previous studies showing that an acute sustained LV dilatation caused a shortening of action potential duration and myocardial refractoriness (188,189) and that a stretching of cardiac muscle decreased conduction velocity (190).

The clinical implications of the results of this pilot study are not known at present. However, it would be of interest to evaluate possible benefits of a more frequent HD on LV dyssynchrony. Such a notion is supported by the results of an animal study (191) in which increase in preload applied over seconds to minutes was found to affect action potential duration, whereas sustained increases in load (over days to weeks) resulted in heterogeneous action potential duration prolongation in LV, with interventricular dispersion of repolarization and increased arrhythmia susceptibility.

Solute retention. Calcium–phosphate metabolism disorders, particularly high serum phosphorus levels, contribute significantly to the high rates of CV mortality among CKD patients (192). Acutely, changes in phosphate concentration can affect myocardial contraction and may justify the inverse correlation between myocardial contractility (IVCV) and contraction (PSV) and phosphate observed in Study III. Inorganic phosphate (Pi) concentrations have been reported to have depressant effects on myofilaments (193) and inhibition of the Ca$^{2+}$-activated force (Fmax) in skinned fibers (194,195). Free energy available from ATP hydrolysis has been shown to be reduced when Pi is increased (196), and
inorganic phosphate may combine with Ca\(^{2+}\) and form an insoluble precipitate of calcium phosphate (CaPi) leading to reduced sarcoplasmic reticulum Ca\(^{2+}\) release which may also result in a reduction of contraction force (197).

7. Limitations

To be able to have less confounding factors in Studies III and V, we excluded patients with previous myocardial infarction, arrhythmia, conduction disturbances, severe valvular heart disease, and symptoms of congestive heart failure (New York Heart Association class III and IV), signs of pericardial disease and clinical or electrocardiographic signs of coronary artery disease. This selection, allowed us to analyze the presence of disturbed cardiac function and LV dyssynchrony without the influences of other factors that could cause misinterpretation of the results. Mainly, ischemic heart disease that by causing diffuse and regional tissue injury can result in low myocardial velocities and LV dyssynchrony (198). As a consequence of these exclusion criteria, it does not appear likely that the results were affected by coronary heart disease to a significant extent. However, this influence cannot be completely ruled out. This selection, on the other hand, made it difficult to find a larger number of patients, as the majority of the dialysis patients met at least one of these criteria. Therefore, with the resulting small sample size it was not possible to draw any definitive conclusions regarding the associations between risk factors and myocardial velocities and LV dyssynchrony.

One limitation of TVI is the fact that myocardial velocities may have heterogeneous distribution and can be affected by heart translation and tethering of adjacent myocardial segments (60,199). We therefore used strain rate as an additional marker of contraction.
8. Future projects

With the results obtained in the studies presented in this thesis, new projects are in development with the aim to determine: 1. the cause of the observed improvement of cardiac function after HD (solute removal or fluid removal), 2. the acute effect of HD on cardiac function and dyssynchrony in patients with different degrees of cardiac dysfunction, 3. the prevalence of LV dyssynchrony and its prognostic value in patients at different stages of CKD and 4. the prognostic value of TVI variables in CKD patients.
In the studied population of patients with chronic kidney disease the results obtained allow the following conclusions:

1. Despite the good correlation between the results of mitral annulus motion measurements obtained with colour tissue velocity imaging technique, M-mode, anatomical M-mode and 2-dimensional strain imaging, the methods are not interchangeable. The M-mode method is insonation angle-dependent and may produce over- or underestimation of the true annulus movement whereas this movement is usually underestimated when using colour tissue velocity imaging.

The two dimensional strain imaging allows the imaging and tracking of natural acoustic markers (reflectors/speckles) in the myocardium and has the advantage of being insonation angle independent and theoretically provides a true measure of local tissue motion.
2. Colour tissue velocity imaging complements the echocardiographic evaluation of cardiac function in patients at different stages of chronic kidney disease. In patients with predialysis chronic kidney disease (Stages IV and V), TVI revealed more accentuated diastolic dysfunction than in patients with less advanced CKD (Stages I, II and III). In hemodialysis patients, TVI measurements obtained before the start of the hemodialysis procedures, revealed systolic and diastolic disturbances.

In predialysis chronic kidney disease (Stages IV and V) with left ventricular hypertrophy and hemodialysis patients, both with normal ejection fraction, colour tissue velocity imaging detected disturbances in myocardial contractility and contraction.

Furthermore, the results suggest that left ventricular disturbances could be related to hyperparathyroidism, phosphate levels, hypertension and left ventricular hypertrophy.

3. Using tissue synchronization imaging, left ventricular dyssynchrony was detected in hemodialysis patients with left ventricular hypertrophy.

4. Colour tissue velocity imaging technique allows objective quantification of acute changes in cardiac function induced by different therapeutic interventions in hemodialysis patients. Using colour tissue velocity imaging it was possible to detect an increase of myocardial contraction and contractility and also an improvement of left ventricular dyssynchrony after hemodialysis, suggesting a mechanistic relevance of volume overload, and perhaps accumulation of uremic toxins.

Using colour tissue velocity imaging it was also possible to demonstrate that low and high doses of furosemide do not have any significant effects on central cardiac hemodynamics. Therefore, the use of furosemide infusion in anuric end stage renal disease patients with acute pulmonary oedema is not supported by the results of Study IV.
Summary

The results of the present thesis demonstrate that the addition of colour tissue velocity imaging improves the ability of echocardiography to detect earlier stages of cardiac dysfunction, to diagnose left ventricular dyssynchrony, as well as to objectively quantify changes in cardiac function induced by different interventions, in chronic kidney disease patients.

In the chronic kidney disease population, the advances in the echocardiographic examination obtained by the use of colour tissue velocity imaging may contribute to improve clinical outcome as the more sensitive and reliable the diagnostic method, the better the possibilities become to optimize patient’s management.

Therefore, extended studies applying colour tissue velocity imaging in larger populations of chronic kidney disease patients should be performed, to better define its potential role for improving detection and characterization of abnormalities in cardiac function. Such studies if integrated and linked with studies on traditional, non-traditional and uraemic risk factors are
likely to contribute significantly to new strategies for prevention and treatment of cardiac
disorders in patients with chronic kidney disease.
References


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