LEFT VENTRICULAR REMODELLING IN CHRONIC KIDNEY DISEASE : CORRELATION WITH AORTIC STIFFNESS

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ABSTRACT OF THE THESIS

Title **Left ventricular remodelling in chronic kidney disease: Correlation with aortic stiffness**

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Chronic kidney disease (CKD) is associated with increased cardiovascular (CV) morbidity and mortality. Early detection of CV disease (CVD) can enable therapeutic interventions in earlier stages and potentially slow disease progression, reduce complications related to CV risk, and improve quality of life and survival rates.

This prospective, observational study was designed to assess the use of novel speckle tracking echocardiography (STE) and arterial tonometry in a clinical setting. It focuses primarily on the assessment of CKD patients who have no clinical evidence of CVD and have normal left ventricular (LV) ejection fraction (LVEF) as assessed by conventional echocardiography. Our cohort comprised 106 CKD patients of all stages, 38 healthy controls and 38 risk factor matched controls that were hypertensive and/or diabetic with normal renal function

The first aim of this study was to investigate the association between STE markers with worsening glomerular filtration rate (eGFR). Our data showed that worsening eGFR is associated with early and asymptomatic impairment of systolic (reduced global longitudinal strain (GLS), global longitudinal strain rate (GLSRs) and increased LV twist) and diastolic (segmental diastolic dysfunction) myocardial function. In addition, we found that both LV twist and GLS can be useful to differentiate CV involvement in patients with CKD in the presence of risk factors such as hypertension (HT) and/or diabetes mellitus (DM).

The second aim was to explore a possible association between aortic pulse wave velocity (aPWV), a marker of arterial stiffness, and STE markers. The results showed that arterial stiffness was inversely and independently associated with eGFR in our cohort. Moreover, among the STE parameters, only LV twist was significantly associated with the presence of arterial stiffness. These findings suggest a close interaction between arterial and LV twist mechanics early in the CV disease continuum.

Finally, we aimed to investigate the prognostic value of STE and aPWV in our cohort. Our primary endpoint was a composite of major adverse CV events (MACE). Over a median follow-up period of 49 months, 26 patients (24.5%) reached the primary endpoint. A multivariate Cox proportional hazards model showed that GLS and

aPWV were significant independent predictors of outcomes. Moreover, patients with increased (more impaired) GLS and higher aPWV had reduced MACE-free rates.

In summary, this study shows that, in CKD patients who have no previous history of CVD and who have normal LVEF as assessed by conventional echocardiography, early subclinical abnormalities in cardiac structure and function are associated with kidney function decline. Moreover, we demonstrated, for the first time, an association between aPWV and LV twist in CKD patients. In addition, we found that GLS and aPWV improves the characterisation of the risk in this cohort, as they are associated with the occurrence of adverse cardiac events.

PUBLICATIONS ARISING FROM THIS WORK

Peer-reviewed papers

1. Sulemane, S.*, Panoulas V.F., and Nihoyannopoulos, P., **2017.** Echocardiography in patients with chronic kidney disease: Current update**.** *Echocardiography***,** Apr, 34(4), pp. 594-602

2. Sulemane, S.*, Panoulas V.F., Bratsas, A., Grapsa, J., Tam, F.W., Brown, E.A. and Nihoyannopoulos, P., **2017.** Subclinical markers of cardiovascular disease predict adverse outcomes in chronic kidney disease patients with normal left ventricular ejection fraction. *Int J Cardiovasc Imaging***,** Jan, 33(5), pp. 687-698

3. Sulemane, S.*, Panoulas, V.F., Konstantinou, K., Bratsas, A., Grapsa, J., Tam, F.W., Brown, E.A. and Nihoyannopoulos, P., **2016**. Left ventricular twist mechanics and its relation with aortic stiffness in chronic kidney disease patients without overt cardiovascular disease. *Cardiovasc Ultrasound,* Mar 9, 14:10. Erratum in: Cardiovasc Ultrasound 2016 14:15

4. Panoulas, V.F., **Sulemane, S.**, Konstantinou, K., Bratsas, A., Elliott, S.J., Dawson, D., Frankel, A. and Nihoyannopoulos, P., **2014**. Early detection of subclinical left ventricular myocardial dysfunction in patients with chronic kidney disease. *Eur Heart J Cardiovasc Imaging***,** May, 16(5), pp. 539-48.

Published abstracts

1. Sulemane, S.*, Panoulas, V., Brown, E. and Nihoyannopoulos, P., 2015. Subclinical abnormalities of two-dimensional speckle tracking echocardiography associate with early peripheral vascular disease in patients with chronic kidney disease. *Eur Heart Journal* 36 (Supplement 1), pp. 939-940.
Presented at the European Society of Cardiology Congress, London, 2015

2. Sulemane, S.*, Panoulas, V., Brown, E. and Nihoyannopoulos, P., 2013. Can myocardial deformation imaging provide insights in early detection of chronic kidney disease. *Eur Heart Journal – Cardiovascular Imaging,* 14 (Supplement 2), pp. ii233.
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Presented at the American College of Cardiology Conference, San Francisco, 2013

5. Panoulas, V., **Sulemane, S.**, Frankel, A. and Nihoyannopoulos, P., 2012. The role of left ventricular systolic twisting and peak diastolic untwisting velocity as novel markers of early diastolic dysfunction in CKD patients. *Eur Heart Journal – Cardiovascular Imaging*, 33 (Abstract Supplement) 932.

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DECLARATION OF ORIGINALITY

I hereby declare that this thesis, and the research to which it refers, is a product of my own work. I also declare that all sources of information have been specifically acknowledged.

Samir Sulemane

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DEDICATION

I dedicate this thesis to my daughter Mia Sophia Hurley Sulemane.

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LIST OF ABBREVIATIONS

A

AF	Atrial Fibrillation
AFI	Automated Function Imaging
AV	Aortic Valve

B

BP	Blood Pressure
BMI	Body Mass Index
BSA	Body Surface Area
β	Standardised Beta

C

CABG	Coronary Artery Bypass Graft
CAD	Coronary Artery Disease
CI	Confidence Interval
CKD	Chronic Kidney Disease
CT	Computer Tomography
CV	Cardiovascular
CVD	Cardiovascular Disease
4ch	Four Chamber
2ch	Two Chamber
3ch	Three Chamber
CW	Continuous Wave

D

DD	Diastolic Dysfunction
DM	Diabetes Mellitus
2D	Two-Dimensional
2DSTE	Two-Dimensional Speckle Tracking Echocardiography
3DSTE	Three-Dimensional Speckle Tracking Echocardiography

E

ED	End-Diastolic
ES	End-Systolic
ECG	Electrocardiogram
EDV	End-Diastolic Volume
ESV	End-Systolic Volume
ESRD	End Stage Renal Disease

F

FS	Fractional Shortening

G

eGFR	Estimated Glomerular Filtration Rate
GLS	Global Longitudinal Strain
GCS	Global Circumferential Strain
GRS	Global Radial Strain
GLSRs	Global Systolic Longitudinal Strain Rate
GLSRe	Early Diastolic Longitudinal Strain Rate
GLSRa	Late Diastolic Longitudinal Strain Rate

H

HT	Hypertension
HR	Hazard Ratio

I

IVSd	Interventricular Septum Diastole
IAS	Intratrial Septum
ICC	Intraclass Correlation Coefficient

L

LA	Left Atrium
LAV	Left Atrial Volume
LAVI	Left Atrial Volume Indexed to Body Surface Area
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
LVMI	Left Ventricular Mass Index
LVIDd	Left Ventricular Internal Dimension diastole
LVIDs	Left Ventricular Internal Dimension systole
LVOT	Left Ventricular Outflow Tract

M

MDRD	Modification of Diet in Renal Disease
MRI	Magnetic Resonance Imaging
MV	Mitral Valve
MI	Myocardial Infarction
MACE	Major Adverse Cardiovascular Event

N

NFS	Nephrogenic Systemic Fibrosis

P

PA	Pulmonary Artery
PASP	Pulmonary Artery Systolic Pressure
PCI	Percutaneous Coronary Intervention
PLAX	Parasternal Long Axis
PWT	Posterior Wall Thickness
aPWV	Aortic Pulse Wave Velocity

R

RA	Right Atrium
RV	Right Ventricle
RWMA	Regional Wall Motion Abnormalities
RWT	Regional Wall Thickness

S

SR	Strain Rate
SD	Standard Deviation
STE	Speckle Tracking Echocardiography
STEMI	ST Elevation Myocardial Infarction

T

TDI	Tissue Doppler Imaging
TV	Tricuspid Valve

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CHAPTER 1

INTRODUCTION

1.1 Problem statement

Cardiac and arterial changes such as arterial stiffness, left ventricular hypertrophy (LVH), and LV diastolic dysfunction (DD) have been associated with an unfavourable prognosis in CKD patients[1-3]. Despite the prevalence of underlying CV abnormalities, symptoms might not manifest in many CKD patients[4]. Early detection of these abnormalities might allow therapeutic interventions in the early stages to slow down disease progression, reduce complications, and improve quality of life and survival rates. A range of emerging cardiac and vascular imaging tools may overcome the limitations of current imaging modalities in assessing subclinical CVD. Speckle tracking echocardiography is a relatively new ultrasonic imaging modality that has shown to be a sensitive tool to detect early and subtle changes in LV function[5,6]. Aortic pulse wave velocity, determined by arterial tonometry, is a measure of arterial stiffness and it is a marker of subclinical arteriosclerosis in patients with mild to moderate CKD[7].

This prospective, observational study was designed to assess the use of novel STE and arterial tonometry in a clinical setting. It focuses primarily on the assessment of CKD patients who have no clinical evidence of CVD and have normal LVEF as assessed by conventional echocardiography. We recruited 182 individuals which were divided in 3 groups: 106 CKD patients of all stages, 38 healthy controls and 38 risk

factor matched controls that were hypertensive and/or diabetic with normal renal function. We examined the association between early myocardial disease and declining eGFR, and the association between aortic stiffness and early myocardial dysfunction. Furthermore, we aimed to study the prognostic value of these markers in our cohort.

As the author, I was responsible for all aspects regarding management of the research, including protocol development, day-to-day running of the study, participant recruitment, database management, statistical analysis, and presentation of results.

1.2 Research objectives

1.2.1 Research gap

Currently, limited data exists using STE in CKD patients without previous CVD. In 2012, two studies that compared STE markers in CKD patients with those of controls (both with normal LVEF) reported reduced GLS in the CKD group[8,9]. Although of interest, the studies mentioned above were underpowered, and some novel elements of deformation (e.g. LV twist) were not investigated. In 2014, Krishnasamy et al. investigated the association between GLS and all-cause mortality by examining 447 CKD patients from a single centre. In the five-year follow-up period, GLS was a significant independent predictor of mortality (hazard ratio [HR] 1.08, 95% confidence interval [CI] 1.01–1.15)[10]. In that study, however, a considerable portion of patients with previous myocardial infarction (MI) (~40%) and reduced LVEF <45% (15%) were included, hence confounding the significance of GLS in predicting mortality in asymptomatic patients without previous CV history; the exact significance of STE markers in predicting CV outcomes among patients without a

history of CVD remains unclear. Prior to our study and to our knowledge, no research has used STE to predict outcomes in CKD patients with no overt CVD.

Arterial stiffness in CKD patients has been associated with the presence of DD[11] and increased LV mass[12]. However, its relationship with systolic function remains poorly investigated and largely unknown. In 2015 a study by Krishnasamy and colleagues established an association between GLS assessed by STE and arterial stiffness in patients with moderate CKD (stages 3 and 4)[13]. Once more, in the aforementioned study, a considerable portion of patients with previous CV events was included.

1.2.2 Research questions

New imaging modalities are able to assess subclinical dysfunction before CVD becomes clinically relevant. Such imaging therefore allows mechanistic insights into the early pathogenesis of clinical disease. The three principle research questions of the study are as follows:

i) Can STE detect early myocardial abnormalities in CKD patients with no previous history of CVD? Additionally, can STE be a discriminator for the effects of renal disease beyond those of associated risk factors such as HT and/or DM?

ii) Is there any interaction between arterial remodelling and early myocardial disease in CKD patients with no evidence of CVD?

iii) Can STE and arterial tonometry markers predict MACE in CKD patients with preserved LVEF?

1.2.3. Research hypothesis

Hypothesis 1: Chronic kidney disease patients, who are asymptomatic from a CV point of view, may present early myocardial dysfunction before CVD becomes clinically relevant. Additionally, STE can potentially distinguish the effects of subclinical myocardial dysfunction in renal disease beyond those of associated risk factors such as HT and or DM.

Hypothesis 2: Arterial stiffness is associated with subclinical myocardial disease.

Hypothesis 3: Subclinical markers of myocardial and vascular disease can provide important prognostic value in predicting MACE in our cohort.

1.3 Thesis overview

An introduction to the problem, research objectives and a thesis overview are described in chapter 1. Chapter 2 introduces the reader to CKD and its association with CVD. Chapter 3 explores the pathogenesis of arterial and cardiac remodelling in CKD while chapter 4 provides insight into current and new imaging modalities in assessing cardiac and vascular disease in CKD. The methods used in the study are then described in chapter 5, after which the results are presented and discussed in Chapters 6,7 and 8. Chapter 9 outlines the main conclusion and identifies study limitations. Finally chapter 10 discusses future directions.

CHAPTER 2

CHRONIC KIDNEY DISEASE

2.1 Introduction

Chronic kidney disease is defined by reduced eGFR, increased urinary albumin excretion, or both. This type of disease is of increasing concern in the public health sphere[14]. In 2002, the clinical practice guidelines of the US-based National Kidney Foundation Disease Outcomes Quality Initiative defined CKD as kidney damage or an eGFR measurement of lower than 60 ml/min/1.73 m^2 that persists for three months or longer[15]. Analyses conducted in 2012 showed that albuminuria also has an important effect on disease outcomes[16]. This finding prompted the Kidney Disease: Improving Global Outcomes (KDIGO) workgroup to include albuminuria in the revised 2012 classification[17] (see ***Table 1***).

***Table 1**. Criteria for the definition of CKD[17].*

Criteria
1. Kidney damage for ≥3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifest by *either*: • Pathological abnormalities; or • Markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests
2. GFR <60 mL/min/1.73 m^2 for ≥3 months, with or without kidney damage

Glomerular filtration rate is commonly estimated using the four-variable formula for 'modification of diet in renal disease' (MDRD). The four variables are age, sex, ethnicity, and serum creatinine, and the formula is as follows: eGFR = 186.3 (serum creatinine$^{-1.154}$) x (age $^{-0.203}$) [x1.212 (if black) or x 0.742 (if female)][18]. Based on the eGFR, CKD is classified into six stages: 1, 2, 3A, 3B, 4, and 5. Stage 1 represents normal values, whereas the later stages represent more severe and advanced disease (***Table 2***).

***Table 2**. Classification of chronic kidney disease[18].*

Stage	Description	Classification by severity	
		GFR mL/min/1.73 m^2	Related terms
1	Kidney damage with normal or ↑ GFR	≥90	Albuminuria, proteinuria, hematuria
2	Kidney damage with mild ↓ GFR	60–89	Albuminuria, proteinuria, hematuria
3	Moderate ↓ GFR	30–59	Chronic renal insufficiency, early renal insufficiency
4	Severe ↓ GFR	15–29	Chronic renal insufficiency, late renal insufficiency, pre-ESRD
5	Kidney failure	<15 (or dialysis)	Renal failure, uremia, end-stage renal disease

2.2 Epidemiology of CKD

According to the Global Burden of Disease Study, CKD ranked 18th in the list of causes of death, with an annual rate of 15.7 deaths per 100,000 people[19]. Evaluating the incidence and prevalence of early and less symptomatic or asymptomatic CKD, which accounts for between 80% and 90% of all cases, is difficult[15]. Although data have been published, they are confounded by issues such as heterogeneity among the populations screened and in the methods used to determine eGFR and proteinuria. The estimates are usually based on a single measurement, rather than on sustained demonstration of abnormality.

The demographics of people with CKD vary widely from region to region. The mean age of 9,614 patients in India who were diagnosed with stage 3 CKD was 51.0 years (standard deviation [SD] 13.6 years)[20], whereas the mean age of 1,185 similar patients

in China was 63.6 years (SD 14.7 years)[21]. In India, patients with CKD of unknown origin were younger, poorer, and more likely to be diagnosed with advanced CKD than were people with known causes of the disease[20]. In sub-Saharan Africa, young adults aged 20–50 years mainly develop CKD owing to HT and glomerulonephritis[22]. The population prevalence of CKD in the UK was reported in 2009 and 2010 for the first time, using data on eGFR and albuminuria, in the nationally representative Health Surveys for England (HSEs). In the combined 2009/2010 survey, 6% of men and 7% of women had eGFR <60 ml/min/1.73 m^2 (equivalent to CKD stage 3–5 if chronic).

2.3 Causes of CKD

Diabetes mellitus and HT are the leading causes of CKD in all developed and many developing countries. However, primary diseases such as glomerulonephritis are more common in countries in Asia and sub-Saharan Africa[22]. This difference is related mainly to the burden of disease among populations in developed countries moving away from infections towards lifestyle-related diseases, along with decreased birth rates and increased life expectancy[14]. In low-income countries, CKD originates as an infectious disease, and is often caused by poor sanitation, inadequate supply of safe water, or environmental pollution – all factors that contribute most heavily to the burden of the disease[23].

2.4 CKD and CVD

It is well known that patients with CKD have a strong risk of developing CVD[24]. Co-morbidities such as HT or DM are frequently associated with CKD, and until recently the increased risk of CVD was assumed to be a result of these underlying pathologies. The discrepancies were resolved by two landmark meta-analyses that showed that impaired kidney function and raised albuminuria are risk factors for CVD, independently of HT and DM[25,26]. This finding was supported by the fact that roughly 30% of the studied patients did not have HT or DM. More importantly, the associations between eGFR and CV mortality in patients without DM and HT are similar to the associations observed in patients who do have DM and HT[25,26].

2.4.1 CKD is associated with an increase in CVD risk factors

Recently, the American Heart Association reviewed the significance of kidney disease as a risk factor for the development of CVD. The Association described two types of CVD risk factors in patients with CKD: traditional and non-traditional[27] (***Table 3***[28]). Traditional CVD risk factors—such as older age, DM, higher total cholesterol level, LVH, smoking, and higher systolic blood pressure (SBP)—have each been defined in the Framingham Heart Study and used to predict coronary heart disease outcomes in the general population[29]. Several of these risk factors increase in prevalence as kidney function declines[25,30-32].

Non-traditional risk factors—such as inflammation, oxidative stress, and elevated lipoprotein levels—have been shown to play a role in promoting CVD risk. They also

display a relationship with the degree of risk and the severity of CKD[33,34]. However, the relative importance of non-traditional risk factors, in comparison to traditional risk factors, remains unknown.

Table 3. *Traditional and non-traditional risk factors for CVD in CKD[28].*

Traditional Risk Factors	Nontraditional Risk Factors
Older age	Albuminuria/proteinuria
Male sex	Homocysteine
Hypertension	Lipoprotein(a) and apolipoprotein(a) isoforms
Higher LDL cholesterol	Lipoprotein remnants
Low HDL cholesterol	Anemia
Diabetes	Abnormal calcium-phosphate metabolism
Smoking	Extracellular fluid overload
Physical inactivity	Oxidative stress
Menopause	Inflammation (C-reactive protein)
Family history of CVD	Malnutrition
Left ventricular hypertrophy	Thrombogenic factors
	Sleep disturbances
	Altered nitric oxide/endothelin balance

2.4.2 CKD is an independent risk factor for CVD

An abundance of evidence has emerged in recent years that establish minor reductions in eGFR as an independent risk factor for CV mortality[35,36]. Furthermore, analysis of data from several population-based epidemiological studies points to an association between reduced kidney function and risk of all-cause and CV mortality, which persists after adjustment for traditional CVD risk factors. These population-based

studies have included, for example, the Atherosclerosis Risk in Communities study (ARIC)[37], the Cardiovascular Health Study[38,39], and the Hoorn Study[40]. In the National Health and Nutrition Examination Survey (NHANES) II, patients with a eGFR less than 70 mL/min had a 68% greater risk for CV mortality, relative to participants with a normal eGFR. Additionally, the risk of developing congestive heart failure (CHF), atrial fibrillation (AF), stroke, coronary artery disease (CAD), and peripheral artery disease is increased twofold in patients with eGFR <70 mL/min/1.73m^2 [31,32]

Cross-sectional studies have also shown that microalbuminuria and albuminuria are associated with CVD risk factors[41,42] and with surrogate markers of CVD[43,44] in clinically healthy participants and patients—both with and without DM. Prospective studies, including the Prevention of Renal and Vascular End-Stage Disease Intervention Study[45] and the Heart Outcomes Prevention Evaluation Study[46], have established microalbuminuria as an independent predictor of clinical CV adverse outcomes.

2.4.3 The impact of DM and HT in subclinical myocardial disease

In many CKD patients with associated DM and HT, myocardial changes are amplified by the existence of these co-morbidities. According to some studies, these co-morbidities augment the development of LVH, increase the susceptibility of the heart to ischemic injury, and increase the overall likelihood of heart failure[47,48]. The classic paradigm of hypertensive heart disease is that the LV wall thickens in response to elevated blood pressure as a compensatory mechanism to minimize wall stress.

Subsequently, after a series of poorly characterized events ("transition to failure"), the LV dilates and LVEF declines[49]. From subclinical perspective, a few studies explored the impact of HT on myocardial remodelling. In the Coronary Artery Risk Development in Young Adults study[50], among 2,479 patients, HT was not associated with the reduction of LVEF, however it was associated with lower GLS as shown by STE. In that study, the average entry age was 25 years and cumulative exposure occurred over 25 years.

The pathogenesis of diabetic cardiomyopathy is likely to be multifactorial, including microvascular disease, altered myocardial metabolism, and altered myocardial structure with fibrosis. Patients with early diabetic cardiomyopathy often have evidence of global DD but preserved systolic function, as reflected by a normal LVEF[51]. However recently, a landmark study by Ng et al.[6] showed for the first time that GLS was impaired in patients with uncomplicated Type 2 DM, suggesting the presence of subclinical dysfunction before the reduction of LVEF.

As such, I aim to explore if STE can be a discriminator for the effects of renal disease beyond those of associated risk factors such as HT and/or DM.

2.5 Conclusion

The problem of CKD is a serious public health concern. The association between CVD and CKD (even in its early stage) has been extensively documented in the literature. Both CKD and CVD share common traditional risk factors, such as smoking, obesity, HT, DM, and dyslipidaemia. However, CVD remains frequently

underdiagnosed und undertreated in patients with CKD. Additional studies, devoted to further understanding early structural and functional abnormalities within the CV system, are necessary to develop and institute preventative and treatment strategies to reduce the high rates of morbidity and mortality associated with CVD. Accordingly, with the use of STE, I investigate the potential associations between markers of subclinical myocardial disease and worsening eGFR.

CHAPTER 3

CARDIAC AND ARTERIAL REMODELLING IN CHRONIC KIDNEY DISEASE

3.1 Introduction

Cardiovascular disease in patients with CKD can primarily be attributed to two distinct but overlapping pathological processes: *atherosclerosis* and *arteriosclerosis*[52]. The presence of CVD is associated with an elevated risk of athero-thrombotic events, such as MI. However, *arteriosclerosis* is the predominant pathophysiological process involving fibrosis and thickening of the medial arterial layer[52]. This process can result in increased arterial stiffness, causing LVH, fibrosis and microvascular disease. These pathophysiological features are manifested by a high risk of lethal arrhythmias, CHF, MI, and stroke. Renal-specific disturbances, including oxidative stress and inflammation, have also recently been associated with both atherosclerotic and arteriosclerotic remodelling in CKD[53]

3.2 Atheroma in CKD

The morphological characteristics of atheroma in patients with CKD are distinct. They include increased plaque calcification as well as increased intimal and medial

thickness[54].

Although the prevalence of atheroma in CKD patients is high, it is unclear whether this is a direct result of renal dysfunction or due to the clustering of risk factors—such as HT, DM, and inflammation—that invariably accompany CKD. Leskien and colleagues showed a fourfold greater plaque score among CKD patients (pre-dialysis, dialysis, and post-transplant) compared with controls[55]. Tonneli et al. demonstrated that compared with a reference control group, patients with non-diabetic CKD had an adjusted relative risk for MI of 1.4, whereas patients with diabetic stages 3 and 4 CKD had an adjusted relative risk of 2.7. The equivalent figures were 2.0 among patients with DM and 3.8 among patients with previous MI. However, Tonneli et al. also showed that when severe proteinuria was present, the relative risk of MI among patients with non-diabetic CKD equalled that of the diabetic population[56]. Outcomes after acute coronary syndrome and stroke are much worse among CKD patients than among the general population[57,58]. Following an ST elevation myocardial infarction (STEMI), people on dialysis (undergoing percutaneous coronary intervention) had a one-year death rate of almost 60%, with a one-year cardiac mortality of 41%[57].

Studies evaluating the pathogenesis of atheroma in patients with early CKD have yielded contradictory results. Previously, researchers had measured endothelial function and suggested that patients with early CKD showed deranged endothelial function[59-61]. More recent work has suggested that this finding might have been the result of including patients with other risk factors—such as HT, smoking, and even established vascular disease[45]. If such factors are excluded as far as possible, endothelial function might be abnormal only in more advanced (stage 5) CKD[62].

The precise relationship between eGFR and endothelial dysfunction remains to be elucidated. A number of potential mechanisms are under investigation, and further prospective and longitudinal studies are required to clarify this association.

3.3 Myocardial disease in CKD

Large epidemiological studies suggest that although roughly 40% of all deaths in patients with end-stage renal disease (ESRD) are due to CVD, most CV deaths are attributable to sudden cardiac death, arrhythmia, or CHF. Relatively few deaths occur because of vasculo-occlusive events such as MI[63]. ***Figure 1*** shows the relevant data for the US for the period 2007–2009.

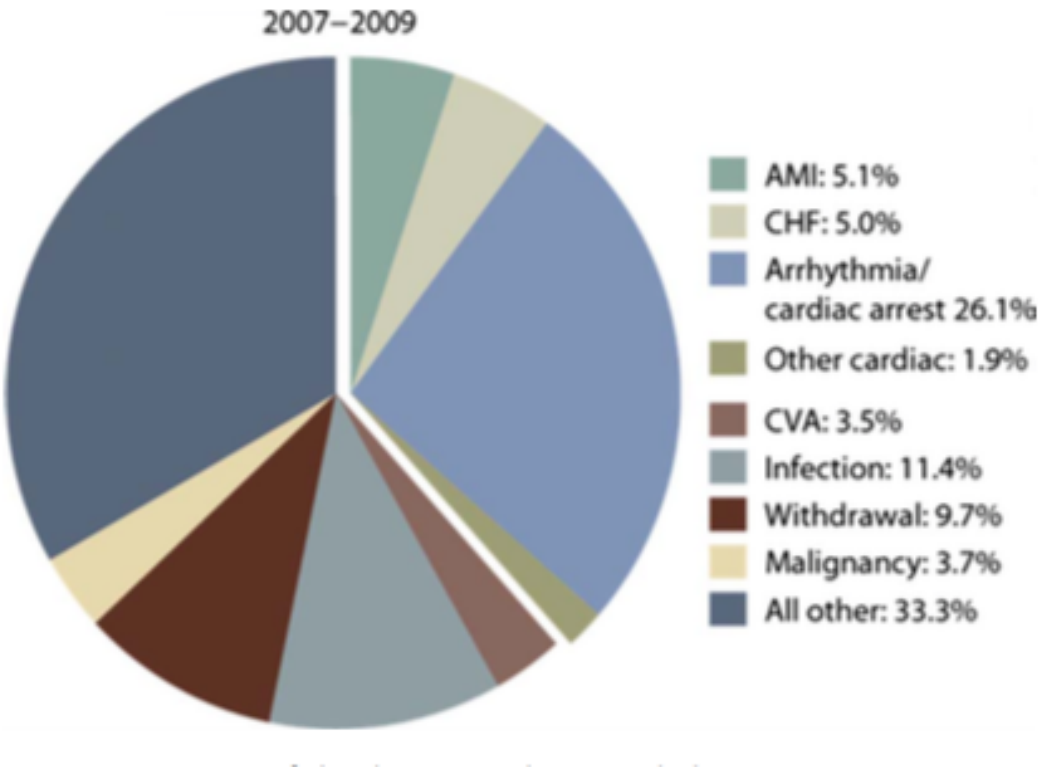


Figure 1. *Causes of death in US dialysis patients*[63]

AMI, acute myocardial infarction; CVA, cerebrovascular accident.

Heart failure is a major cause of morbidity and mortality in both early stage CKD and ESRD, with incident rates three to four times higher than in non-CKD participants[64]. Accordingly, the main cause of CV-related death and disease among CKD patients appears not to be atheromous CAD but myocardial disease (LVH, LVH with fibrosis or DD, often accompanied by systolic dysfunction).

3.3.1 Left ventricular hypertrophy

Left ventricular hypertrophy is the principal myocardial alteration in patients with CKD[65]. Left ventricular hypertrophy develops early during the progression of kidney

dysfunction and is present in roughly 20% of patients in CKD stages 1, 2, or 3[66]. This proportion increases to as much as 70% or 80% (based on echocardiography and cardiac magnetic resonance imaging, cMRI) among patients with end-stage renal disease[67-69]. In a cross-sectional study of 3,487 CKD patients, the prevalence of LVH was 32%, 48%, 57%, and 75% in patients with eGFRs of ≥60 ml/min/1.73 m^2, 45–59 ml/min/1.73 m^2, 30–44 ml/min/1.73 m^2, and <30 ml/min/1.73 m^2, respectively[65].

A number of echocardiographic studies have examined the prognostic value of LVH and LV geometry. Paoletti et al. investigated the prognostics of LV geometry for 445 patients with hypertension and CKD (stages 2 to 5)[70]. The authors showed that concentric and eccentric LVH were associated with higher risk of CV outcomes (concentric LVH: HR 2.59, 95% CI 1.39–4.84, eccentric LVH: HR 2.79, 95% CI 1.47–5.26). In the same study, the researchers analysed the sensitivity of these indicators, using LVH and relative wall thickness (RWT) separately. The results showed that, of these factors, only LVH was associated with elevated cardiorenal risk. This finding suggests that LVH is a strong independent predictor of risk, independent of LV geometry. A study of 433 patients receiving dialysis showed that the adjusted relative risk (RR) of death after two years of dialysis was greatest for patients with eccentric hypertrophy (RR 17.14), and lowest for patients with concentric hypertrophy (RR 3.29); patients with 'normal' volume and left ventricular mass (LVM) were used as the reference category[71].

3.3.2 Fibrosis

Apart from LVH, uremic cardiomyopathy is characterised by myocardial fibrosis, occurring independently of blood pressure and LVH itself[72]. Typically, CKD patients exhibit diffuse myocardial fibrosis with collagen deposition between capillaries and cardiomyocytes. These features in turn contribute to the maladaptive ventricular hypertrophy with subsequent dilatation of the heart. Recently, strain parameters assessed by STE have been used to detect uremic fibrosis in CKD patients, and these parameters were predictive for mortality in dialysis patients[73].

3.3.3 Sudden cardiac death

In the general population, the risk of sudden cardiac death is 1 in 1,000 patient-years. This risk increases to 59 in 1,000 patient-years among CKD patients[63]. Among 4,465 community-based participants in the Cardiovascular Health Study who had no history of heart failure or MI, the incidence of sudden cardiac death was more than 2.5-fold higher among patients who had lower levels of kidney function[74]. Further analysis from that study utilised creatinine and cystatin C measures to identify a subgroup with preclinical kidney disease, defined as creatinine-based eGFR of ≥60 ml/min per 1.73 m^2 and cystatin C of ≥1.0 mg/L. After multivariate adjustment, the risk of sudden cardiac death was twice as high in the preclinical kidney disease group compared with the normal kidney function referent group. (The referent group had creatinine-based eGFR ≥60 ml/min per 1.73 m^2 and cystatin C <1.0 mg/L). These findings suggest that

even mild reductions in kidney function can increase the risk of sudden cardiac death, especially within susceptible populations such as elderly people.

3.4 Arteriosclerosis in CKD

Arteriosclerosis is characterised by thickening and calcification of the medial arterial layer, and is a hallmark feature of arterial disease in CKD[52]. The pathophysiological effects of arteriosclerosis and arterial stiffening are best understood through an appreciation of the normal physiology of the aorta and large arteries. Their function is to deliver blood around the body and to buffer the oscillatory changes in blood pressure that result from intermittent ventricular ejection. Loss of arterial distensibility results in a relatively rigid aorta that is less able to accommodate the volume of blood ejected by the left ventricle[56].

Increased arterial stiffness results in the pressure waves travelling faster and the reflected pressure wave returning earlier to the heart. This earlier return means that the reflected wave will affect the central arteries during systole rather than diastole, thus amplifying aortic and LV pressures during systole. This pattern effectively increases pulse pressures and reduces coronary perfusion (which preferentially occurs during diastole).

A study was conducted with a cohort of 241 participants with ESRD, with an average follow-up of 11 years[75]. Logistic regression and Cox analyses identified two dominant predictors of CV and all-cause mortality: aPWV(a marker of arterial stiffness) and age at inclusion in the study. After adjustment for confounding variables—notably

age, LVH, and SBP—the odds ratio for aPWV (>12.00 m/s *vs* <9.4 m/s, p<0.001) was 5.4 for all-cause mortality (95% CI 2.4–11.9, p<0.001) and 5.9 for CV mortality (95% CI 2.3–15.5, p=0.001). ***Figure 2*** shows the results. These results provide the earliest evidence that, in patients with ESRD who undergo haemodialysis, increased aortic stiffness is a major and independent predictor of CV and all-cause mortality.

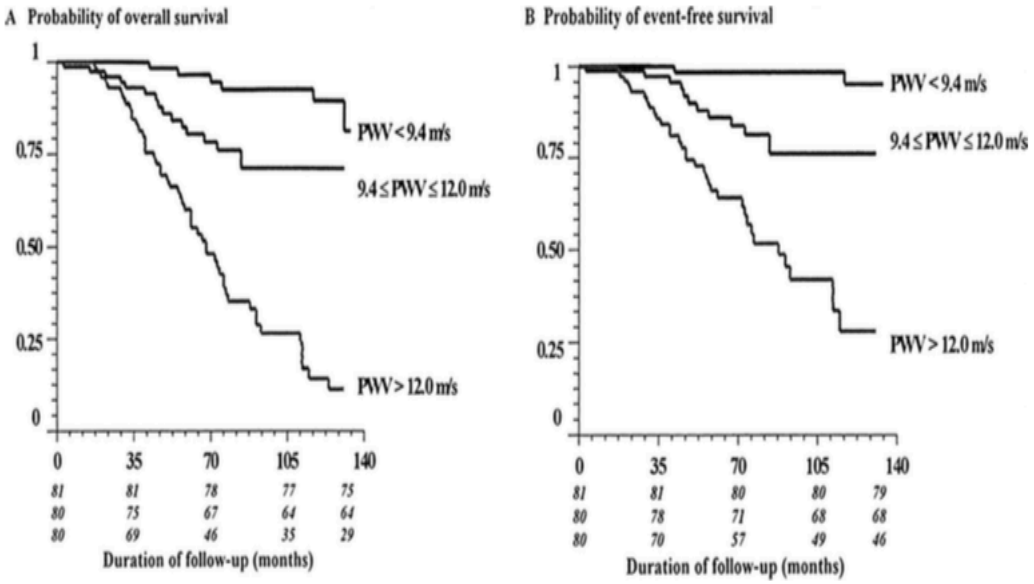


Figure 2. *Probability of overall survival (**A**) and event-free survival CV mortality (**B**) in patients with ESRD (N=241)[75]. Probability is shown by level of PWV, divided into tertiles. Patients with aPWV >12m/s had reduced overall survival and reduced event-free survival rates.*

Arterial remodelling has already been observed in early stage CKD[7]. Compared with normotensive and hypertensive controls, patients with CKD (stages 2–4) displayed significantly larger internal carotid artery diameters but comparable intima-media

thickness, resulting in significantly increased circumferential wall stress[7]. For CKD populations, conflicting results have been published as to whether aortic stiffness is associated with CKD severity or not. Cross-sectional studies, including the CRIC study that included 2,564 CKD patients, demonstrated an independent association between aortic stiffness and CKD progression[76]. More recently, however, Lilitkarntakul et al. reported that CKD patients' blood pressure, not renal function, was the major determinant of arterial stiffness[62].

3.5 Conclusion

Although CKD is associated with a high risk of *atherosclerotic* disease, *arteriosclerosis* is the predominant arterial disease. Medial inflammation, fibrosis, and calcification collectively result in increased arterial stiffness. This stiffness in turn accelerates the normal process of vascular ageing, contributing to the development of LV fibrosis and hypertrophy and precipitating further renal damage—and ultimately causing a high rate of premature mortality from heart failure and arrhythmias. Despite the growing evidence that these changes start to develop early in the uremic milieu, there is a paucity of data regarding CV imaging studies to investigate the interaction between myocardial and arterial remodelling within the CKD population.

In view of this, I will explore the potential associations between arterial stiffness and subclinical myocardial disease

CHAPTER 4

IMAGING CARDIOVASCULAR DISEASE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

A recent meta-analysis examined research priority-setting in relation to kidney disease. The efficacy of imaging methods for detecting diabetic renal and CVD, along with the benefits and harms of screening, were regarded as research priorities[77]. In the next chapter the reader will find a brief description of current and emerging imaging modalities to assess cardiac disease and arterial stiffness in CKD patients.

4.1 Current cardiac imaging modalities for CKD patients

Two-dimensional transthoracic echocardiography is usually the first-line investigation for suspected cardiac disease in patients with CKD. Other imaging modalities exist, such as cardiac computer tomography (CT) and cMRI, which can detect cardiac disease that might otherwise be missed by echocardiography. These modalities are accompanied, however, by several limitations that restrict their applicability in current clinical practice. Exposure to ionising radiation—with a resulting increase in the risk of cancer—and high costs are considered the main limitations for cardiac CT. Cardiac MRI examinations are extremely time-consuming, and there have been reports on nephrogenic systemic fibrosis (NSF) resulting from the use of gadolinium-based contrast media in patients with renal disease. However no case of NSF have been

reported in patients with eGFR greater than 60ml/min.1,73^2. In patients with CKD stages 4-5 (eGFR <30 ml/min.1,73^2) it is recommended to give the smallest amount of contrast agent in order to achieve an adequate diagnostic examination.

The next sub-chapters of this thesis focus on current and new echocardiographic modalities. These are widely available, inexpensive, and non-invasive; the use of contrast agents or exposure to ionising radiation is unnecessary. These characteristics render such modalities the first choice for tests to assess cardiac disease in CKD.

4.2 Two-dimensional transthoracic echocardiography and CKD

Two-dimensional (2D) transthoracic echocardiography is a portable and readily available diagnostic tool for investigating cardiac disease. Applications of 2D echocardiography in CKD include the following:

i. *Pre-transplantation workup.* Guidelines suggest screening for CVD in high-risk patients with renal disease before renal transplantation. Two-dimensional transthoracic echocardiography is a simple screening tool for detecting abnormalities, helping to diagnose cardiac dysfunction, stratifying risk, and enabling early referral to cardiology for any necessary cardiac intervention.

ii. *Chronic kidney disease stage 5 cardiovascular risk monitoring.* It is recommended that all patients on dialysis undergo 2D transthoracic echocardiography at the start of haemodialysis and every three years

thereafter[78]. This screening enables early identification of necessary cardiac prescriptions, referrals, or intervention.

iii. *Haemodialysis prescription.* Two-dimensional echocardiography may aid in the assessment of fluid balance, for example, in determining inferior vena cava collapsibility. Diuretics or dialysis prescriptions can be altered accordingly.

iv. *Prognostication and therapeutic monitoring*. Various cardiac structural parameters are predictive of poor prognosis, and 2D transthoracic echocardiography can be used as a risk stratification tool. Once abnormalities are identified, treating physicians might be more vigilant in administering certain treatments. For example, they might adjust treatment to avoid fluid overload in patients with severe systolic dysfunction. Furthermore, 2D transthoracic echocardiography can be used for diagnosis and monitoring after treatment.

Certain limitations, however, need to be addressed. Two dimensional echocardiography is operator-dependent, and image quality can vary depending on the operator's experience and the patient's acoustic window. These limitations can restrict its use with all CKD patients. Furthermore, conventional echocardiographic measures to assess LV volumes and function –

i. rely on geometric assumptions that might not be met by patients with CKD;

ii. are limited as a measure of contractility by load dependency (which can affect dialysis patients).

The section below presents a brief description of the most important abnormalities in CKD that can be diagnosed using 2D echocardiography.

4.2.1 LV hypertrophy

The topic of LVH is discussed in Chapter 2 (section 2.3.1) and the information is not repeated here. The interested reader may refer to that section of the thesis.

4.2.2 LV diastolic dysfunction

Left ventricular DD, characterised by abnormalities of ventricular filling—including decreased diastolic distensibility and impaired relaxation—is a very common structural abnormality in patients with CKD[79]. In current literature, impaired LV diastolic function and increased LV filling pressures are seen in some[80-82] but not all[83] CKD patients. Hayashi et al., in a small study of 22 CKD stage 1–3 and 18 CKD stage 4–5 patients, identified increased prevalence of DD (~63% and ~66%, respectively), where the prevalence of DD was defined as e' septal <8 cm/s[81]. A prospective observational study of 57 patients on dialysis and 96 patients with non-dialysis CKD (mean eGFR 22.3±7.4 ml/min/1.73 m^2) found diastolic dysfunction in 85% of the total cohort, with 35% exhibiting severe diastolic dysfunction (grade ≥2, elevated left atrial [LA] pressure)[2 68]. In multivariate analysis, DD grade >1 remained a significant independent predictor of all-cause mortality after a mean follow-up of 2.6 years (HR 3.42, 95% CI 1.66–7.08, p=0.001)[84].

An enlarged LA volume (LAV) is a surrogate marker for chronic DD. This marker is present in patients with CKD and is predictive of all-cause mortality[2,85]. In 2016, a study recruited 76 patients with stage 3 CKD (eGFR 30–59 ml/min/1.73 m^2), without any previous cardiac illness. Measured LAV was significantly larger in the CKD group compared with the risk-factor matched group and healthy control

participants (38.5±10 ml/m^2 *vs* 31.2±9 ml/m^2 *vs* 22.3±5 ml/m^2 respectively; p<0001). In addition, forward logistic regression analysis showed LAV to be a sensitive predictor for the presence of CKD, suggesting that this parameter may be useful for detecting myocardial involvement in stage 3C[2].

4.2.3 Left ventricular systolic function

Evaluation of global systolic function using 2D echocardiography has traditionally been limited to volume-based assessment of LVEF, assessment of regional wall motion, or visual estimation of regional thickening and fractional shortening (FS) measured at the endocardium[86].

A few studies have demonstrated an association between LVEF and renal disease in non-dialysis patients. A study by Ahmed et al.[87] identified increased mortality among CKD patients with reduced LVEF, though mortality was found to be even higher in patients with preserved LV systolic function but with presence of diastolic heart failure. Curtis et al. showed that lower LVEF was associated with a linear increase in mortality in nearly 8,000 non-haemodialysis patients with stable heart failure[88]. The association between LVEF and mortality is most commonly seen in end-stage renal disease or dialysis patients. A recent prospective cohort study of 254 asymptomatic haemodialysis patients, with a mean follow-up of 41±22 months, assessed the prognostic value of LVEF. Although these patients were asymptomatic, LV systolic dysfunction was present in 22% of cases when measured by LVEF[3]. In the same study, measures of systolic function were predictive of cardiovascular events; the HRs

for a 1% decrease in LV function were 1.04 for LVEF measurements (95% CI 1.02–1.06) and 1.06 for FS measurements (95% CI 1.01–1.08)[3].

Although LVEF is one of the most powerful echocardiographic predictors of death or CV morbidity, it is limited as a measure of contractility by load dependency (which may affect dialysis patients). In addition, it might be not sufficiently sensitive to identify mild or subclinical degrees of systolic dysfunction (common in early stages of CKD). Analysis of LVEF was unable to identify a gradation of risk in patients with LVEF >45%[88]. Furthermore, it suffers from poor reproducibility and standardisation, which limits its use in clinical practice[89]. Finally, LVEF reflect merely the change in LV volume but does not take into consideration ultra structural changes that may occur at the myocardial level and that may impair LV systolic performance. These limitations have led to an interest in new echocardiographic techniques that provide more objective and reproducible measures of myocardial function in CKD.

4.3 Myocardial deformation imaging

Even when LVEF is in the normal range, segmental contraction-relaxation abnormalities can exist within the myocardium. The LV myocardium undergoes a complex multi-dimensional deformation during the cardiac cycle. During systole, the ventricular myocardium simultaneously shortens in the longitudinal and circumferential planes and thickens in the radial plane, with reciprocal changes in diastole. ***Figure 3*** provides a graphical representation of this process.

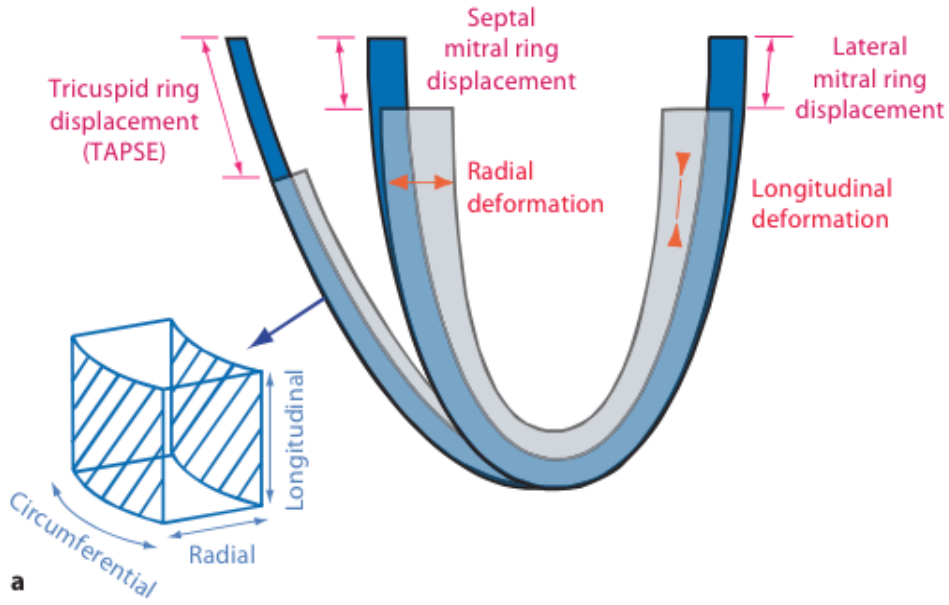


Figure 3. *Myocardial motion and deformation components.*

Deformation imaging, in the broadest sense, allows for fairly direct assessment of myocardial muscle shortening and lengthening throughout the cardiac cycle, through assessment of myocardial strain and strain rate. *Strain* is defined as the change in length of a segment of myocardium, relative to its resting length, and is expressed as a percentage (***Figure 4***). By contrast, *strain rate* (SR) is the rate of this deformation. The longitudinal strain denotes shortening of the LV along its long axis. The circumferential strain depicts reduction in the circumference of the LV cavity during the cardiac cycle. Radial strain is the passive movement of the LV fibres that arises from the incompressible nature of the myocardium. Longitudinal and circumferential shortening result in negative strain values, whereas radial thickening results in a positive strain value.



Figure 4. *Calculation of deformation. Calculation of strain (ε), with L_0 as the original length (grey bar in figure), ΔL the change in length (orange bar), and L as the total length (grey and orange bar).*

In addition to contracting and relaxing, the LV also undergoes rotation around its long axis. When viewed from the apex, the apex rotates in an anticlockwise direction during systole, whereas the base rotates in a clockwise direction. This opposite rotation of the LV apex and base during systole produces a wringing motion—or twist—of the LV, which is critical to its normal systolic functioning.[90] ***Figure 5*** depicts this process in graphical format.

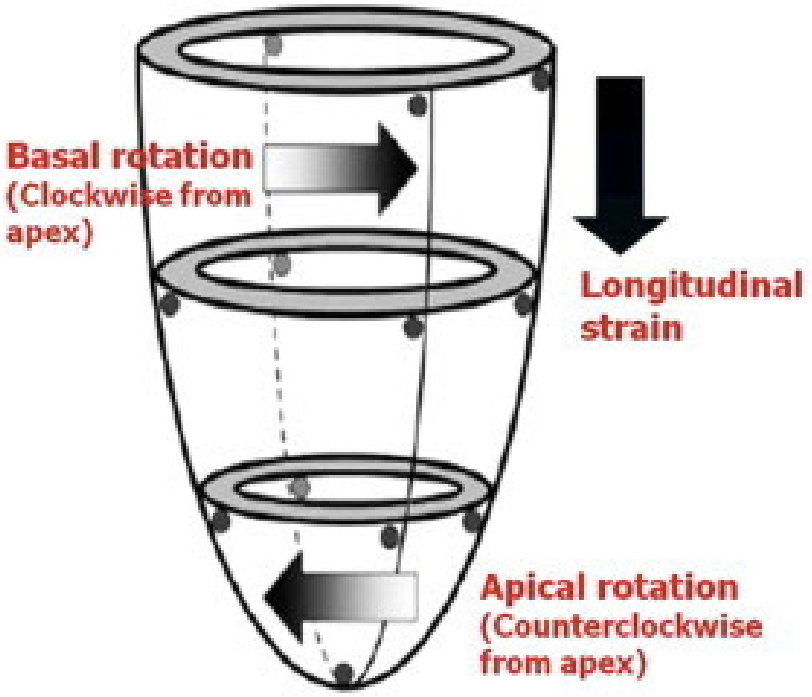


Figure 5. *Myofibre architecture of LV twist.*

Previously, assessment of myocardial strain could be performed exclusively through the use of sonomicrometry crystals, which can only be used experimentally[91] or with MRI-based myocardial tagging[92]. However, MRI tagging is restricted by its relatively low temporal resolution, limited availability, and high cost. The extension of these techniques to echocardiography has allowed for substantially greater utility in a broader range of patients.

4.4 Speckle tracking echocardiography

A relatively new echocardiographic application for measuring strain patterns is STE. This technique is an offline application that follows the motion of myocardial tissue

throughout the cardiac cycle by tracking acoustic reflections—known as 'speckles'—in previously obtained echocardiographic images (***Figure 6***). Most currently available STE software is semi-automated.

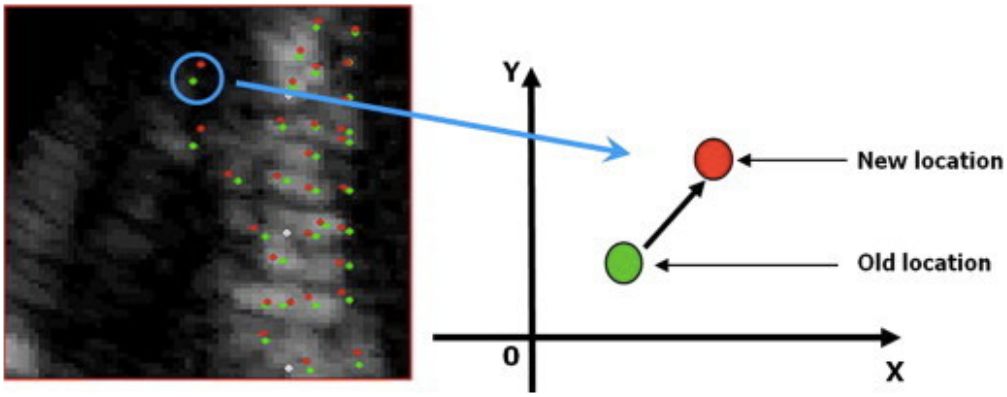


Figure 6. *The fundamental basis of speckle-tracking echocardiography. Myocardial speckles in a greyscale image are tracked frame-by-frame to resolve myocardial deformation in different directions.*

Strain and SR derived from STE have been validated against sonomicrometry[93-95] and tagged cMRI[93,96,97] results. Moreover, Helle-Valle et al. showed that the LV twist could be accurately measured using STE[98]. The same study validated the capability of STE to measure LV twist by comparing it with sonomicrometry ($r=0.94$, $p<0.001$) and tagged MRI ($r=0.85$, $p<0.001$) in healthy adults. Overall, STE is highly reproducible and minimally affected by intra-observer and inter-observer variability[99]. Myocardial deformational measures, such as strain and SR, may have greater utility in terms of predictive power for patients who have LVEF >45% and have no wall motion abnormalities[100].

4.4.1 Speckle tracking to assess subclinical systolic function

Liu et al. were the first researchers to investigate subclinical systolic dysfunction using STE among uremic patients. Their sample consisted of 153 patients who were categorised into three subgroups: the controls, 56 participants without CKD; 37 patients with moderate-to-advanced CKD (stages 3, 4 or 5); and 60 patients with ESRD who were receiving maintenance haemodialysis. Liu et al. found that compared with the controls, among the CKD group the GLS, global circumferential strain (GCS) and SR were decreased. Along with the decline of renal function, GLS deteriorated.[9]

A separate study examined 36 dialysis patients, including 17 pre-dialysis CKD patients, and 18 healthy controls, all with LVEF >60%. Patients with renal disease showed reduced GLS, GCS, and radial strain[8]. In addition, a GLS ≥-15% was predictive of mortality in haemodialysis patients with LVEF ≥50% (HR 3.57, 95% CI 1.41–9.04, p=0.01)[9].

Speckle tracking has a potential role as a non-invasive screening tool for CAD in CKD patients. It was used in a cohort study of 102 patients receiving haemodialysis, of whom 38% had a history of CAD (previous MI, angiographically significant stenosis, or past coronary revascularisation). Having more than six LV segments with decreased GLS was associated with CAD (HR 12.88, 95% CI 3.98–41.59, p<0.001)[101]. Speckle tracking has also shown that strain pattern is affected by dialysis. In 29 patients receiving haemodialysis, peak systolic longitudinal strain was reduced after dialysis (-18±2.9% before dialysis vs -16.9±3.2% after dialysis, p<0.001)[102].

Although these studies provide some insight into the relationship between renal dysfunction and subclinical myocardial disease, they are limited by the fact that novel elements of deformation, such as LV twist, were not studied. LV twist has in fact been used in a variety of clinical settings[103-108]; however, prior to our study, its use in CKD patients was yet to be investigated. In addition, a large proportion of the sample had experienced previous cardiac events, confounding the significance of STE parameters in asymptomatic patients without a previous CV history.

4.4.2 Speckle tracking in detecting subclinical diastolic dysfunction

Speckle tracking echocardiography also has the potential to assess segmental relaxation patterns (segmental DD). Takemoto and colleagues found a wide range of altered segmental relaxation (number of segments with DD) in elderly patients and in patients with normal global diastolic function, when assessed through conventional echocardiography[109]. The authors used an early-to-late diastolic SR ratio of ≤1.1 (SRa/SRe≤1.1) to signify altered segmental relaxation. Currently, there are no data on assessing segmental relaxation patterns in CKD patients.

4.4.3 Association of STE with clinical outcomes

Several studies have investigated the relationship between STE measures and clinical outcomes. Significant associations with mortality have been noted among patients with acute decompensated and symptomatic heart failure[110], with stable heart failure with reduced LVEF and wide QRS[111], after MI[112], and among unselected patients

referred for echocardiography[100]. In general, GLS measures tend to perform better than LVEF in predicting risk[100,113], and at least two studies suggest that GLS measures offer incremental value beyond clinical information and LVEF[110,112]. Regarding non-dialysis CKD patients, very limited data exist. In 2014, Krishnasamy et al. investigated the association between GLS and all-cause mortality by examining 447 CKD patients from a single centre. In the five-year follow-up period, GLS was a significant independent predictor of mortality (HR 1.08, 95% CI 1.01–1.15)[10]. In that study, however, a considerable portion of patients with previous MI (~40%) and reduced LVEF <45% (15%) were included, which confounded the results; the exact significance of GLS in predicting mortality among patients without a history of CVD remains unclear. Prior to our study and to our knowledge, no research has used STE to predict outcomes in CKD patients with no overt CVD.

4.5 Assessing arterial stiffness in CKD patients

Early research explored multiple methods for assessing arterial stiffness and led to a consensus that carotid-femoral aortic aPWV should be regarded as the 'gold standard'[114]. This was mainly because aPWV has been validated based on clinical and epidemiological studies with different populations, including CKD patients, and the reference values have been published[115-117]. Briefly, aPWV is the velocity of propagation of a pressure wave (forward pressure wave) generated by the ejection of blood from the left ventricle. The wave is reflected at arterial branching points, which generates reflected waves that travel back towards the ascending aorta. As arterial

stiffness increases, so too does the velocity of the forward and reflected waves. The accelerated return of the reflected waves imposes an increase in afterload (pressure work) on the LV, resulting in LVH and reduced coronary perfusion.

Numerous studies have determined aPWV as an important risk factor for CV events and mortality in patients with CKD at all stages[1,76,118]. A recent study by Karras et al.[1] identified aPWV velocity as an independent predictor of all-cause mortality and of CV events, both fatal and non-fatal, among 439 patients with CKD stages 3–5. However, in that study, 28% of the population had a history of CVD.

In the current literature, there exists no prospective data analysing the role of aPWV in predicting MACE in CKD patients who do not have overt CVD. On that basis, I aim to examine the potential effect of STE and aPWV in predicting MACE in our cohort.

4.6 Arterial-ventricular interaction in CKD

The central role of arterial stiffening in CKD has also focused attention on the interaction between the LV and the arterial tree, termed arterial–ventricular coupling. In healthy people, cardiac function is physiologically matched or coupled with arterial function to ensure maximum cardiac work and efficiency. This coupling is most commonly investigated and quantified by the measurement of arterial and ventricular elastances, using an invasive procedure that involves conductance catheters. End-systolic elastance (Ees) is a measure of LV intrinsic contractility, which is minimally affected by preload and afterload.[12] Effective arterial elastance (Ea) reflects the net

impact of arterial afterload on the heart, which is related to systemic vascular resistance and inversely related to total arterial compliance. The ratio of Ea to Ees (Ea/Ees) represents the interaction between the heart and large arteries, which is called ventricular-arterial coupling.[10] The coupling is important because it demonstrates mechanical efficiency in transferring blood from the heart to the arterial system.

When LV function is normal, the coupling ratio is generally between 0.7 and 1.0 (Ea is smaller than Ees), so that work efficiency is maximised but stroke work is submaximal for a given preload. With systolic dysfunction, Ees falls, such that in mild dysfunction the ventricle performs at maximum stroke work. When the heart is coupled to a stiff vascular system it must increase its systolic stiffness (Ees) to maintain cardiac metabolic efficiency, ensuring transfer of blood to the arterial tree without excessive changes in pressure. Thus, the arterial-to-ventricular coupling ratio is maintained, but both Ea and Ees are considerably elevated. These compensatory adaptations maintain cardiac performance with enhanced contractility at rest, but at a price: cardiac reserve is reduced, diastolic function is impaired, and the cardiovascular responses to alterations in pressure and volume load are blunted. These features lead to haemodynamic instability.

The abnormalities of arterial and LV function that are so prevalent in ESRD can begin in early stage CKD. In a population-based study of 742 individuals, mildly reduced eGFR was significantly associated with greater LV mass in a process that appeared dependent upon increased arterial stiffness[12]. In a cross-sectional study of patients with stages 2 and 3 CKD, the authors found delayed ventricular relaxation, increased

LV end-diastolic stiffness, and elevated LAV compared with healthy matched controls[11]. The Ea was also elevated such that the arterial-to-ventricular coupling ratio was preserved. This finding suggests that the two processes (LV and arterial stiffness) occur in parallel, and further lends support to the theory that aortic stiffness drives the development of LV stiffness in CKD[11].

4.7 Conclusion

Two-dimensional echocardiography is the first choice of modality to assess cardiac dysfunction in CKD patients, whereas aPWV is considered the 'gold standard' for assessing arterial stiffness. Current echocardiography methods for assessing cardiac function in CKD patients present a number of limitations, particularly for milder stages of systolic dysfunction. In practice, STE can overcome some of these limitations, and may be readily applied to echocardiographic images to provide additional information on myocardial strain patterns in patients with renal disease. Speckle tracking could play a role in assessing CKD patients who i) are asymptomatic from a CV point of view, and ii) have symptoms of heart failure and preserved LVEF, as myocardial strain abnormalities are associated with a relatively poor prognosis[10].

The interaction between arterial and sub clinical myocardial function is largely unknown. The use of STE and measures of arterial stiffness to explore this avenue of enquiry might provide a new non-invasive methodological approach to the assessment of ventricular–arterial coupling.

CHAPTER 5

METHODOLOGY

5.1 Concept, ethics, and funding

The study was designed by the principal investigator Professor Petros Nihoyannopoulos and co-investigators Dr Vasileus Panoulas, and Dr Atanasios Bratsas and myself, with the aim of answering the research questions. As the author, I was responsible for all aspects regarding management of the research, including protocol development, day-to-day running of the study, participant recruitment, database management, statistical analysis, and presentation of results.

The study was approved by the UK National Research Ethics Committee Service on 25/09/2010 (REC 10/H0704/81) and by the Joint Research Office at Imperial College Healthcare NHS, with the reference number JROHH0031. A substantial amendment was submitted on 04/11/2013 and was signed for approval on 14/01/2014 (REC reference: 10/H0704/81). Written informed consent was obtained from all participants, and the study has been conducted in accordance with the Declaration of Helsinki (updated 2008). Funding consisted of a research grant from *Fundacao para a Ciencia e Tecnologia* in Portugal, under the grant number SFRH/BD/73646. All data rests with the principal investigator, and there was no commercial involvement in protocol design, running of the study, data analysis, or manuscript preparation.

5.2 Data review and database management

Throughout the study, strict standardised study protocols were adhered to. I performed regular data checks, and my main supervisor did the same, to identify potential outliers and errors. The data were maintained using Microsoft Office Excel (Microsoft Corporation, Redmond) prior to export for statistical analysis.

5.3 Study population

5.3.1 Sample size and statistical power

We calculated our sample size requirements after recruiting 71 CKD patients and 36 controls. We performed the power analysis for differences in LV twist (one of the main variables of interest) amongst three CKD patient groups. The data produced an effect size of 0.269. In order to detect differences in LV twist between CKD groups at statistical power of 80% and at α=0.05, the total sample size should be n=101. We also ran a power analysis for differences in GLS between CKD patients and controls. The effect size (*d*) for this data was 0.546, with power of 90% and α=o.005. The total sample size needed to detect differences between patients and controls was n=125.

We recruited 199 patients. Owing to sub-optimal echodiagnostic windows (n=9), atrial fibrillation (n=4), valvular abnormalities (n=1), and regional wall motion abnormalities (n=1), deformation parameters could not be quantified in 15 patients in the CKD cohort. In addition we had to exclude two healthy controls because of poor imaging quality. The final study group comprised 182 patients, detailed below and in section 5.3.2.

5.3.2 CKD cohort

We prospectively enrolled 106 CKD patients from Imperial College Healthcare NHS Trust renal outpatient clinics between 2011 and 2016. Patients receiving haemodialysis were excluded from this study. We also excluded patients with echocardiographic evidence of LV systolic dysfunction (Simpson biplane LVEF <55% and/or presence of regional wall motion abnormalities), significant valvular abnormalities (moderate or severe), presence of AF or flutter, echocardiographic findings suggestive of pulmonary HT, congenital heart disease, cardiomyopathy, pericardial disease, or inadequate echocardiographic acoustic windows. All our patients were asymptomatic from the CV point of view, and had normal systolic function assessed by conventional echocardiography (Simpson's biplane LVEF ≥55% and absence of regional wall motion abnormalities). We carefully reviewed patients' clinical notes, and if any evidence of signs or symptoms of heart failure was present then we excluded those patients from the study.

5.3.3 Risk-factor matched and healthy control groups

Owing to the fact that our CKD cohort had a high prevalence of HT and/or DM, a risk-factor matched control group (hypertensive and/or diabetic with normal renal function) of 38 participants was recruited. By doing so, we limited the influence of both these risk factors in our analysis. They were drawn from both Hammersmith and Charing Cross hospitals at Imperial College Healthcare NHS. These patients were participants with HT (essential HT) and Type 2 DM with normal renal function, who were also matched for age and sex to the CKD group. These patients were on antihypertensive agents and oral hypoglycemic agents or insulin, and none of them

had a history of CVD. These participants were treated for their HT and DM by their treating physicians.

We also enrolled 38 healthy control individuals prospectively. Overall, the major recruitment sources for controls were family members and family friends of our patient group (~60%), Imperial College Healthcare NHS intranet website (~20%), and flyers in public settings (~20%). The healthy control participants were carefully screened for history of CVD and for the presence of cardiovascular risk factors including HT and DM. They were normotensive with normal echocardiography, and they were not receiving any cardio-active medications.

Both patient and control groups were clearly informed that they were free to withdraw from the study at any time.

5.4 Data collection

Using a structured questionnaire, anthropometric measurements, and a review of medical notes, we captured the following data: anthropometrics (weight in kg, height in cm, body mass index as BMI kg/m^2, body surface area as BSA g/m^2); CV risk factors (SBP; DBP; sitting position, mmHg); HT (defined as SBP ≥140mmHg and/or DBP ≥90mmHg, or receiving antihypertensive treatment); diagnosis of DM; treated hypercholesterolemia (use of statin, fibrate, or ezetimibe); family history of ischaemic heart disease (IHD) (first-degree relative with myocardial infarction, angina, or coronary artery bypass surgery [CABG], percutaneous coronary intervention [PCI], or sudden/cardiac death before

age 55 years for men and 65 years for women); smoking status (current, ex, never); and detailed records of current medical treatment. The medical records of each participant were reviewed carefully, with specific attention to past medical history, including diagnosis of CAD, HT, and DM. A detailed drug history was acquired from current prescriptions. Biochemical results were obtained from the most recent renal clinic review (within one month of recruitment), provided there was no evidence of superimposed acute kidney injury during the time of blood sampling. The value of eGFR was calculated using the four-variable equation in the MDRD study[23]. Proteinuria was examined using dipsticks (Hema-Combistix, Bayer Diagnostics); a test result of 1+ or higher was defined as positive.

5.5 Follow-up

All participants were followed up for a median time of 49 months (interquartile range 11–63 months). The primary endpoint was a composite of all-cause mortality, acute coronary syndromes, stable angina requiring revascularisation (either PCI or CABG), hospitalisation for heart failure, and stroke. Deaths were identified from the Office of National Statistics. All patients were followed up either in outpatient clinics or through telephone conversations.

5.6 2D Transthoracic echocardiography

5.6.1 Hardware for imaging

All the patients in this study were scanned using the GE Vivid 7 ultrasound system (***Figure 7***). The GE7 system is able to use 2D black-and-white imaging, 2D colour flow, M-mode black-and-white imaging, colour M-mode, Doppler, real-time longitudinal strain (automated function imaging [AFI]), and real-time 3D measurements (4D). The system was equipped with a phased array M3S 1.5–4-MHz transducer probe for adult cardiac applications. Briefly, each probe produces signals using quartz piezoelectric crystals that, when electric current is applied, change shape and oscillate. The transducer then emits short, strong electrical impulses at a preselected frequency. The sound waves are directed by the shape of the transducer, producing an arched array of ultrasound, which penetrates tissue at a required depth. Phased arrays are commonly used in echocardiography, as they are able to sweep the sound beam in an arc across a predetermined field. A water-based gel is applied to all transducers, providing a higher quality image owing to better contact impedance between the probe and the surface of the skin. A three-lead electrocardiogram (ECG) (I, II, III) was also attached to the patient. Electrodes were carefully positioned to maximise positive R wave deflection. The ECG was very helpful in establishing the timing of various cardiac events, especially the aortic valve closure, when analysing STE longitudinal deformation parameters.



Figure 7. *GE Vivid 7 system.*

5.6.2 Imaging protocol

The imaging protocol was conducted in all patients in keeping with the guidelines of the British Society of Echocardiography[119]. I carried out all the studies. I am accredited by the European Society of Cardiovascular Imaging (formerly the European Society of Echocardiography), a branch of the European Society of Cardiology (Aug 2010). A brief description is presented below of how to perform the

echocardiographic test in a standardised fashion, according to this research protocol.

5.6.3 Initial test setup

In the waiting room, the participant was given a set of instructions to read about the echocardiographic test that is available at the Echocardiography Department of Hammersmith Hospital. The participant was then taken to the test room, where he or she completed a structured questionnaire and anthropometric data were recorded. After this, but prior to the examination, the participant was asked to sign three research consent forms: patient copy, patient notes copy, and study file copy (Appendix II). An additional study information sheet was also sent to the general practitioner of each participant. The local research committee approved all information sheets and consent forms.

5.6.4 Acquisition

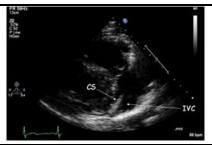
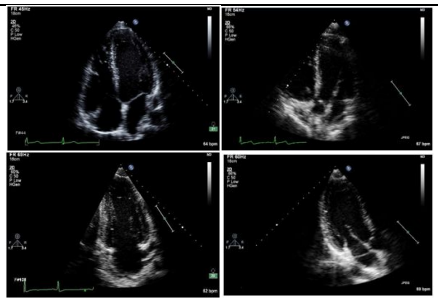
Each participant's specific research ID number, date of birth, and date of scanning were entered on the GE Vivid 7 ultrasound system.

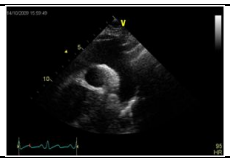
Participant setup

After the patient had lain down, three electrodes were placed on his or her chest, ensuring good tracings to facilitate the acquisition of complete digital loops. Electrode placement was carefully positioned to maximise positive R wave deflection. We asked the patient to lie on his or her left side, with the left arm positioned on the pillow. We then started the echocardiographic examination following the scanning protocol[119].

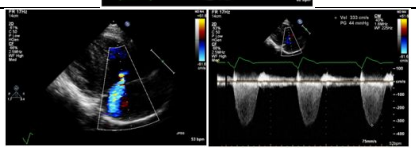
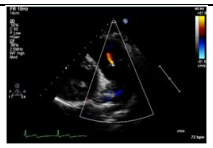
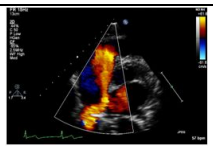
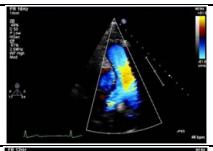
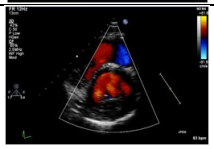
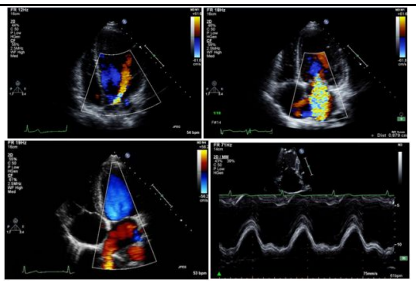
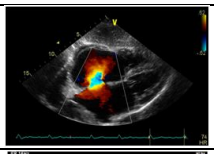
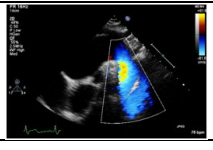
Transthoracic echocardiography protocol

2D imaging

View	Assessment	Image
Parasternal long axis (PLAX) view	LV cavity size, wall thickness, LA size, mitral valve (MV) and aortic valve (AV) appearance, LV outflow tract, aortic root	
PLAX right ventricular (RV) inflow	RV cavity size and function, right atrium and tricuspid valve (TV) appearance	
PLAX RV outflow	RV outflow tract, pulmonary artery	
Parasternal short axis (PSAX) outflow	RV outflow tract, AV, LA, TV	
PSAX outflow	Pulmonary artery (PA), pulmonic valve, PA branches	
PSAX base, mid, and apical	MV and annulus (thickness, mobility, calcification), radial LV function, regional wall motion abnormalities (RWMA)	
Apical 4ch, 5ch, 2ch, and 3ch	LV cavity size, longitudinal function, RWMA, MV, TV and AV appearance, atrial septum mobility, LV end-systolic and end-diastolic volumes, LV biplane Simpsons	
Subcostal	Atrial septum, inferior vena cava, hepatic vein	

Aortic arch	Aortic arch	

M-Mode, spectral, and colour Doppler imaging

View	Assessment	Image
PLAX view	MV and AV function	
PLAX RV inflow	TV function, continuous wave (CW) Doppler across TR to determine pulmonary artery systolic pressures (PASP)	
PLAX RV outflow	PV function, CW across PV	
PSAX outflow	Colour flow across Aorta/LA, atrial septum, TV	
PSAX outflow	Colour flow across PA	
PSAX base, mid and apical	Colour flow (sweep beam from base to apex) to assess ventricular septum	
Apical 4ch, 5ch, 2ch, 3ch	Colour flow across MV, LVOT, TV and aortic septum, mitral inflow PW Doppler, LVOT PW and CW Doppler, CW TV inflow if presence of TR, M-mode RV septum (tricuspid annular plane excursion)	
Subcostal	Atrial septum	
Aortic arch	Abnormal colour flow (flow reversal), CW across descending aorta	

5.6.5 Imaging software and analysis

Offline analysis was performed using the EchoPac PC v.7.0.1 clinical software package (GE Healthcare, Horten, Norway). *All echocardiographic parametres were measured in batches, and the assessor (myself) was blinded to the patients' clinical data*.

Measurements were obtained in keeping with the guidelines in the recommendations issued jointly by the European and American Societies of Echocardiography[120-122]. Linear measurements (interventricular septal wall thickness [IVS], posterior wall thickness [PWT], and LV internal dimensions) were recorded from the 2D parasternal long-axis acoustic window. They were measured at the level of the LV minor axis, approximately at the MV leaflet tips. Aortic root measurements were also obtained from the same acoustic view. Linear RV measurements were obtained on the apical 4ch view at basal and mid RV level. Left ventricular systolic function was assessed using the biplane Simpson's method, in which end-diastolic volume (EDV) and end-systolic volume (ESV) are calculated, and then LVEF is calculated as follows: Ejection Fraction (%) = (EDV - ESV) / EDV × 100. ***Figure 8*** shows 2D measurements for volume calculations using the Biplane methods of disk (Simpson's biplane)

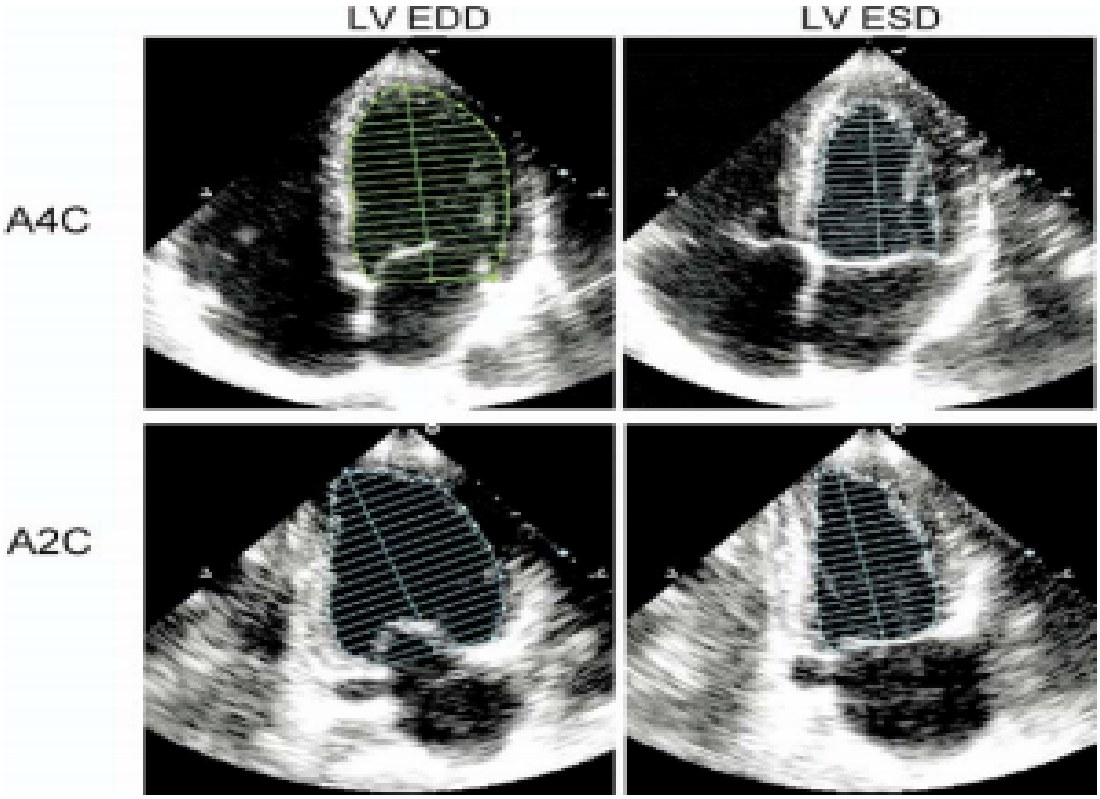


Figure 8. *Two-dimensional measurements for volume calculations using biplane method of disks (modified Simpson's rule) in apical 4-chamber (A4C) and apical 2-chamber (A2C) views, at end-diastole (LV EDD) and at end-systole (LV ESD).*

Left ventricular mass (LVM) was calculated according to Devereux's formula[123]. The RWT was calculated using the following formula: [2 × PWT]/[LV internal diameter in diastole (LVIDd)]. We used linear measurements and defined concentric hypertrophy as a left ventricular mass index (LVMI) of >115 g/m^2 in men and >95 g/m^2 in women, with RWT >0.42. Eccentric LVH was defined as LVMI >115 g/m^2 in men and >95g/m^2 in women, with RWT ≤0.42. Patients with normal LVMI can have either concentric remodelling (normal LV mass with RWT >0.42) or normal

geometry (RWT ≤0.42) and normal LV mass[120](***Figure 9***).

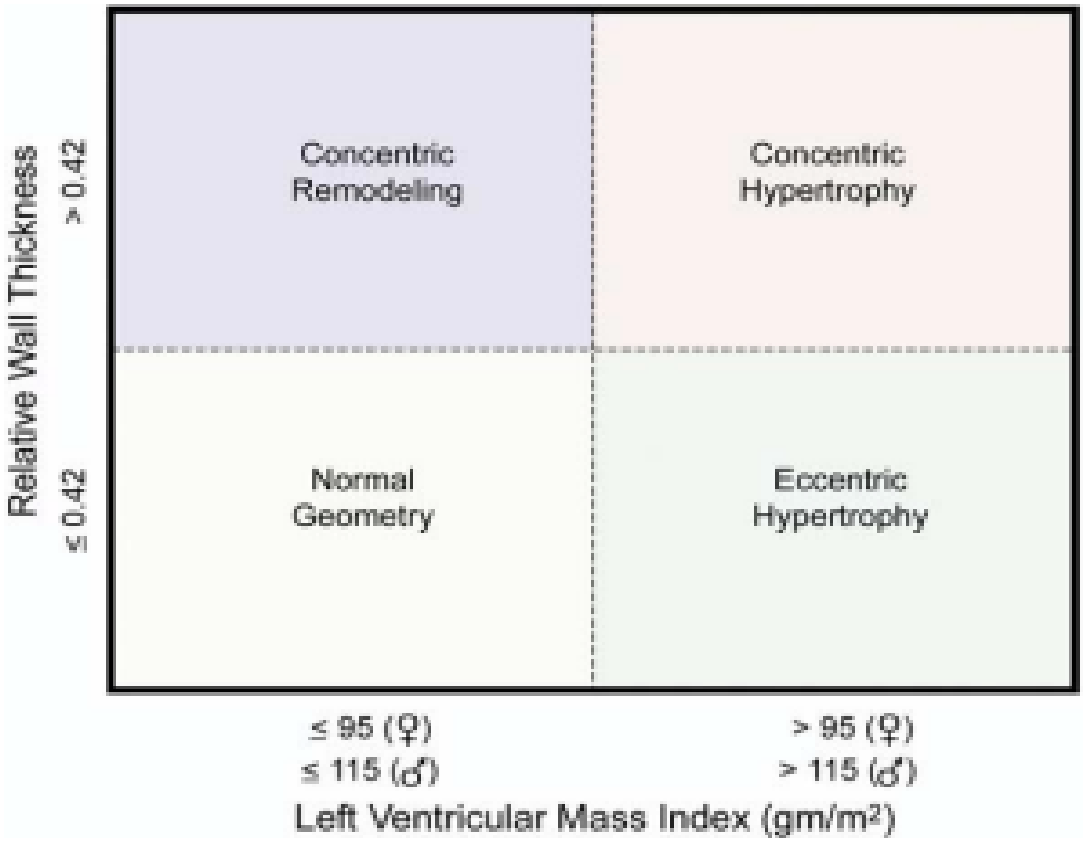


Figure 9*. Comparison of relative wall thickness*[120].

Diagnosis and types of global DD (***Figure 11***) were based on the current guidelines[124] using the following parameters:

i. *Mitral Inflow*. PW Doppler was performed in the apical 4ch view to obtain mitral inflow velocities. A 1-mm to 3-mm sample volume was placed between the mitral leaflet tips during diastole to record a laminated velocity profile. Measurements of mitral inflow include the peak early filling (E-wave) and late

diastolic filling (A-wave) velocities, the E/A ratio, and deceleration time of early filling velocity.

ii. *Tissue Doppler annular velocities*. PW tissue Doppler imaging was performed in the apical views to acquire mitral annular velocities. The sample volume was positioned at 1 cm within the septal and lateral insertion sites of the mitral leaflets. It was adjusted as necessary (usually 5mm – 10 mm) to cover the longitudinal excursion of the mitral annulus in both systole and diastole. Primary measurements include the early diastolic (e') and late diastolic (a') velocities. The mitral inflow E velocity-to-tissue Doppler e' (E/e') ratio has been considered a robust method for assessing estimated LV filling pressures[125-127] (***Figure 10***).

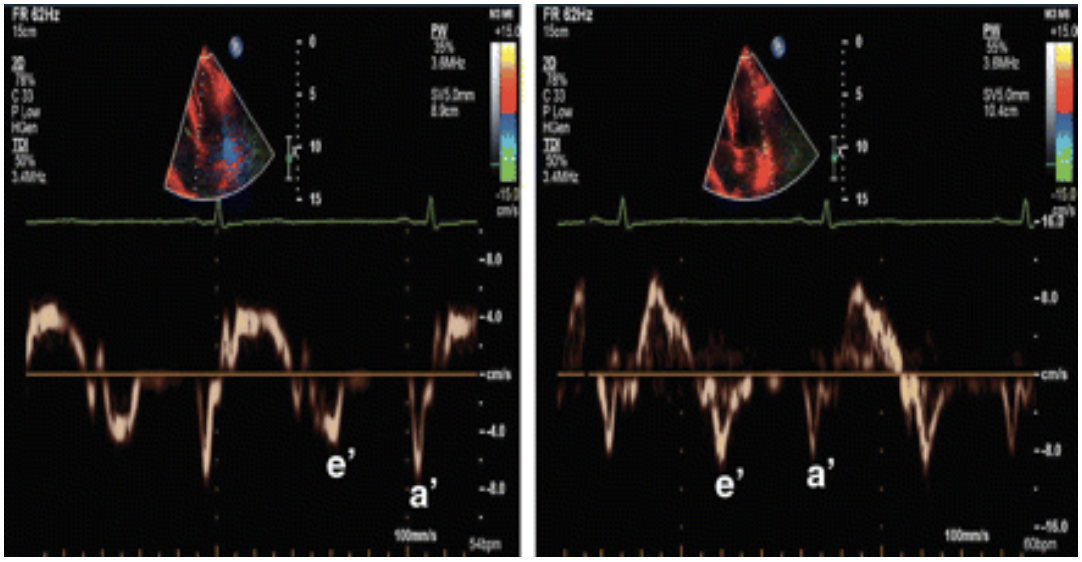


Figure 10. *Septal (**left**) and lateral (**right**) tissue Doppler. Septal e' (5 cm/s) and lateral e' (10 cm/s). We used the average of septal and lateral e' velocities to arrive at more reliable assessments of LV relaxation and filling pressures.*

iii. *Pulmonary venous flow*. Doppler of pulmonary venous flow was performed in the apical 4ch view. Recordings were obtained by angulating the transducer superiorly such that the aortic valve is seen. A 2-mm to 3-mm sample volume was placed >0.5 cm into the pulmonary vein for optimal recording of the spectral waveforms. Measurements included peak S and D velocities, the S/D ratio, and peak A reversal (Ar) velocity in late diastole.

iv. *Left atrium*. Assessment of LA was done by i) measuring LA diameter at the end-ventricular systole when the LA chamber is at its greatest dimension on the parasternal long axis view, and ii) measuring LA volume (LAV) using the area-length method. LAV was then indexed to BSA (LAVI).

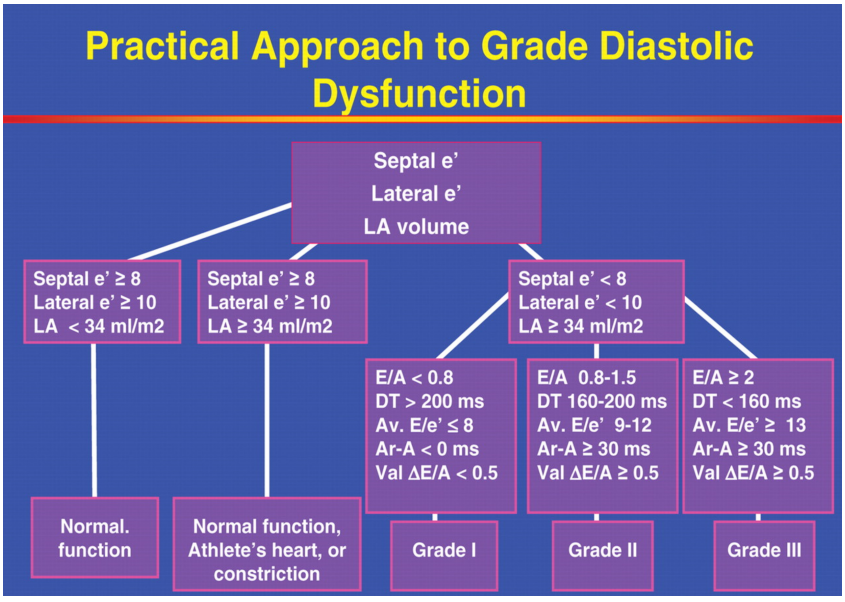


Figure 11. *Scheme for grading diastolic dysfunction*[117]*. Av.Average; LA, left atrium; Val.,Valsalva*

5.7 Speckle tracking echocardiography

Image acquisition and post-process analysis of the STE indices were conducted in keeping with the current guidelines of the European Society of Cardiovascular Imaging and the American Society of Echocardiography; the guidelines deal with evaluating cardiac mechanics and standardising deformation imaging data[128,129].

5.7.1 Imaging acquisition

Images were obtained in the left lateral decubitus position during the echocardiographic test, using the same ultrasound system (Vivid 7, GE Vingmed, Ultrasound AS, Horten, Norway) with phased array M3S 1.5–4MHz-transducer probe. Two-dimensional greyscale harmonic images were obtained at a frame rate of 60 to 70 frames per second. The focus was positioned at an intermediate depth to optimise the images, and the sector depth and width were adjusted to include as little as possible outside the region of interest[128]. Longitudinal deformation values (strain and SR) were obtained from the three apical views and circumferential values from the basal, mid, and apical short-axis planes.

For the assessment of LV twist, LV short-axis planes were obtained at basal and apical levels. The basal plane was defined as that plane which allows visualisation of the mitral valve with the cross-section appearing as circular as possible—defined as the long-axis position in which the LV and aorta were most in line with the mitral valve tips in the middle of the sector. The apical plane was acquired distally to the papillary muscles. When obtaining the latter plane, care was taken to ensure the

transducer was placed one or two intercostal spaces inferiorly, as previously described[130]. The LV twist was defined as the net difference in degrees between the basal and apical rotations.

5.7.2 Post acquisition analysis

Images were directly recorded from the ultrasound system and transferred to the speckle tracking analysis system (2D-strain EchoPac PC v.7.0.1, GE Healthcare, Horten, Norway). A set of measurements was subsequently obtained.

Global longitudinal strain and strain rate

We first manually delineated the endocardial border on the apical 3ch view, using the GE semi-automated tracking algorithm. The reason we chose the apical 3ch first is that we were able to manually define aortic valve closure (the systolic duration was defined as lasting from the onset of QRS of the ECG to aortic valve closure). If aortic valve closure was difficult to recognise accurately, a spectral Doppler display of the LV outflow was used. The endocardial border was considered to be the inner contour of the myocardium, whereas the epicardial border was considered as the outer contour of the myocardium (***Figure 12).*** Care was taken not to include the pericardium, as this would result in underestimation of strain values.

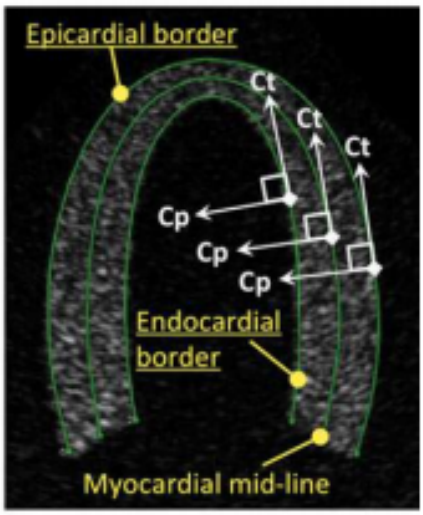


***Figure 12**. Speckle tracking-derived parameters are reported with reference to the endo–epicardial or mid-myocardial line or to the full wall independently of the way they are achieved. The longitudinal or circumferential component of any parameter is then directed tangentially to the respective line (C_t), while the radial component is directed perpendicular to it (C_p).*

The software then analyses how well the tracking line follows the endocardial motion. If the operator agrees with the software regarding endocardial tracking and contour, the operator validates it and accepts the reading. This procedure results in a set of measurements that include regional longitudinal strain and SR.

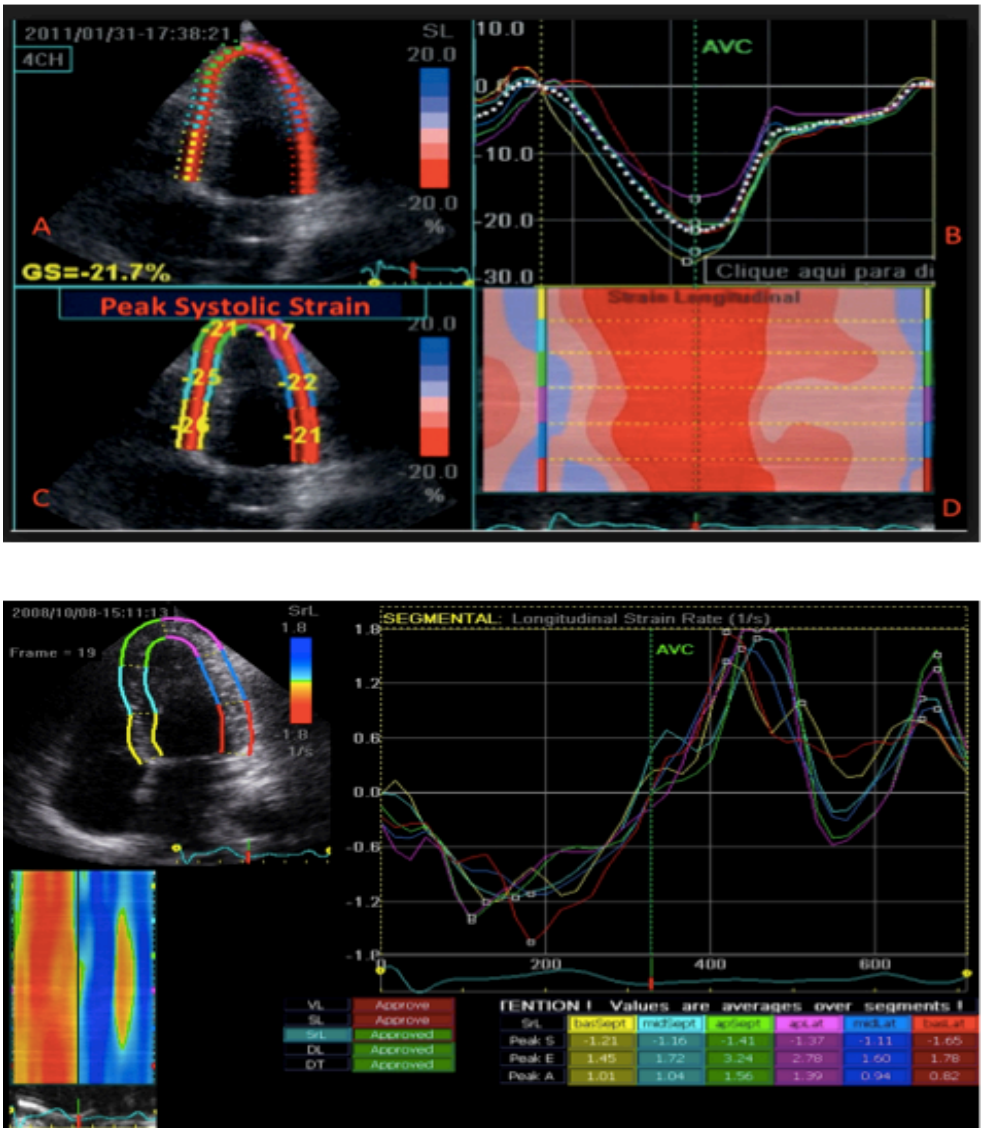


***Figure 13**. Global longitudinal strain with a strain value of -21.7% (**top**) and segmental longitudinal strain rate GLSRe and GLSRa (**bottom**), analysed on a 4ch view.*

Secondly, myocardial speckle tracking of apical 4ch (***Figure 13***) and 2ch was performed in a similar way. When all three apical views are analysed, the software delivers GLS. Global strain values were calculated from the average of the 18 segments in the longitudinal planes (***Figure 14***). A GLS absolute value of -16% was considered pathological[131]. Furthermore, the bull's-eye view of longitudinal systolic strain was used to support regional function analysis (***Figure 15***).

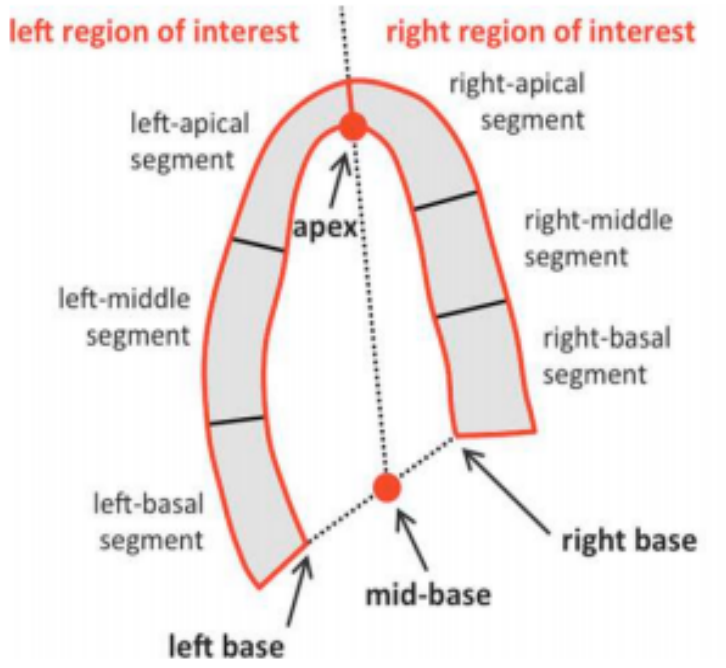


Figure 14. *Segmentation of the region of interest in apical views, applicable for a 16- or 18-segment model.*

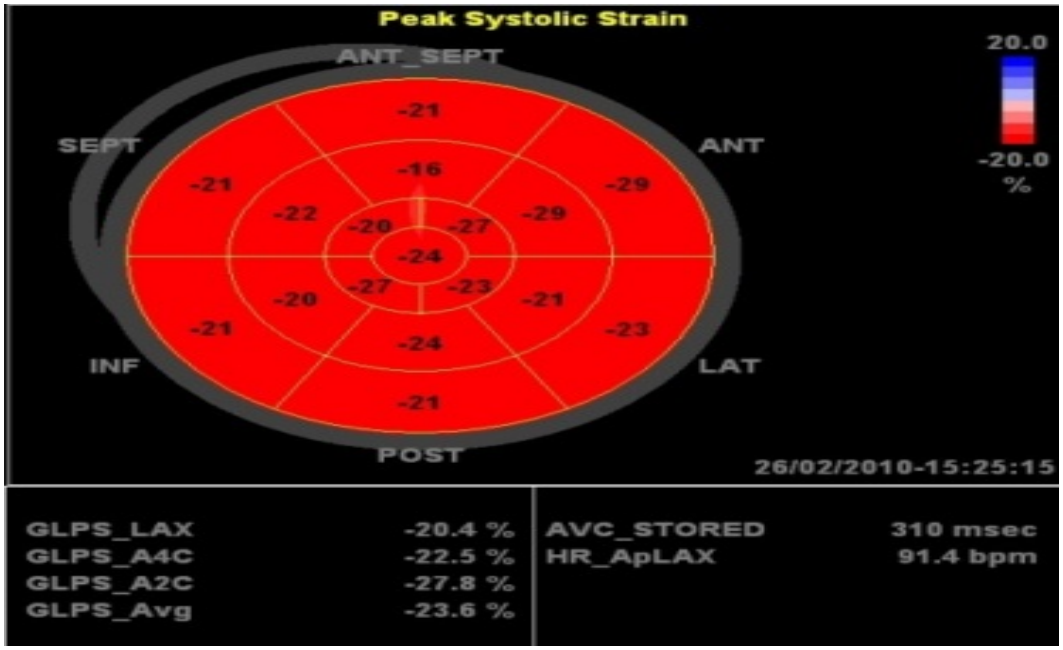


Figure 15. *Speckle tracking-based strain bull's-eye map showing segmental and global longitudinal strain values on a healthy control. The GLS value of -23.6% and absence of regional deformation abnormalities suggest normal myocardial deformation patterns.*

Circumferential strain

Circumferential strain was calculated on a short axis recording at the level of the mitral valve, papillary muscle, and apex. Similar to the longitudinal analysis, the endocardial border was manually traced on the short axis views, and—after validation—the GCS values were displayed (***Figure 16***).

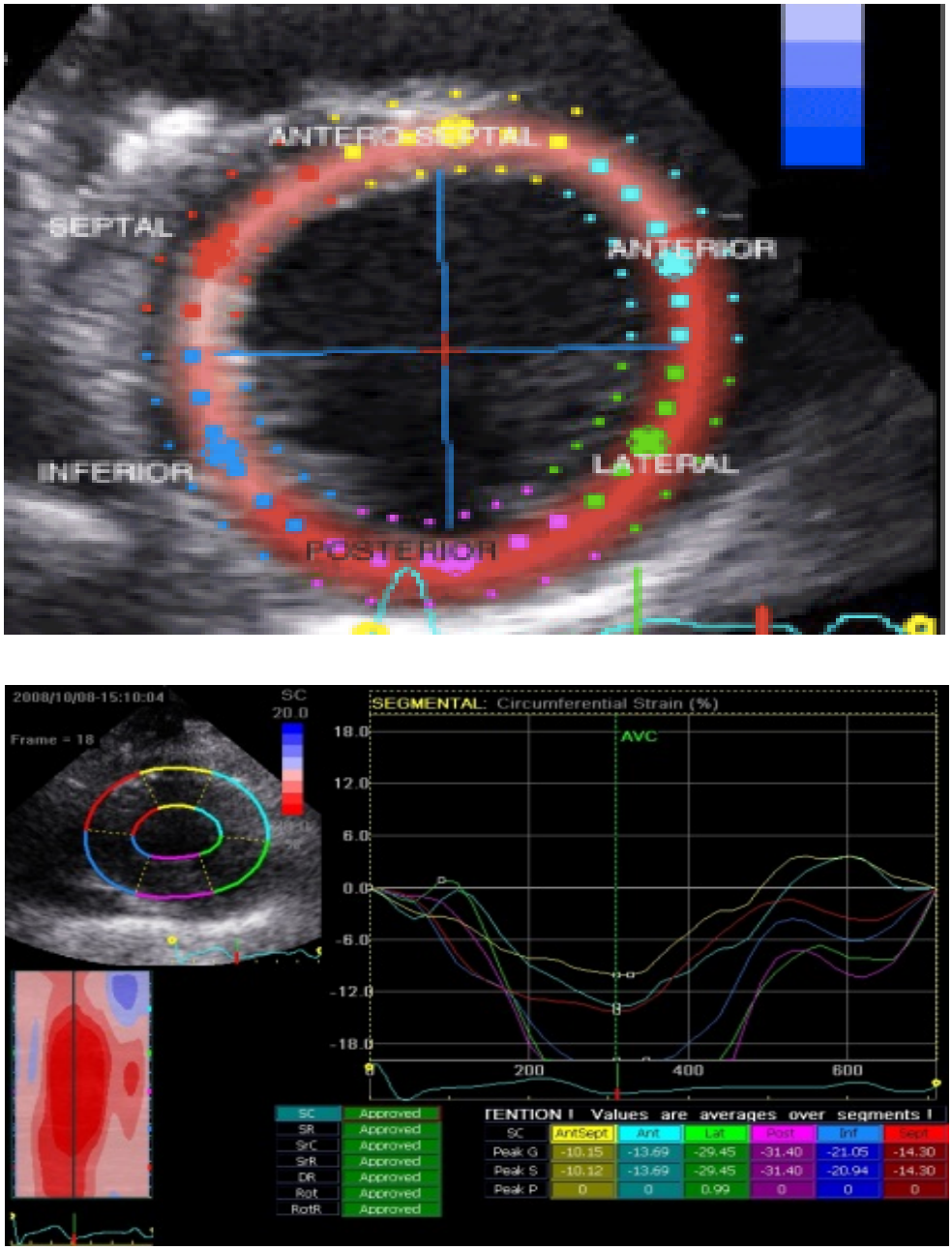


Figure 16. *Segmental circumferential strain on a short axis view (**upper view**) at the level of the papillary muscles. This patient was excluded, as he presented anterior and septal LV regional wall motion abnormalities, which were confirmed by STE (**lower view**).*

LV Twist

LV twist was determined using basal and apical short axis views. The difference in the systolic rotation of the myocardium in an apical and basal short-axis plane is commonly referred to as twist, and is reported in degrees (***Figure 17***).

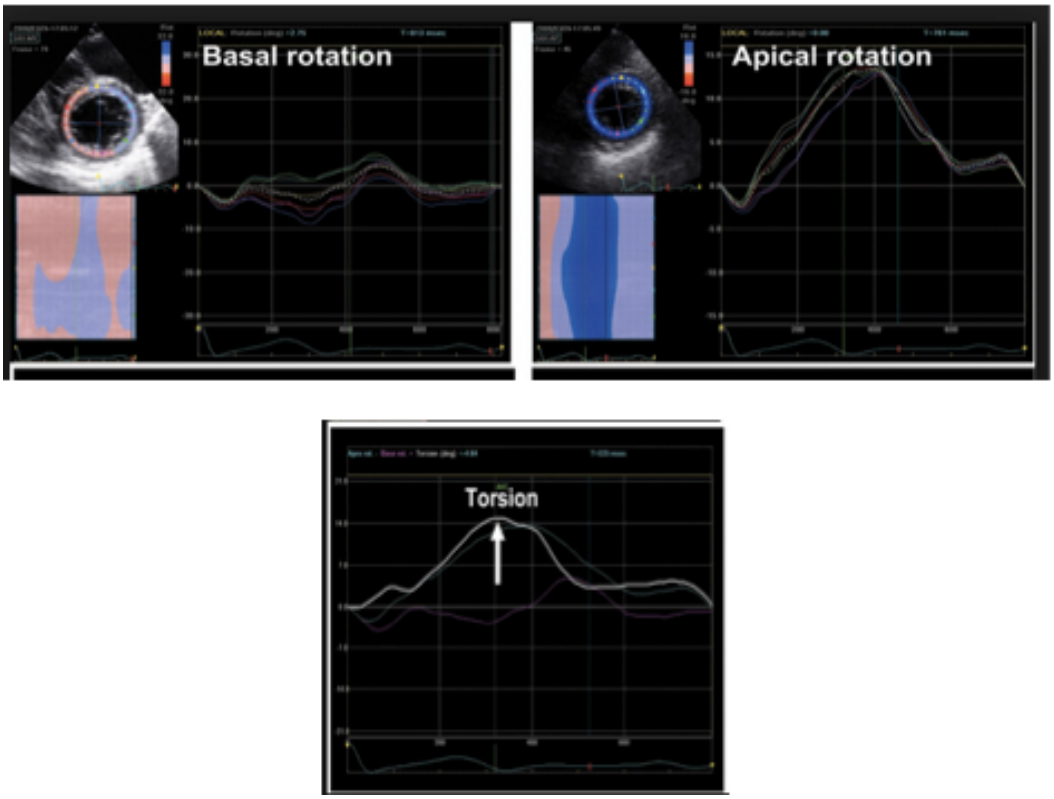


Figure 17. *Measurement of LV twist. Peak systolic LV twist was calculated as the maximum instantaneous difference between peak systolic apical and basal rotation.*

LV segmental diastolic function / segmental relaxation

Early and late diastolic SR parameters (SRe and SRa) were calculated from 18 segments in the longitudinal direction (***Figure 18***). As previously described, SRe/SRa

≤1.1 was regarded as an index of altered segmental relaxation–segmental DD[109]. The total number of segmental DD was calculated for all the participants.

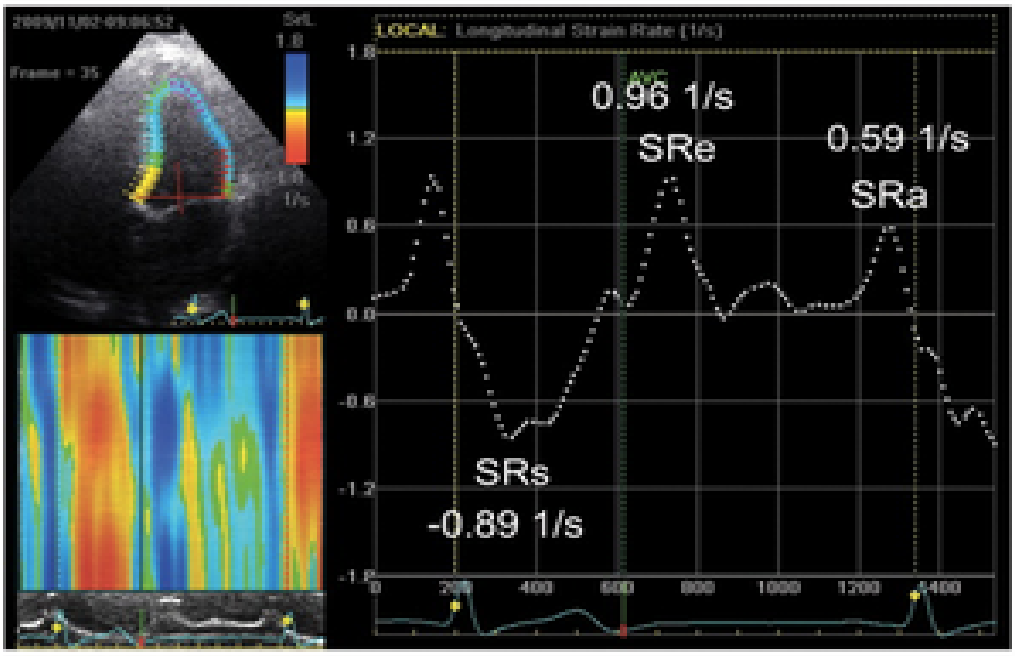


***Figure 18**. Example of segmental relaxation pattern using strain rate.*

5.8 Pulse wave velocity analysis

On the same day, roughly 10 minutes after the echocardiogram had been performed, BP was measured with the participants still in supine position. Pressure waveforms were recorded on the carotid and femoral arteries using applanation tonometry[132] to estimate carotid-femoral aPWV. We utilised a commercially available device (SphygmoCor, Pulse Wave Analysis System, AtCor Medical) with a high-fidelity Millar strain-gauge transducer (Millar Instruments, Houston, TX) (***Figure 19***).

Applanation tonometry allows the detection of peripheral pulse signals, using an extremely sensitive pressure sensor. Experimental trials showed that if this element is pressed on the outside of the skin over an artery, and the artery is compressed slightly against a firm structure (such as bone), the signal produced is similar to that of the intravascular pulse.



Figure 19. SphygmoCor, Pulse Wave Analysis System, AtCor Medical.

5.8.1 Pulse wave velocity

For assessment of aPWV, the operator located the carotid and femoral arteries using applanation tonometry, and marked the point for capturing the corresponding pressure curves. The distance travelled by the pulse waveforms was measured between the two recording sites directly on the body surface, and was corrected automatically according to the equation '0.8 × direct distance', subtracting the manubrium-to-carotid distance as recommended previously[114]. Brachial blood pressure was then measured, averaged (across three recordings), and entered into the SphygmoCor

system. I located the sensor in the femoral artery and, when I recognised pulse waveforms of sufficient quality, the pressure wave was recorded. The same procedure was repeated for the carotid artery. At least ten seconds of recording was required for both carotid and femoral traces.

As described previously[117], the time delay (aortic transit time) between the two pulse waveforms was calculated according to the intersecting tangent algorithm. The PWV was then calculated using measurements of transit time and distance travelled by the pulse wave between the two recording sites[114] (***Figure 20***). We obtained multiple sets of waveforms where feasible, and overall PWV was taken as the average of the two highest quality recordings.

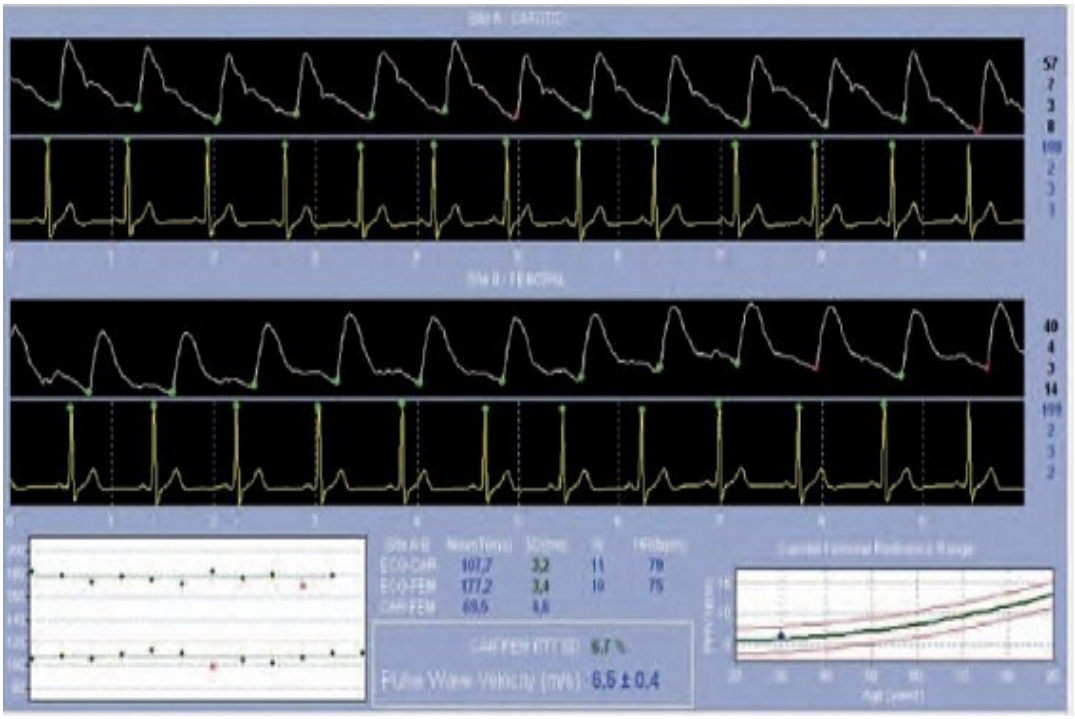


Figure 20. *Measurement of carotid-femoral PWV with the SphygmoCor® device. Using the electrocardiogram's R wave as gating, pressure curves are recorded at the carotid artery (**upper panel**) and at the femoral artery (**lower panel**), consecutively. For each QRS complex over a 10 sec–20 sec recording, the difference in time to the foot of each waveform is calculated (ΔT). PWV = distance/mean ΔT. The foot of the pressure wave is identified automatically, using a predefined algorithm (the intersecting tangents method). The system calculates transit time. Travel distance is measured on the surface of the body and entered into the system, which computes pulse wave velocity (in this example, distance was 430 mm and ΔT = 66 ± 7.3 m/s; PWV = 6.5 m/s). Age-specific reference values are shown in the right lower panel.*

5.9 Reproducibility of STE and arterial stiffness parameters

In order to explore inter- and intra-observer variability for both STE and arterial stiffness indices, we reassessed the measurements with a subgroup of 20 patients. Shortly after the first assessment, a second operator (who was experienced in strain analysis) analysed the arterial (aPWV) and myocardial (GLS, GLSR, GCS, and LV twist) indices. The second operator was fully blinded to the results of the analysis. Subsequently, the first operator re-measured the same indices. Intra-class correlation

coefficient (ICC)[133] values, together with Bland-Altman[134] and absolute average mean difference + standard deviation (SD) plots, were constructed for the data. The results are reported in the reproducibility sections of each experimental chapter.

5.10 Statistical analysis

All statistical analyses were performed by the author and reviewed by Dr Vasielios Panoulas and Dr Joseph Eliahoo, Senior Statistician at Imperial College London. Statistical analysis was performed using the SPSS V22 (IBM Corp., Armonk, NY, 2015) and STATA V12 (Stata Corp., 2011) software.

The criterion for statistical significance for our data was a two-tailed *p*-value of ≤0.05 with a 95% confidence interval. The following section describes the statistical tests we used for each results chapter.

5.10.1 Chapter 6

Values were expressed as means ± SD, medians (25^{th}–75th percentile values), or percentages, as appropriate. Group differences were tested using ANOVA and Kruskal–Wallis tests for normally and non-normally distributed variables, respectively. Chi-square tests or Fisher's exact test was used (as appropriate) to examine the group differences in proportions. The ICC and mean differences were used to assess the inter- and intra-operator variability of speckle tracking measurements. A general linear model was used to adjust for potential confounders (e.g. HT, DM, relevant medication, and others) among CKD groups and controls.

5.10.2 Chapter 7

First, analysis was performed by dividing the patients into two groups, one with aPWV <10m/s and the other with aPWV ≥10m/s. Differences between the two groups were analysed using the chi-square test for categorical data, the *t*-test for continuous normally distributed data, and the Wilcoxon rank-sum test for continuous non-normally distributed data. Correlations were assessed for normal and non-normal variables using Pearson's and Spearman's coefficients, respectively. Univariate and multivariate analyses were used to evaluate the relationship between arterial stiffness and a range of variables, including general demographics, cardiovascular risk factors, and conventional echocardiographic and speckle tracking parameters. The ICC and Bland-Altman plots were determined to assess the inter- and intra-operator variability of speckle tracking and arterial stiffness measurements.

5.10.3 Chapter 8

Receiver operating characteristic curves were generated to assess the overall performance of the imaging parameters in predicting outcomes. Univariate and multivariate Cox proportional hazards regression analyses were performed to relate clinical and imaging parameters to the primary endpoint and, at the same, to adjust for potential confounders. Survival curves were constructed using the Kaplan–Meier method, and the log-rank test was used to compare survival curves.

RESULTS

CHAPTER 6

EARLY DETECTION OF SUBCLINICAL MYOCARDIAL DYSFUNCTION IN PATIENTS WITH CKD AND NORMAL LVEF

6.1 Introduction

Chronic kidney disease is associated with CV morbidity and mortality[24]. Interestingly, LVEF predominantly remains preserved in these patients, and fewer than 15% of patients with ESRF have evident systolic dysfunction when assessed by conventional methods[135,136]. Novel echocardiographic STE permits accurate assessment of subclinical myocardial function, and may provide mechanistic insights into the early pathogenesis of clinical CVD in CKD with normal LVEF. Currently, limited data exists using STE in CKD patients who are asymptomatic from a CV point of view. In 2012, two studies that compared STE markers in CKD patients with those of controls (both with normal LVEF) reported reduced GLS and GLC in the CKD group[8,9]. Previously, in 2008, Edwards et al.[137] used tissue Doppler imaging to demonstrate reduced GLS and GLSR among patients in early stages of CKD. Although of interest, the studies mentioned above were small and underpowered, and some novel elements of deformation (e.g. twist) were not investigated.

In this chapter, our purpose is to conduct an extensive analysis of conventional and

STE parameters in patients who have CKD (at all stages) with preserved LVEF and no known history or symptoms of CVD.

6.2 Results

6.2.1 Inter- and intra-operator variability

Intraclass coefficient correlation is a measure of agreement between two or more measurements. Based on the 95% confident interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively

Inter-operator variability (n=20)

The ICC for GLS was 0.82 ($p<0.001$), for GCS it was 0.71 ($p=0.001$), and for LV twist it was 0.79 ($p<0.001$). The absolute average mean difference $\pm$ SD for GLS was 0.10 ± 2.25, for GCS it was 0.11 ± 4.06, and for LV twist it was 0.69 ± 4.51 (***Figure 21***).

Intra-operator variability (n=20)

The ICC for GLS was 0.79, $p<0.001$, GCS 0.85, $p<0.001$, and LV twist 0.89, $p<0.001$. Absolute average mean difference ± SD for GLS was 0.65 ± 2.99, GCS 0.27 ± 3.56, and LV twist 0.18 ± 3.49.

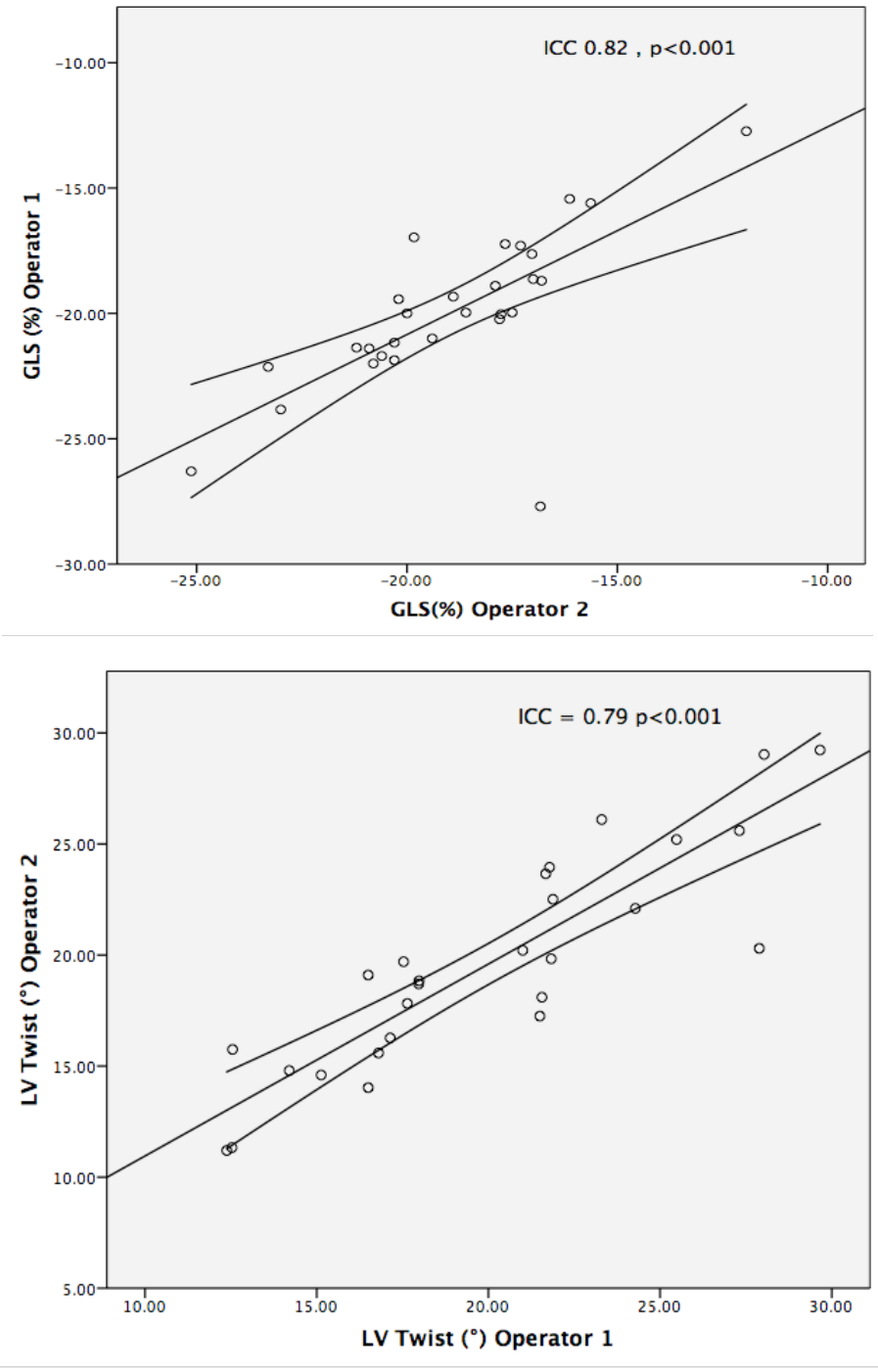


Figure 21. *ICC of GLS (**upper figure**) and LV twist (**lower figure**).*

6.2.2 Demographics and patient characteristics

The mean age of our CKD patients was 54 ± 15.1 years, which did not differ significantly from that of the two comparison groups (p=0.09). The number of women in the groups was as follows: 52 (49.1%) women in the CKD group, 22 (55.6%) in the control group, and 19 (50%) in the risk factor group (p=0.521).

Among the total sample, 95 CKD patients underwent renal biopsy to identify CKD aetiology. The most common CKD aetiology was diabetes (n=30), followed by vasculitis (n=18), post renal causes (n=10), IgA nephropathy (n=7), focal glomerulosclerosis (n=6), membranous glomerulonephritis (n=5), hypertension (n=4), adult polycystic kidney disease (n=3), renovascular disease (n=3), and minimal change disease (n=3). In six patients, the CKD aetiology was unknown. Study participants were then stratified into five groups: 38 (20.9%) healthy controls, 38 risk factor controls (20.9%), 29 (15.9%) patients with CKD stages 1 or 2, 38 (20.9%) patients with CKD stage 3, and 39 (21.4%) patients with CKD stages 4 and 5. Demographics, CV risk factors, and medication across the five groups are summarised in ***Table 4***. Data on micro- and macro-albuminuria was available for 79 (74.5%) CKD patients within a month after recruitment. Albuminuria (micro/macro) was more prevalent among late-stage CKD groups (69.6% *vs* 72.4% *vs* 85.2% for CKD stages 1–2, stage 3, and stages 4–5, respectively).

6.2.3 Conventional 2D echocardiography

Conventional echocardiographic statistics for the study sample are presented in ***Table 5***. No significant difference was identified for LVEF between the CKD patients,

controls, and risk factor group (p=0.571). Compared with healthy controls, CKD patients displayed an increased LV wall thickness (p<0.001) and increased LVMI with concentric remodelling (p<0.001). As seen in ***Table 5***, CKD patients had a higher prevalence of DD(p<0.001).

Table 4. *General demographics, CV risk factors, and drug therapy across the five groups.*

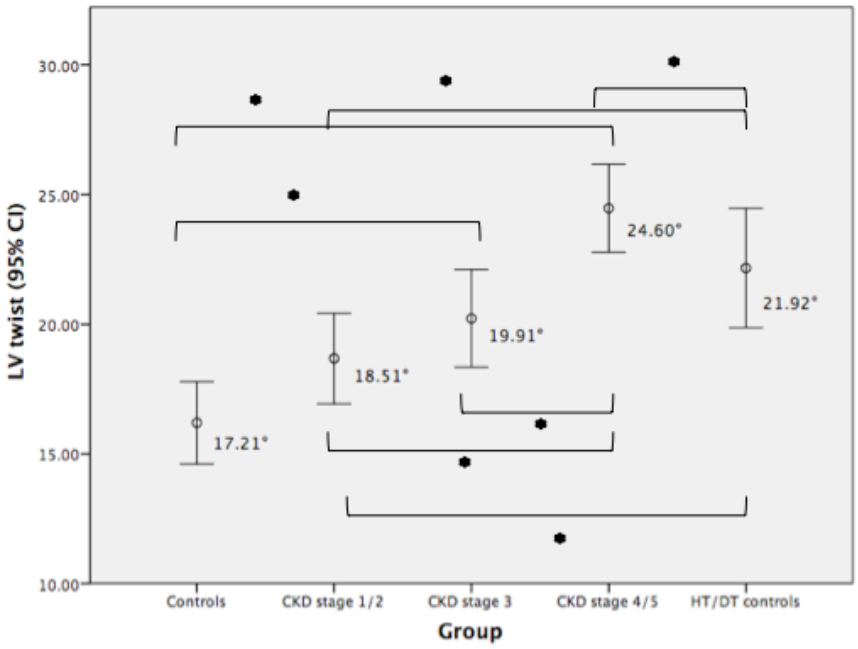
	Controls (n=38)	Risk-factor controls (n=38)	CKD A (n=29)	CKD B (n=38)	CKD C (n=39)	p-value[1]
General demographics						
Age in years (M±SD)	51.7±13.3	52.5±11.4	44.9±13.1	58.8±13.9	57.4±14.6	<0.09
Male sex (%)	44.4	50	41.4	55.3	53.8	0.521
BSA in g/m^2	1.85±0.21	1.80±0.21	1.81±0.21	1.94±0.23	1.89±0.21	0.181
Cardiovascular risk factors						
BMI (kg/m^2)	25.3±4.1	24.1±4.1	25.6±4.5	28.5±5.3	26.9±4.7	0.043 [gh]
GFR (ml/min/1.73mm^2)	-	81.2±17.4	70.8±17.4	45.3±9.3	18.0±5.5	<0.001 [cdfgh]
Systolic BP (mmHg)	116±14.4	150±18	128.0±26.1	134.6±21.8	135.5±17.0	<0.001 [afghi]
Diastolic BP (mmHg)	73.9±8.1	97	76.5±12.1	78.4±9.3	79.9±10.9	0.001 [fghi]
DM (%)	0	59	20.7	60.5	43.6	<0.001
HT (%)	2.6	89	41.4	89.5	79.5	<0.001
Hb (g/dL)	-	11.1±2.4	14.2± 2.1	11.9±2.7	9.7±3.3	<0.001
Treated dyslipidaemia (%)	2.6	45	53.6	70.3	69.4	<0.001
Family history of IHD (%)	0	23	34.5	34.2	20.5	0.001
Smoking status (%) Current Ex Never	 3.9 7.9 88.2	 7.3 0 92.7	 3.5 17.2 79.3	 10.5 13.2 76.3	 7.7 2.6 89.7	0.323
Medication						
Aspirin (%)	2.6	21.2	20.7	42.1	28.2	<0.001
Clopidogrel (%)	0	0	0	10.5	2.6	0.039
ACE-I (%)	2.6	72.1	37.9	57.9	45.9	<0.001
ARBs (%)	0	51.7	44.8	57.9	54.1	<0.001
Beta-blockers (%)	0	8	13.8	13.2	25.6	0.011
CCB dihydropyridine (%)	0	61.2	10.3	23.7	51.4	<0.001
CCB non-dihydropyridine (%)	0	0	0	7.9	0	0.027
Loop diuretic (%)	0	11.4	17.2	28.9	30.8	0.002
Thiazide diuretic (%)	0	0	0	2.6	7.7	0.112
Statins (%)	2.6	38.6	55.2	63.2	69.2	<0.001
Prednisolone (%)	0	0	20.7	21.1	10.3	0.019
Metformin (%)	0	41.1	13.8	18.9	7.7	0.053
Gliclazide (%)	0	25.7	3.4	5.3	20.5	0.004
Insulin (%)	0	59	3.4	34.2	20.5	<0.001

Results represent mean ± standard deviation unless otherwise specified.
[1]Statistically significant difference between: [a]controls and group B, [b]controls and group C, [c]groups A and B, [d]groups A and C, [e]groups B and C, [f]risk factor controls and group A, [g]risk factor controls and group B, [h]risk factor controls and group C, [i]risk factor controls and healthy controls
ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCB, calcium channel blocker , Hb haemoglobin

6.2.4 Speckle tracking echocardiography analysis

The STE statistics are shown in ***Table 6***. A gradual reduction in GLS, GCS, and GLSRs is noted among the groups, moving from healthy controls to late-stage CKD patients.

A GLS absolute value of -16% was considered pathological (GLS >-16%)[131]. Subclinical GLS impairment was found in 2.6% of healthy controls, 21% in the risk factor control group, and 3.4% among CKD stage 1–2 patients. A significantly higher prevalence of impaired GLS was noted amongst CKD stage 3 patients (39.5%) and stages 4–5 (25.6%) when compared with healthy controls and CKD stages 1-2. Moving from early to late-stage CKD, there was a gradual increase in LV twist (18.51±4.36, 19.91±5.35, 24.60±5.35; $p<0.001$) and twist rate (110.4±30.1, 121.0±31.4, 154.8±36.7; $p<0.001$). These trends are shown in ***Figure 22.*** The number of segments with DD increased significantly, again moving healthy controls to late-stage CKD patients (3, 6, 8, 9; $p<0.003$).



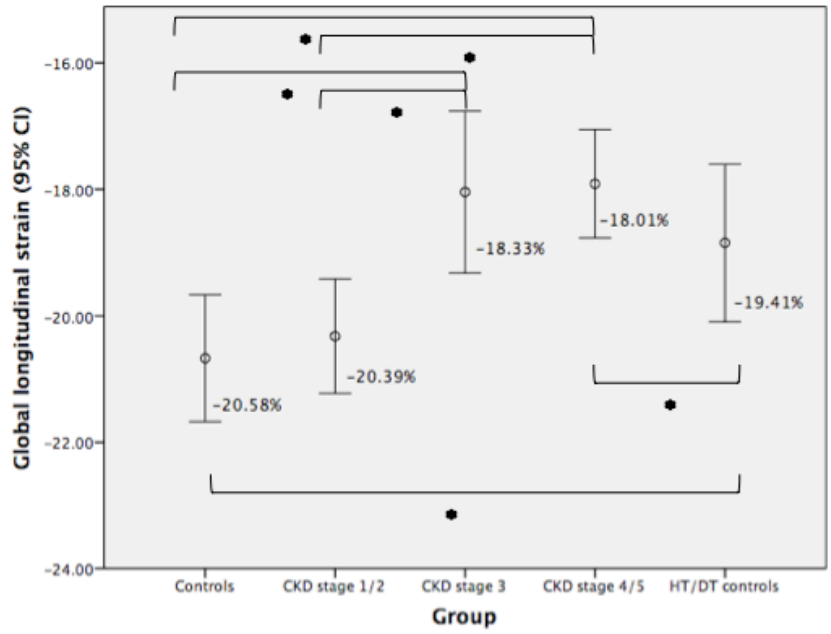


Figure 22. *LV twist (upper graph) and GLS (lower graph) in our cohort (observed values: 95% CI, *p<0.05 after Bonferroni correction in the univariate model).*

Table 5. *Conventional echocardiographic parameters across the five groups.*

	Controls (n=38)	Risk factor control (n=38)	CKD A (n=29)	CKD B (n=38)	CKD C (n=39)	Crude p-value[1]	Adjusted p-value[2]	Adjusted p-value[3]
IVSd (cm)	0.81±0.14	1.19±0.32	0.87±0.21	1.04±0.23	1.04±0.25	<0.001[abfi]	0.449	0.589
LVIDd (cm)	4.71±0.49	4.11±0.59	4.45±0.44	4.42±0.54	4.50±0.55	0.011[ih]	0.391	0.507
LVPWd (cm)	0.87±0.13	1.17±0.29	0.85±0.15	0.94±0.18	0.97±0.14	<0.001[fghi]	0.213	0.390
RWT	0.33±0.06	0.47±0.05	0.38±0.08	0.43±0.10	0.44±0.07	<0.001[abi]	0.352	0.419
Eccentric remodelling (%) RWT <0.42	89.5	39.2	69	47.4	46.2	<0.001	0.093	0.196
LVMI(g/m^2)	60.1±10.1	81.7±20.9	60.5±20.0	70.0±19.8	75.6±24.1	0.001[abfi]	0.035[i]	0.178
LVH (%)	0	12.6	3.4	7.9	10.3	0.030	0.067	0.653
Simpson's biplane EF %	63.1±5.5	59.9±4.1	63.7±5.5	62.3±4.9	62.5±4.9	0.317	0.638	0.743
LAVI (mL/m^2)	22.3±6.2	30.1±10.5	24±7.2	28.6±8.48	31.4±10.5	0.02[bf]	0.04[b]	0.11
E/A ratio	1.41±0.48	0.92±0.13	1.25±0.42	1.12±0.61	1.03±0.43	0.003[abfi]	0.176	0.605
MV DT (msec)	197.8±45.1	221.9±53.1	222.0±74.5	218.5±59.1	218.4±47.1	0.257	0.405	0.445
IVRT (msec)	93.2±15.1	115.9±23.0	99.8±17.0	111.1±25.8	106.4±23.0	0.009[afi]	0.532	0.712
E/e' (septal)	7.40±2.53	10.5±2.13	8.12±2.24	11.00±4.72	12.10±4.58	0.003[abfi]	0.023[b]	0.035[b]
E/e' (average)	6.41±2.21	11.1±4.22	6.96±2.25	9.41±3.87	9.92±3.96	0.002[abfgi]	0.044[bi]	0.039[bi]
e' (septal) <8 (%)	18.1	69.5	24.1	76.3	74.4	0.001[abfi]	0.013[abi]	0.03[ab]
Diastolic dysfunction (%)								
Not present	80.2	21.6	75.9	26.3	25.6	<0.001		
Type 1	17.2	50.3	17.2	39.5	43.6			
Type 2	2.6	28.1	6.9	31.6	30.8			
Type 3	0	0	0	2.6	0			

Results represent mean ± standard deviation unless otherwise specified; [1]Statistically significant difference between: [a]controls and group B, [b]controls and group C, [c]groups A and B, [d]groups A and C, [e]groups B and C, [f]risk factor controls and group A, [g]risk factor controls and group B, [h]risk factor controls and group C, [i]risk factor controls and healthy controls;
[2]Adjusted for age, sex, diabetes, Hb, systolic BP, BMI, treated hyperlipidaemia (variables that differed significantly across CKD groups in univariate analyses).
[3]Adjusted for factors in Model 2 plus antihypertensive medication (b-blockers, dihydropyridine calcium channel blockers, ACE inhibitors, angiotensin II receptor blockers, and thiazide diuretics).

***Table 6**. Two-dimensional speckle tracking parameters across the five groups.*

	Controls (n=38)	Risk factor control (n=38)	CKD A (n=29)	CKD B (n=38)	CKD C (n=39)	p-value[1]	Adjusted p-value[2]	Adjusted p-value[3]
Global longitudinal systolic strain (%)	-20.58±3.01	-19.41±2.64	-20.39±2.29	-18.33±3.81	-18.01±2.64	<0.001[abcdfi]	0.017[bd]	0.054
Global circumferential systolic strain (%)	-24.14±3.52	-24.79±4.56	-25.70±3.86	-23.38±4.92	-22.79±4.56	0.014[bdh]	0.037[d]	0.061
Global longitudinal peak systolic SR (s^{-1})	-1.01±0.13	-1.01±0.15	-1.06±0.17	-0.99±0.22	-0.88±0.52	0.047[bdh]	0.031[bdf]	0.058
Global circumferential peak systolic SR (s^{-1})	-1.38±0.23	-1.49±0.27	-1.56±0.31	-1.46±0.35	-1.46±0.31	0.512	0.313	0.255
Global longitudinal SRe (s^{-1})	1.23±0.31	0.97±0.21	1.35±0.38	1.03±0.38	1.02±0.24	0.007[abfi]	0.052	0.096
Global circumferential SRe (s^{-1})	1.93±0.53	1.85±0.43	2.15±0.61	1.87±0.54	1.79±0.50	0.011[fi]	0.185	0.212
Global longitudinal SRa (s^{-1})	0.89±0.28	0.95±0.18	0.91±0.20	0.88±0.31	0.99±0.18	0.064	0.345	0.386
Global circumferential SRa(s^{-1})	0.81±0.35	1.14±0.31	0.86±0.29	1.03±0.50	1.11±0.31	0.002[bi]	0.482	0.512
LV twist (°)	17.21±4.95	21.92±3.96	18.51±4.36	19.91±5.35	24.60±5.35	<0.001[**abdhei**]	<0.001[bdhi]	<0.001[bdh]
LV twist rate(°/s)	106.7±30.5	122.5±28.4	110.4±30.1	121.0±31.4	154.8±36.7	<0.001[**abdhefi**]	<0.001[bdih]	<0.001[bid]
Number of segments with DD	3 (0 to 5)	9 (4 to 11)	6 (1.5 to 8)	8 (1.5 to 12)	9 (5 to 12)	<0.003[b]	-	-

Results represent mean ± standard deviation unless otherwise specified;
[1]Statistically significant difference between: [a]controls and group B, [b]controls and group C, [c]groups A and B, [d]groups A and C, [e]groups B and C, [f]risk factor controls and group A, [g] risk factor controls and group B, [h]risk factor controls and group C, [i]risk factor controls and healthy controls
[2]Adjusted for age, sex, diabetes, SBP, BMI, treated hyperlipidaemia, Hb (variables that differed significantly across CKD groups in univariate analyses)
[3]Adjusted for factors in Model 2 plus antihypertensive medication (*b*-blockers, dihydropyridine calcium channel blockers, ACE inhibitors, angiotensin II receptor blockers, and thiazide diuretics)

6.2.5 Relationship between renal function, conventional echocardiography and STE

Conventional echocardiography

No association was found between LVEF and eGFR. After adjusting for risk factors (HT, hyperlipidaemia, BMI, and DM), family history of IHD, and hypertensive medication, eGFR was inversely associated with LV wall thickness, LVMI, and E/e' ratio. Estimated GFR was independently associated with E/A (***Table 7***).

2D STE

The eGFR variable was associated independently with absolute GLS, GLSRs, and GLSRe. Strong, independent inverse associations were observed between eGFR and LV twist, LV twist rate, and number of segments with DD. As shown in ***Table 7***, the strongest echocardiographic associations (standardised $\beta>0.3$) with eGFR were noted for LV twist rate, LV twist, LVMI, E/e' (average), and E/e' (septal).

Association of LVMI with speckle tracking parameters

Left ventricular mass index correlated significantly with LV twist ($r=0.214$, $p=0.011$), twist rate ($r=0.263$, $p=0.005$), and number of LV segments with DD ($r=0.433$, $p<0.001$). Left ventricular mass index was inversely correlated with absolute GLS ($r=-0.184$ $p=0.030$), absolute GLSRs ($r=-0.182$, $p=0.031$), and GLSRe ($r=-0.251$, $p=0.003$).

Table 7. *Crude and adjusted correlations between eGFR and conventional and STE parameters (CKD patients).*

	Crude		Adjusted[1]	
	Standardised beta	**p-value**	**Standardised beta**	**p-value**
Conventional echocardiography				
IVSd	-0.412	<0.001	-0.362	0.001
LVIDd	-0.079	0.424	-0.042	0.679
PWTd	-0.364	<0.001	-0.257	0.022
RWT	-0.291	0.002	-0.196	0.060
LVMI[3]	-0.423	<0.001	-0.362	<0.001
Simpson's biplane EF	0.117	0.235	0.120	0.219
LAVI	-0.218	0.025	-0.078	0.455
E/A ratio	0.248	0.010	0.201	0.036
E/e' (septal)	-0.374	<0.001	-0.319	0.002
E/e' (average)	-0.365	<0.001	-0.330	0.002
e'/a' (average)	0.321	0.001	0.269	0.006
2D speckle tracking echocardiography				
Global longitudinal systolic strain	-0.261	0.007	-0.214	0.035
Global circumferential systolic strain	-0.186	0.056	-0.130	0.184
Global longitudinal peak systolic SR	-0.210	0.031	-0.208	0.034
Global circumferential peak systolic SR	-0.112	0.254	-0.139	0.148
Global longitudinal SRe	0.296	0.002	0.247	0.049
Global circumferential SRe	0.224	0.021	0.175	0.067
Global longitudinal Sra	-0.201	0.039	-0.153	0.115
Global circumferential SRa	-0.206	0.034	-0.125	0.204
LV twist	-0.480	<0.001	-0.412	<0.001
LV twist rate	-0.468	<0.001	-0381	<0.001
Segments with diastolic dysfunction	-0.340	<0.001	-0.246	0.017

[1]Adjusted for diabetes, systolic BP, BMI, treated hyperlipidaemia (eGFR incorporates age/sex). Standardised coefficients (b) refer to how many SDs the dependent variable (eGFR) will change by, with each SD increase in a predictor variable.

6.2.6 The influence of HT and DM in assessing STE

Among our CKD cohort, 81 patients (76%) had concomitant HT and/or DM. The HT and/or DM CKD patients showed significantly lower GLS compared with the risk factor control group (HT and/or DM with normal renal function), as shown in Table 8 (-18.05±2.25 vs. -19.41±2.64). However, after adjustments for relevant medication GLS lost it's significance. The LV twist (23.61±5.13 vs. 21.92±3.96, p=0.034) and twist rate (138.8±34.3 vs. 122.5±28.4, p=0.032) were significantly higher in the CKD cohort compared with the risk factor group, even after adjustments. All other STE parameters did not show a statistically significant difference between the two groups.

Table 8*. LV strain parameters in CKD and risk factor–matched groups*

	CKD group n=81	**Risk factor matched group n=38**	***p*-value**	***p*-value [1]**	***p*-value [2]**
Global longitudinal strain (%)	-18.05±2.25	-19.41±2.64	0.021•	0.044•	0.068
Global circumferential strain(%)	-23.98±4.56	-24.79±4.56	0.120	0.331	0.453
Global longitudinal strain rate (s^{-1})	-0.92±0.19	-1.01±0.15	0.198	0.251	0.321
Global circumferential strain rate (s^{-1})	-1.39±0.21	-1.49±0.27	0.166	0.247	0.376
LV Twist(°)	23.61±5.13	21.92±3.96	0.004•	0.034•	0.042*
LV Twist rate(°/s)	138.8±34.3	122.5±28.4	0.011•	0.039•	0.05*
Number of segments with DD	8 (2.5 to 12)	9 (4 to 11)	0.131	0.252	0.281

Results represent mean ± standard deviation unless otherwise specified
[a] CKD group with HT and/or DM (n=81); [b] HT and/or DB group with normal renal function (n=38); • Statistically significant difference between groups;[1]Adjusted for age, sex, BMI, treated hyperlipidaemia and Hb; [2] Adjusted for relevant medication (significantly different between groups in table 4)

6.3 Discussion

To our knowledge, this is the first comprehensive study of subclinical myocardial abnormalities identified with STE in CKD patients with normal LVEF and no previous history of CVD. Despite normal LVEF assessed by conventional echocardiography, STE showed a reduced GLS and GLSR and increased LV twist and twist rate among CKD patients, variables that were significantly associated with eGFR. Around a third of patients with advanced CKD (stages 3–5) had impaired GLS (<16%), while DD was more extensive with worsening eGFR. The bulk of CKD patients had normal LV systolic function when assessed with conventional echocardiography (±90%)[81,138]. Ahmed et al.[87] showed increased mortality in CKD patients with low LVEF and, more importantly, in patients who have preserved LV systolic function with the presence of DD. In CKD patients, conventional echocardiography shows a high prevalence of LVH[65,139,140], especially in late stages[9]. The presence of LVH is correlated with eGFR[141] and predicts mortality and progression to ESRF. Our data coincide with the findings of previous studies, implying an inverse association between eGFR and LVMI.

Impaired LV diastolic function with increased LV filling pressures has been reported by some[80,81] but not all[83] previous researchers. Hayashi et al.[65], in a small study with 22 patients who had CKD stage 1–3 and 18 who had CKD stage 4–5, identified an increased prevalence of DD (63% and 66%, respectively), defined as e' septal<8 cm/s. However, most studies[64,65] that present data on DD have omitted the grading according to the established guidelines[122]. In our cohort, DD[122] was most common in

CKD stages 3–5 (70%), with Grade I (impaired relaxation) being the most prevalent (40%), followed closely by Grade II (pseudonormal; 30%).

Very few studies have attempted to identify STE abnormalities in CKD. Among a small number of pre-dialysis CKD patients (n=20) with normal LVEF, Yan et al. found reduced GLS and GCS compared with healthy volunteers[8]. Liu at al. found similar results when comparing a cohort of 37 CKD patients (stages 3–5) with healthy controls. Using tissue Doppler imaging in 40 CKD stage 2–3 patients, Edwards and colleagues found a reduction in GLS compared with 30 healthy controls. Again, although both groups had normal LVEF, CKD patients displayed reduced GLS and GLSR. Limitations of the above studies included the small numbers of patients and the fact that some interesting elements of deformation, such as twist or twist rate, were not examined.

We demonstrated new patterns of LV deformation amongst asymptomatic CKD patients. Throughout systole, reduced GLS and GLSRs were compensated for by increased LV twist and twist rate. This finding suggests a pattern of subendocardial ischaemia, the effects of which are in turn compensated for by the epicardial myocardial fibres (increased LV twist). Throughout diastole, GLSRe was independently associated with eGFR, suggesting impaired early diastolic filling on CKD. Segmental STE analysis demonstrated i) an inverse independent association between eGFR and number of segments with DD, and ii) progression of number of segments with DD when moving from early to late CKD stages. These findings suggest the presence of subendocardial damage, a myocardial layer that is commonly impaired soonest after ischaemic insults. Ischaemia can be macrovascular or

microvascular in origin. The absence of LV RWMA and normal LVEF, and the global pattern of subendocardial injury suggest that microvascular disease is the most likely cause of our findings.

In many CKD patients with associated DM and HT, myocardial changes are amplified by the existence of these co-morbidities. According to some studies, these co-morbidities augment the development of LVH, increase the susceptibility of the heart to ischemic injury, and increase the overall likelihood of heart failure[47,48]. Accordingly, in this chapter we also sought to identify echocardiographic parameters that are sensitive to detect myocardial involvement in CKD patients, and which could be a discriminator for the effects of renal disease beyond those of associated risk factors such as HT and/or DM. We showed that patients with CKD and HT/DM display significantly reduced GLS and increased LV twist compared with risk factor–matched control participants (DM and/or HT with normal renal function); this implies a further deterioration in subclinical longitudinal function in CKD. These findings suggest that both LV twist and GLS can be useful parameters for differentiating cardiovascular involvement in patients with CKD in the presence of HT and/or DM. Non-traditional CVD risk factors, which are commonly present in CKD patients, might explain the reduction in the values of these parameters in the CKD HT and/or DM population, as they have shown to promote CVD risk[33,34].

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6.4 Conclusions

With the use of STE, our CKD patients with normal LVEF and no previous history of

CVD displayed subclinical abnormalities in LV global longitudinal systolic function (GLS and GLSRs), which is compensated for by increased LV twist and twist rate. Speckle tracking echocardiography also showed a high prevalence of segmental DD in CKD, and further revealed that DD becomes more extensive as CKD progresses. In addition, both LV twist and GLS can be useful to differentiate cardiovascular involvement in patients with CKD in the presence of HT and/or DM.

CHAPTER 7

SUBCLINICAL MYOCARDIAL DISEASE AND ITS RELATION WITH AORTIC STIFFNESS

7.1 Introduction

Alterations in both LV and aortic physiology may play an important role in predisposition to heart failure, especially heart failure with preserved ejection fraction (HFpEF)[142]. Interestingly, HFpEF has been related to both reduced myocardial strain and increased aortic stiffness in a number of prior studies[143-140]. Furthermore, both HFpEF[144,145] and increased aortic stiffness[75,118,146] are prevalent in late-stage CKD or dialysis patients. This finding suggests possible pathophysiological links between aortic stiffness and subclinical alterations in LV systolic function, which might promote the development of HFpEF in this population. To further clarify the interrelations between large-artery haemodynamics and alterations in subclinical LV systolic function, we investigated the association between aortic stiffness and STE in a group of CKD patients who were free of overt CVD and had normal LVEF by echocardiography.

7.2 Results

7.2.1 Inter- and intra-operator variability of speckle tracking and arterial stiffness parameters

The strength of agreement for intra- and inter-observer measurements of aPWV and LV twist is shown in ***Figure 23***. ***Figure 24*** is a Bland-Altman plot showing inter-observer variability for aPWV and LV twist.

	PWV 1	PWV2	ICC	P
Intra-observer (n=20)	9.7±2.8	9.6±3.1	0.94	<0.001
Inter-observer (n=20)	9.7±2.8	9.35±2.9	0.89	<0.001

ICC. Intraclass correlation; PWV, Pulse wave velocity (m/s); PVW1, first measured; PWV2, second measured

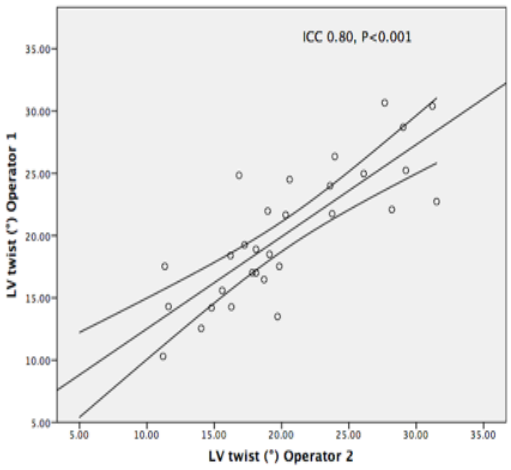


Figure 23. *Intra- and inter-observer strength of agreement for aPWV (**left**); inter-class correlation for LV twist (**right**).*

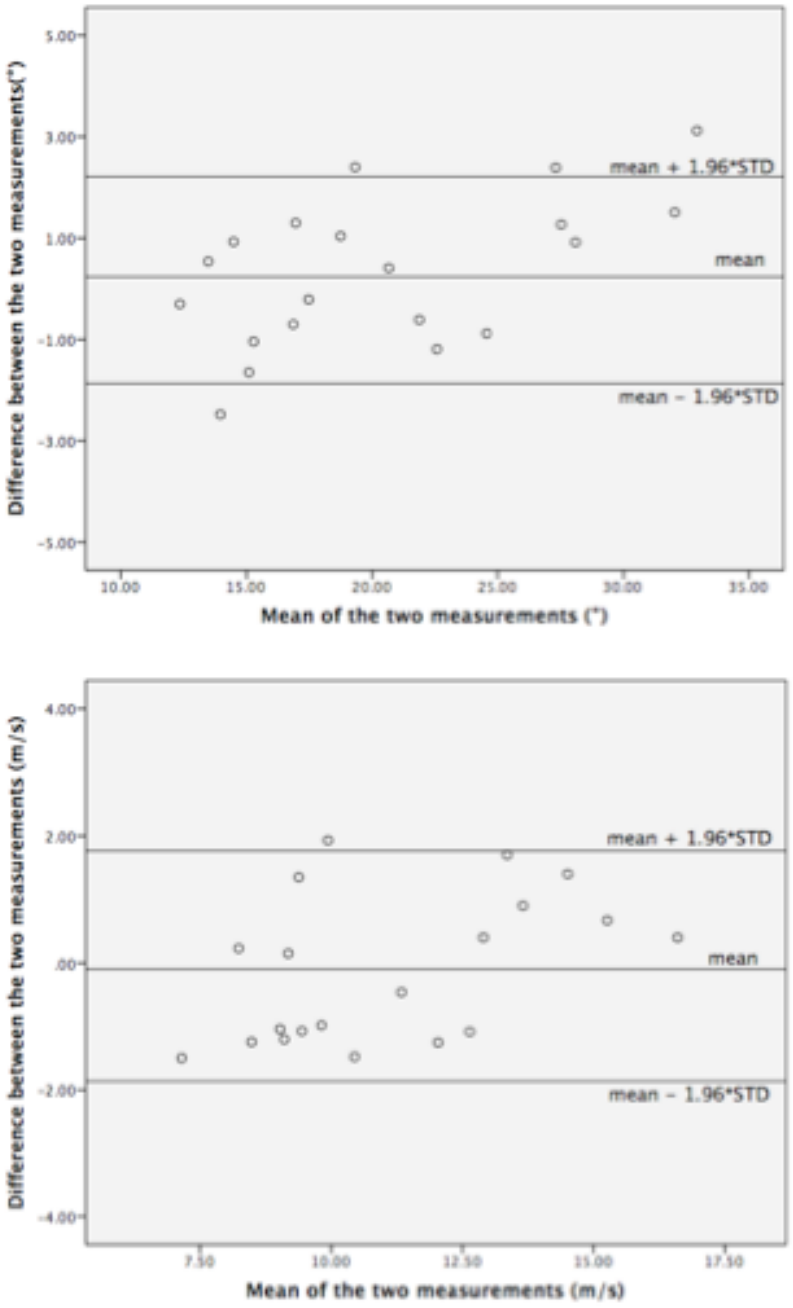


Figure 24. *Bland-Altman plot showing inter-operator variability for PWV (**lower graph**) and LV twist (**upper graph**).*

7.2.2 Clinical and echocardiographic characteristics

Previous studies have shown that an aPWV cut-off of 10m/s provides risk stratification and prognostic value for CKD patients[147]. This value has also been addressed as the clinically relevant level at which CV risk rises, and has recently been recommended as cut-off in the guidelines of the European Society of Hypertension[114].

Therefore, our patients were stratified into two groups according to aPWV: normal aPWV (<10m/s) and increased aPWV (≥10m/s). ***Table 9*** shows patients' clinical characteristics. Participants with increased aPWV were more likely to have DM (p<0.001), HT (p<0.001), and low eGFR (p<0.001). ***Table 10*** shows the values for echocardiographic parameters in the two groups. Patients with increased aPWV had lower TDI S' (p=0.03) and GLS (p=0.002), as well as higher LVMI (p=0.01), E/e' (p=0.01), LV twist (p<0.001) (***Figure 25***), but similar LVEF (p=0.482), relative to patients with normal aPWV.

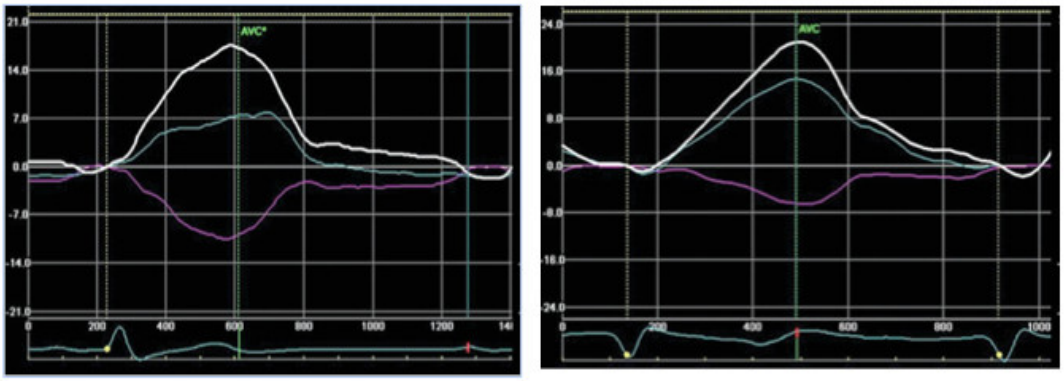


Figure 25. *LV twist in a patient with normal and increased PWV.*

Table 9. *Clinical characteristics according to aPWV.*

	Normal PWV (<10m/s) n=64	**Increased PWV (≥10m/s) n=42**	***p*-value**
Clinical demographics			
Age in years	51.2±14	63±12	<0.001*
Male sex (%)	45.4	41.2	0.455
BSA (g/m^2)	1.88±0.21	1.87±0.22	0.906
Cardiovascular risk factors			
BMI (g/m^2)	26.9±5	27.8±5.1	0.367
eGFR (mL/min/1.73 m^2)	48.95±20.1	30.5±15.4	<0.001*
Systolic BP (mmHg)	127.2±20.4	140.5±18.5	0.01*
Diastolic BP (mmHg)	77.9±10.6	79.5±10.5	0.451
Diabetes (%)	20.3	71	<0.001*
Hypertension (%)	62.5	82.8	<0.001*
Family history of IHD (%)	29.7	26.8	0.755
Smoking status (%)			
Current	6.3	9.8	0.295
Ex	9.4	9.8	
Never	45.3	34.1	
Medication			
Aspirin (%)	28	37	0.367
Clopidogrel (%)	2	7	0.135
ACE-I (%)	34	69	<0.001*
ARBs (%)	53	51	0.858
Beta-blockers (%)	16	22	0.416
CCB dihydropyridine (%)	25	38	0.151
Loop diuretic (%)	22	34	0.112
Thiazide diuretic (%)	2	10	0.06
Any antihypertensive (%)	40.1	87.2	<0.001*
Statins (%)	61	80	0.110
Prednisolone (%)	22	10	0.125
Metformin (%)	9	18	0.081
Gliclazide (%)	9	12	0.649
Insulin (%)	8	41	<0.001*

Results represent mean ± standard deviation unless otherwise specified; * Statistical significant between groups

Table 10. *Conventional and STE characteristics of aPWV groups.*

	Normal PWV (<10m/s) n=64	**Increased PWV (≥10m/s) n=42**	***p*-value**
Conventional echocardiography			
Simpson's biplane EF (%)	62.9±5	61.3±4.7	0.48
TDI S' – septal (cm/s)	10.6±2.4	8.6±1.9	0.03*
LVMI (g/m^2)	63.7±19	78.4±22	0.01*
RWT	0.44±0.09	0.51±0.12	0.001*
E/e' (average)	8.9±3.7	10.8±4.2	0.01*
LA (cm)	3.68±0.58	3.9±0.63	0.001*
Diastolic dysfunction (%)			
Not present	40.6	22.5	0.001*
Type 1	50	40	
Type 2	9.4	34.9	
Speckle tracking echocardiography			
LV twist (°)	19.8±5.4	23.5±5.7	<0.001*
GLS (%)	-19.6±3.3	-17.1±2.9	0.002*
GCS (%)	-23.3±4.3	-22.8±4.9	0.159
Results represent mean ± standard deviation unless otherwise specified *Statistically significant difference between groups			

7.2.3 Univariate association betwen aPWV and other variables

We examined the associations between aPWV and other variables, namely demographics, risk factors, and echocardiographic parameters. ***Table 11*** shows univariate correlations between aPWV and the clinical and echocardiographic parameters. Aortic PWV correlated crudely with age (p<0.001), the presence of DM (p<0.001), hyperlipidaemia (p=0.013), peripheral systolic BP (p<0.001), HT (p<0.001), eGFR (p<0.001), LVMI (p<0.001), E/e' (p<0.001), TDI S' (p=0.03), GLS

(p=0.01), and LV twist (p=0.003). ***Figure 26*** is a scatter plot showing the correlation between aPWV and LV twist.

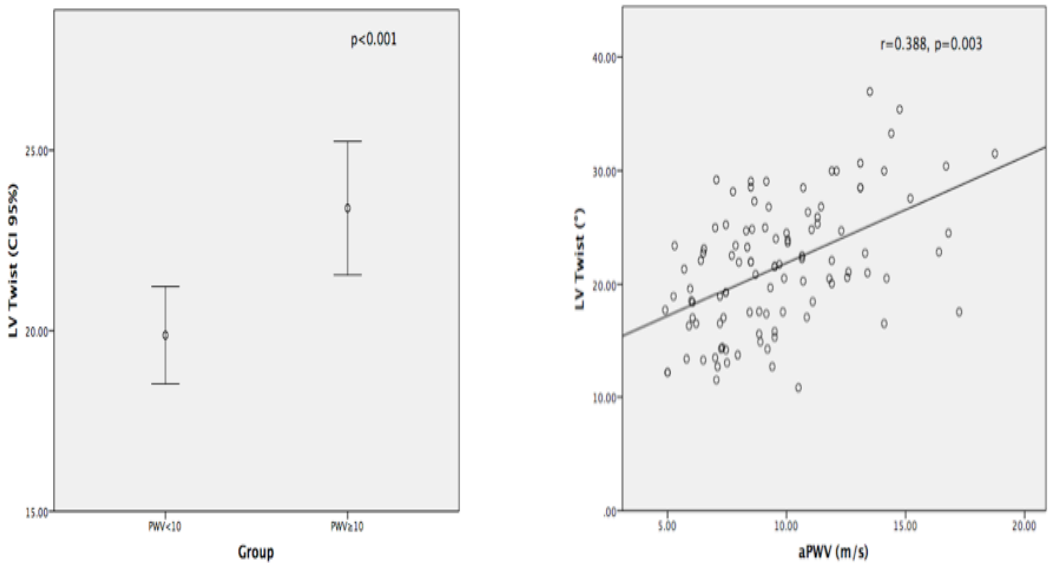


Figure 26. *Increased LV twist in patients with preserved and increased aPWV (**left**); scatter plot correlating aPWV (m/s) and LV twist (°) (**right**).*

7.2.4 Multivariate analysis

In the linear regression model, after covariate adjustments, aPWV was independently associated with age (β=0.467, p<0.001), DM (β=0.532, p<0.001), HT (β=0.158, p=0.05), eGFR (β=0.234, p=0.008), and LVMI (β=0.143, p<0.042). Among the STE parameters, only LV twist (β=0.163, p=0.025) was significantly and independently associated with aPWV (***Table 11***).

Table 11. *Univariate and multivariate analysis for aPWV.*

	Crude		**Adjusted[1]**		**Adjusted[2]**	
	Standardised β	*p*-value	Standardised β	*p*-value	Standardised β	*p*-value
Demographics or Clinical characteristics						
Age	0.635	<0.001*	0.492	<0.001*	0.467	<0.001*
Sex	-0.172	0.079	-0.019	0.654	-0.013	0.691
BSA (g/m^2)	0.27	0.187	0.031	0.238	0.345	0.459
Haemoglobin (g/L)	-0.150	0.131	-0.059	0.481	-0.098	0.221
Hyperlipidaemia	0.242	0.013*	0.118	0.144	0.065	0.433
Peripheral systolic BP (mmHg)	0.397	<0.001*	0.185	0.024*	0.169	0.059
LVMI (g/m^2)	0.379	<0.001*	0.165	0.03*	0.143	0.042*
Diabetes (%)	0.589	<0.001*	0.555	<0.001*	0.532	<0.001*
Hypertension (%)	0.308	<0.001*	0.294	0.013*	0.158	0.050*
BMI (g/m^2)	0.055	0.579	-0.156	0.063	-0.133	0.118
eGFR (mL/min/1.73 m^2)	-0.410	<0.001*	-0.259	0.003*	-0.234	0.008*
Echocardiographic and STE parameters						
Simpson's biplane EF (%)	-0.117	0.237	-0.036	0.646	-0.094	0.634
TDI S' – septal (cm/s)	0.186	0.03*	0.120	0.058	0.101	0.093
E/e'(average)	0.387	<0.001*	0.192	0.022*	0.113	0.089
LV twist (°)	0.388	0.003*	0.181	0.019*	0.163	0.025*
GLS (%)	0.300	0.01*	0.186	0.09	0.060	0.130
GCS (%)	0.046	0.197	0.018	0.458	0.010	0.634

Standardised coefficients (β) refer to how many SDs the dependent variable (aPWV) will change by, per SD increase in a predictor variable. n=106. [1]Adjusted for age, sex, diabetes, systolic BP, BMI, treated hyperlipidaemia, eGFR, and family history of ischaemic heart disease. [2]Adjusted for factors in Model 2 plus antihypertensive medication (b-blockers, calcium channel blockers, ACE inhibitors, angiotensin II receptor blockers), and prednisolone.

7.3 Discussion

To our knowledge, this is the first study to show an independent association between aPWV, a marker of aortic stiffness, with LV twist, a myocardial deformation index. The association between LV twist and aPWV in asymptomatic CKD patients suggests an interplay between arterial and ventricular mechanics early in the CV disease continuum.

Arterial remodelling has been described in early-stage CKD patients[7]. Compared with normotensive and hypertensive controls, patients with CKD stages 2–5 had significantly larger internal carotid artery diameters but comparable intima-media thickness, resulting in significantly increased circumferential wall stress[7]. Arterial stiffness in early-stage CKD patients has been associated with the presence of DD[11] and increased LV mass[12]. However, its relationship with systolic function remains poorly investigated and largely unknown. This might be related to the fact that current methods to assess LV systolic function are not sensitive to detect subclinical LV dysfunction.

A limited number of studies have examined the association between arterial stiffness and LV strain. Fernandes et al.[148] showed a significant correlation between carotid arterial compliance (measured by carotid ultrasonography) and circumferential systolic/diastolic strain rates (measured by cMRI) in 1,100 participants without documented cardiovascular disease. Hwang et al.[149] showed a significant correlation between brachial-ankle PWV and LV systolic GLS in 70 patients with untreated hypertension and normal LVEF. However, Hwang et al. did not perform multivariate analysis, neither did they adjust for the confounding effects of clinical variables.

Krishnasamy et al.[13] demonstrated an independent association between GLS (assessed by STE) and aPWV in patients with CKD stages 3–5. Kim and colleagues[150] also found an independent association between brachial-ankle PWV and GLS among a cohort free of CVD. Our study complements these findings, as we noted a strong independent association between LV twist, which is a marker of systolic dysfunction, and aPWV.

Alterations in LV twist have been linked with subclinical myocardial dysfunction in a variety of clinical settings[103,151,152]. However, the association between LV twist and arterial stiffness had not been studied. To our knowledge, our study is the first to show an independent association between LV twist and aPWV. As arterial stiffness increases, so too does the velocity of the forward and reflected waves. The accelerated return of the reflected waves imposes an increase in afterload (pressure work) on the LV, resulting in LVH and reduced coronary perfusion[153,154]. Myocardial fibre orientation changes continuously from a right-handed helix in the subendocardium to a left-handed helix in the subepicardial region[90]. Subepicardial layers dominate the overall rotation and twist during ejection, and remain preserved in early myocardial disease[90]. Subendocardial fibres counteract subepicardial dominance and are most vulnerable and sensitive to the presence of early myocardial disease. Subendocardial injury would therefore exaggerate overall LV twist, as the subepicardial fibres would function in the absence of the counteracting subendocardial fibres.

Based on the fact that increased arterial stiffness impairs coronary perfusion and elevates LV afterload[155,156], there is a potential pathway whereby increased arterial stiffness precedes and results in increased LV twist movement, rather than vice versa.

This hypothesis is supported by preclinical and clinical studies that have shown that increased afterload leads to a decrease in LV strain[157,158].

In our study, GLS was not independently associated with the presence of arterial stiffness. Our finding contradicts that of Krishnasamy and colleagues, which showed an independent association between GLS and arterial stiffness in a CKD cohort with previous CVD[13]. From a pathophysiological point of view, our findings are difficult to interpret, as GLS is a sensitive marker to detect subendocardial dysfunction—a layer commonly affected by the presence of arterial stiffness[159]. We found, however, that patients with increased arterial stiffness (aPWV ≥10m/s) had significantly impaired GLS. This finding adds to growing evidence that GLS can potentially be used as an early sensitive marker of myocardial disease in the presence of arterial stiffness.

A close pathophysiological link exists between CKD, arterial stiffness, and hypertension[160]. Our findings demonstrated this link, with around 90% of our CKD cohort who had increased arterial stiffness (aPWV≥10m/s) also having HT. The literature provides strong evidence that CKD patients at stages 3–5 have a high prevalence of both HT and arterial stiffness[146,154]. Therefore, the presence of co-morbidities such as HT can influence both myocardial and arterial physiology and cofound results in studies such as ours. However, from a clinical viewpoint, it is challenging if not impossible to find CKD patients (especially at stages 3–5) who have no co-morbidities. To address this issue, we identified potential confounders in our study and used advanced multivariate statistical techniques to handle large

numbers of covariates, as well as confounders, simultaneously. This enabled us to adjust the results of our findings according to relevant factors.

7.4 Conclusion

The current chapter provides—for the first time—evidence of a link between LV twist (assessed by STE) and early peripheral arterial disease among asymptomatic CKD patients with preserved LVEF. This finding supports the longstanding theory that arterial stiffness may impair LV performance.

CHAPTER 8

SUBCLINICAL MARKERS OF CARDIOVASCULAR DISEASE PREDICT ADVERSE OUTCOMES IN CHRONIC KIDNEY DISEASE PATIENTS

8.1 Introduction

Patients who have CKD comprise a group that is substantially at risk for CV events, as a result of prolonged exposure to an ever-growing array of traditional and non-traditional risk factors[24]. Most risk factors exert their influence on the myocardium and arterial wall itself, causing collateral damage to organs when circulatory function is altered sufficiently to result in clinical disease[161]. The process of circulatory damage occurs over many years. Although outcomes such as stroke or MI occur at identifiable discrete times, it is far more difficult to cross-sectionally assess the influence of established biomarkers of CV risk, such as glucose or cholesterol, without some surrogate for CV outcomes[162]. In the last few years a number of surrogate markers of subclinical myocardial and arterial dysfunction have emerged to quantify the degree of subclinical CV impairment. These surrogate markers are particularly relevant to CKD, a population at increased risk for CVD[24].

In this chapter, we hypothesise that subclinical markers of myocardial (STE) and vascular (aPWV) disease can provide important prognostic value in predicting MACE among CKD patients (all stages) with no overt CVD and normal LVEF.

8.2 Results

8.2.1 Baseline clinical and imaging

The median follow-up period in this study was 49 months (interquartile range: 11–63 months). Overall, 26 patients in our total sample (24.5%) reached the primary endpoint. During the follow-up period, five deaths (4.7%) occurred: three were the result of heart failure, one was a sudden death, and one was the result of sepsis. Other events were as follows: four ST elevation MI (3.8%), two non-ST elevation MI (1.9%), three admissions for urgent PCI (2.8%), five admissions for an elective PCI (4.7%), three CABG revascularisations (2.8%), one unstable angina (leading to CABG revascularisation, 0.9%), one admission for heart failure (0.9%), and two strokes (1.9%).

Table 12. *Baseline demographic, clinical, and imaging characteristics, divided into groups according to the occurrence (or lack thereof) of MACE at follow-up.*

	Free of MACE n=80	**Suffered MACE n=26**	***p*-value**
General demographics			
Age, years	54.5±13	60.3±12	0.108
Male sex, n (%)	39 (48.7)	15 (54.3)	0.06
BSA, m^2	1.9±0.21	1.8±0.23	0.854
Clinical characteristics			
BMI, kg/m^2	24.3±4.3	27.5±5.2	0.040*
eGFR, ml/min/1.73 m^2	47.7±9.3	23.2±5.8	<0.001*
Systolic BP, mmHg	130.2±22.1	139.6±24.4	0.392
Diastolic BP, mmHg	78.4±10.1	84.1±13.7	0.764
DM, n (%)	36 (41)	15 (54)	0.265
HT, n (%)	51 (65)	23 (82)	0.01*
Urea, mg/dL	10.7±2.1	16.1±3.8	0.01*
Creatinine	150.6±27.1	269.1±31.9	<0.001*
Total Cholesterol mmol/L	4.4±0.7	4.6±0.6	0.463
LDL, mmol/L	2.2±0.37	2.5±0.42	0.22
HDL, mmol/L	1.4±0.23	1.1±0.20	0.138
Smoking (ever), n (%)	6 (7.9)	5 (15.6)	0.432
Conventional echocardiography			
IVSd, cm	0.98±0.08	1.2±0.13	0.03*
LVIDd, cm	4.4±0.51	4.6±0.68	0.180
LVIDs, cm	2.9±0.36	3.1±0.27	0.445
LVPWd, cm	0.92±0.06	0.97±0.08	0.191
FS, %	34.1±8.1	29.7±5.8	0.238
LVMI, g/m^2	72.5±16.5	88.2±19.1	0.02*
RWT	0.41±0.0.6	0.44±0.07	0.412
Biplane LVEF, %	63.2±9.2	59.1±10.7	0.231
LAVI, mL/m2	24.6±3.8	32.6±4.3	0.02*
E/A ratio	1.06±0.09	1.10±0.07	0.745
E/e' (average)	7.1±1.4	12.5±1.9	0.001*
MV Dec Time, m/s	221±33.1	195±29.4	0.672
Diastolic dysfunction, n (%)			0.001*
Type I	30 (38.4)	11 (60.1)	
Type II	2 (3.1)	2 (7.9)	
Type III	0	1 (1.1)	
Not present	46 (58.5)	14 (30.9)	
Speckle tracking echocardiography parameters			
GCS, %	-24±2.08	-22.9±3.11	0.228
GLS, %	-19.1±3.81	-16.9±3.96	0.01*
GCSRs, s^{-1}	-1.51±0.13	-1.42±0.17	0.231
GLSRs, s^{-1}	-1.03±0.09	-0.78±0.22	0.01*
LV twist, °	16.3±4.82	19.3±5.30	0.05*
Number seg DD	7.1 (1.5-8)	9.9 (1.9-12.1)	0.038*
aPWV, m/s	8.6±1.7	11.5±2.41	0.001*
Data are presented as percentages, mean ± SD, or median (interquartile range), as appropriate. *Statistically significant			

The baseline clinical and imaging characteristics are shown in ***Table 12***. No significant differences were noted for demographic characteristics between patients who suffered MACE and those who did not. Patients who suffered MACE had higher BMI (27.5 vs. 24.3, p=0.04), lower eGFR (23.2 vs. 47.7, p<0.001), and a higher percentage of hypertensive heart disease (82% vs. 65% p=0.01) compared with MACE-free patients. Baseline GLS (-16.8% vs. -19.1%, p<0.001) and GLSr (-0.78m.s vs. -1.03m.s, p=0.01) were significantly decreased in patients who suffered MACE. By contrast, LV twist (19.3° vs. 16.3°, p=0.05) and aPWV (11.5m/s vs. 8.6m/s p < 0.001) were significantly higher in the MACE group compared with the MACE-free group.

8.2.2 Univariate and multivariate predictors of outcome

As shown in ***Table 13***, univariate Cox proportional hazards modelling demonstrated that several variables were significantly associated with the primary end-point. These were as follows: higher LVMI (HR 1.078, 95% CI 1.002–1.035, p=0.027), increased E/e' (HR 1.177, 95% CI 1.091–1.270, p<0.001), impaired GLS (HR 1.239, 95% CI 1.088–1.410, p=0.01), impaired GLSr (HR 3.369, 95% CI 1.710–6.638, p<0.001), and higher aPWV (HR 1.331, 95% CI 1.089–1.692, p=0.001).

In multivariate analysis, GLS (HR 1.12, 95% CI 1.02–1.29, p=0.041) and aPWV (HR 1.31, 95% CI 1.05–1.41, p=0.021) remained significantly and independently associated with the primary end-point. ***Table 13*** also shows the results of the multivariate analysis.

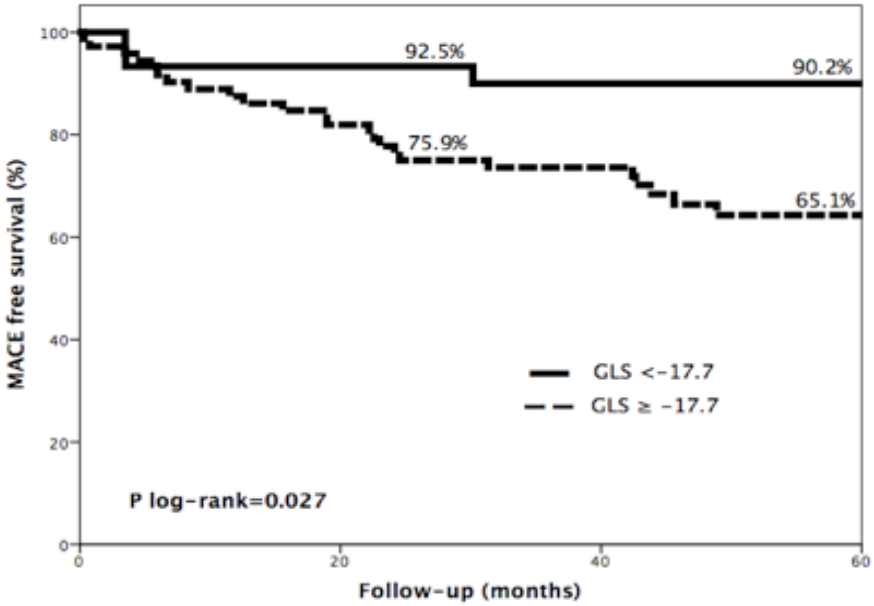
Among the imaging parameters, we identified three that had the best discriminatory power to predict the occurrence of the primary outcome. These were E/e' with a cut-off value of 11.7 (78.1% sensitivity, 82.4% specificity), GLS, with a cut-off value of -17.7% (72.3% sensitivity, 70.3% specificity) and aPWV, with a cut-off value of 10.1m/s (76.1% sensitivity, 69.6% specificity). (***Table 14***). Furthermore, patients with a GLS greater than -17.7% (log rank p=0.027) and aPWV of 10.2m/s or more (log rank p=0.019) had a reduced estimated MACE-free survival at 49 months' follow-up (***Figure 28***).

Table 13. *Univariate and multivariate Cox proportional hazards regression analysis for the primary endpoint.*

	Univariate analysis		**Multivariate analysis**	
	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Demographics/Clinical characteristics				
Age	1.02 (0.99-1.04)	0.128		
Male sex	0.56 (0.26-1.19)	0.136		
HT	2.83 (0.63-12.59)	0.172		
DM	1.66 (1.12-2.51)	0.06		
eGFR	1.10 (1.05-1.15)	0.001*	1.06 (1.02-1.16)	0.01*
Conventional echocardiographic parameters				
IVSd	7.17 (0.91-18.43)	0.083		
LVIDd	1.77 (0.79-3.38)	0.165		
LVIDs	1.43 (0.676-3.06)	0.345		
LVPWd	2.96 (0.67-13.04)	0.151		
LVMI	1.07 (1.00-1.03)	0.027*	1.02 (0.96-1.13)	0.09
RWT	7.96 (1.45-19.65)	0.351		
Biplane LVEF	0.96 (0.89-104)	0.341		
LAVI	1.73 (1.37-1.95)	0.032*	1.46 (0.94-1.13)	0.18
E/A ratio	1.97 (1.21-4.34)	0.121		
E/e' (average)	1.17 (1.09-1.27)	<0.001*	1.13 (1.02-1.21)	0.020*
DD Grade>1	1.25(1.08-1.35)	0.01*	1.10 (0.99-1.03)	0.11
Speckle tracking echocardiographic parameters				
GCS	1.05 (097-1.14)	0.185		
GLS	1.23 (1.08-1.41)	0.01*	1.12 (1.02-1.29)	0.041*
GCSRs	2.05 (0.61-6.86)	0.241		
GLSRs	3.36 (1.71-6.63)	0.04*	1.60 (0.55-4.81)	0.115
LV twist	1.10 (1.03-1.18)	0.06		
Number seg DD	1.07 (0.98-1.17)	0.124		
Arterial stiffness				
aPWV	1.33 (1.08-1.69)	0.001*	1.31 (1.05-1.41)	0.021*
Data is presented as HR, hazard ratio; CI, confidence interval (95%) *Statistically significant at $p<0.05$				

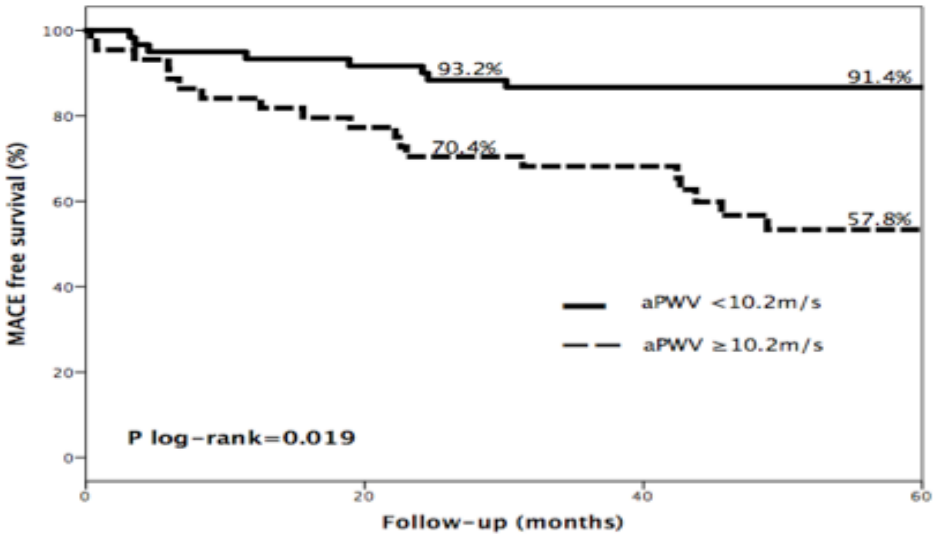
Table 14. *Cut-off values, stratified by best sensitivity and best specificity, to predict MACE.*

	Cut-off value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Area under the curve
GLS (%)	-17.7	72.3 (57.4-81.7)	70.3 (41.3-82.6)	0.71
GCS (%)	-23.4	57.1 (43.1-69.3)	56.8 (26.3-76.5)	0.51
GLSr (s^{-1})	-0.98	67.9 (48.8-75.4)	52.7 (53.0-87.4)	0.58
GCSr (s^{-1})	-1.4	53.6 (41.3-70.3)	49.2 (46.3-64.3)	0.49
LV twist (°)	17.5	62.3 (57.5-79.4)	56.1 (53.6-88.1)	0.61
Number seg DD	7.5	64.3 (46.8-70.9)	52.4 (49.9-76.3)	0.58
aPWV (m/s)	10.2	76.1 (58.4-89.6)	69.6 (55.6-84.2)	0.74
E/e’	11.7	78.1(66.1–89.8)	82.4(70.2–90.1)	0.79



Numbers at risk

Time	0	20	40	60
GLS< -17.7%	79	70	63	43
GLS≥ -17.7 %	17	14	7	2



Numbers t risk

Time	0	20	40	60
aPWV <10.2m/s	82	63	51	37
aPWV ≥ 10.2m/s	14	9	3	0

Figure 27. *Event-free survival according to cut-off values for GLS (**upper graph**) and aPWV (**lower graph**). Kaplan-Meier time to primary endpoint curves stratified according to GLS (-17.7%) and aPWV (10.2m/s).*

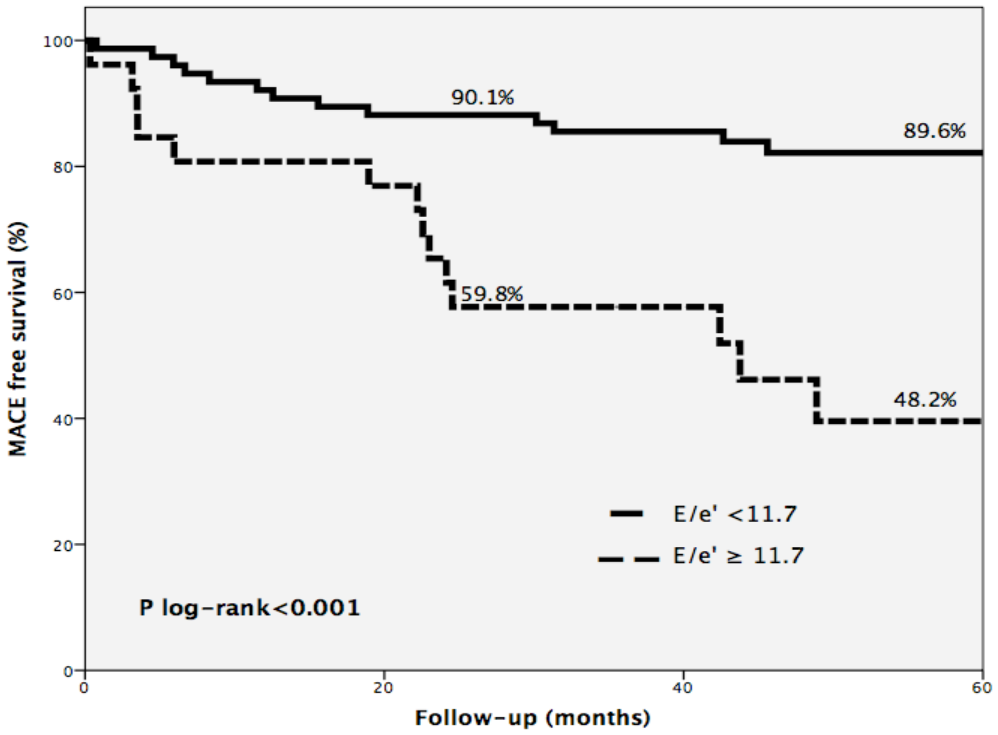


Figure 28. *Event-free survival according to cut-off values for E/e'. Kaplan-Meier time to primary endpoint curves stratified according to E/e' (11.7).*

8.2.3 Sub-analysis according to CKD stages

Further analyses of the data for the CKD groups were conducted using Cox univariate regression models. The results showed that advanced CKD (stages 4–5) was associated with more outcomes compared with earlier stages of CKD (17% *vs* 7.5%, p=0.001). Aortic PWV was a significant predictor in later CKD stages (HR 1.48, 95% CI 1.03–1.79, p=0.022). Although no statistically significant differences among the groups emerged for the speckle tracking parameters, we noted a considerable trend toward significance for GLS (HR 1.189, 95% CI 0.971–1.383, p=0.071) (***Table 15***).

Table 15. *Univariate Cox proportional hazards regression analyses for the primary endpoint according to CKD stages.*

	CKD stages 1-3		**CKD stages 4,5**	
	HR CI (95%)	P-value	HR CI (95%)	P-value
GCS	0.99(0.89 – 1.10)	0.870	1.03(0.90 – 1.18)	0.640
GLS	1.09 (0.95-1.50)	0.228	1.18(0.97-1.38)	0.071
GCSRs	1.32(0.76-6.65)	0.739	1.88(0.92-5.31)	0.538
GLSRs	2.01 (0.61-4.86)	0.141	1.32(1.04-1.42)	0.112
LV Twist	1.05 (0.94-1.18)	0.356	1.04(0.94-1.14)	0.384
Number seg. DD	0.97(0.83-1.17)	0.716	1.06(0.93-1.20)	0.369
aPWV	1.05 (0.88-1.24)	0.279	1.48(1.03-1.79)	0.022*
*Statistically significant at $p<0.05$; Data is presented as HR CI (95%)				

8.3 Discussion

In this chapter we show that GLS and aPWV are significant independent predictors of MACE in CKD patients who lack clinical or conventional echocardiographic evidence of CVD.

Previous observational and prospective studies have shown that, in the general population, GLS provides incremental value above that of conventional imaging[163] and biochemical measurements[164] in predicting survival following MI[164], cardiac surgery[165], or heart failure[110]. Few studies to date have addressed the relationship between GLS and CV mortality among CKD patients. In 2014, a single-centre study by Krishnasamy et al.[10] examined the association between GLS and all-cause mortality among 447 CKD patients. In the five-year follow-up period, GLS was a significant independent predictor of mortality (HR 1.08, 95% CI 1.01–1.15)[10]. More recently, Kramann et al. demonstrated the ability of strain parameters to identify uremic cardiomyopathy and predict CV mortality among dialysis patients[73].

Of interest, however, is the fact that many patients included in the aforementioned studies had already suffered a CV event. This confounds the results of those studies with regard to patients who had no clinical CV disease. In addition, GLS has been shown to be sensitive to loading conditions, especially afterload[166]. As a result, variations in GLS measurements owing to loading conditions occur in dialysis patients, and results from such studies should be viewed with caution. Lastly, novel deformation parameters (i.e. LV twist) were not assessed. Speckle tracking

echocardiography allows direct assessment of myocardial muscle shortening and lengthening by assessing not only strain or strain rate but also LV rotation or twist[90].

We used a wide range of subclinical CV measures—including strain, strain rate, LV twist, number of segments with DD, and aPWV—in an attempt to identify subclinical prognostic information. Our cohort encompassed CKD patients at all stages, with no previous history of CVD, whereas dialysis patients were categorically excluded. Our results demonstrated a significant independent relationship between impaired GLS and MACE among CKD patients showing no CVD and normal LVEF on conventional echocardiography. For our cohort, GLS of -17.7% or less displayed good discriminative power (both sensitive and specific) to predict MACE.

Another interesting finding of our study is that at baseline, patients who reached our composite primary endpoint (MACE) had significantly impaired GLS compared with patients who did not suffer MACE, even though LVEF was similar in both groups. This finding suggests that GLS might be able to detect CKD-related myocardial changes early in their course, as it primarily evaluates the function of subendocardial longitudinal fibres that are particularly sensitive to cardiac injury[167]. Animal and human studies support the idea that changes in myocardial composition and function that are fuelled by traditional (HT, DM) and non-traditional (oxidative stress, inflammation, thrombosis) CKD risk factors occur early in the course of the disease, prior to the onset of clinical manifestations[168,169]. Such reasoning supports the hypothesis that GLS could act as a CV biomarker of outcome in this high-risk cohort.

None of the remaining deformation parameters were significant predictors of CV events. Owing to the derivation of strain, SR is inherently noisier than strain, and

previous correlations between MRI tagging and STE were weaker for SR than they were for strain[170]. This earlier finding may partly explain our findings.

It is also known that GCS can remain relatively preserved to compensate for cardiac function when longitudinal function starts becoming impaired in patients with early-stage heart failure[171]. Left ventricle hypertrophy, which is observed even in early-stage CKD[168], is mainly caused by hypertrophic responses in the mid-myocardial layers that are predominantly circumferentially oriented. This compensates for the reduction in longitudinal function and may explain preserved LVEF and GCS[172] among our cohort.

Left ventricle rotational mechanics are altered with advancing age and with various medical conditions—such as MI, HT, diabetes mellitus, and dilated cardiomyopathies[152,173-175]. By contrast, very little is known about LV twist in CKD patients. The LV twists along its long axis during the cardiac cycle as the result of counter-clockwise and clockwise rotation of the apex and the base, respectively, in systole, and vice versa in diastole[176]. This twisting motion or torsion has been attributed to interaction between the epicardial and endocardial helical myocardial fibre couples[90]. In Chapter 6 of this thesis, we reported that LV twist was shown to be a subclinical marker of early renal impairment[82]. However, no significant independent association between twist and outcomes was identified in this chapter. We nonetheless found that patients who suffered MACE had significantly higher LV twist, adding to the growing evidence that LV rotational mechanics can potentially be used as an early sensitive marker of myocardial disease. Larger outcome-based

studies are required to assess whether this parameter might be useful for predicting cardiac events among CKD patients.

We also wanted to perform a separate analysis to assess predictors of outcome in patients with early (stages 1–3) versus advanced (stages 4–5) CKD. A few authors had already established that cardiac and arterial changes start to develop early in the uremic milieu[147,177]. In our study, however, the absence of significant predictors of outcome among patients with stage 1–3 CKD can be explained by the low number of outcomes for this group compared with patients with CKD stages 4–5 (7.5% vs. 17%, $p_{\log rank} = 0.001$). Nevertheless, for CKD stages 4–5 we found that aPWV is the most robust parameter to predict MACE in our cohort. This finding is in line with previous research[1]. In statistical terms, GLS was not a significant predictor for either group. Larger outcome-based studies are necessary to determine the value of GLS within CKD groups.

Arterial remodelling is known to be present even in the early stages of CKD. Compared with normotensive and hypertensive controls, patients with any CKD (stages 2–5) had significantly larger internal carotid artery diameters, but comparable intima-media thickness, resulting in significantly increased circumferential wall stress[7]. With regard to CV events, our findings complement those of the Nephrotest study[1] regarding the important relationship between aPWV and all-cause mortality and non-fatal CV events in CKD. The Nephrotest findings identified aPWV as an independent predictor of all-cause mortality and both fatal and non-fatal CV events in 439 patients with CKD stages 3–5. However, 28% of that sample had a history of CVD. Our work therefore extends this enquiry by examining a cohort that had no

clinical evidence of CVD. In our study, patients with an aPWV of 10.2m/s or more displayed a reduced estimated MACE-free survival at median follow-up of 49 months (57.8% *vs* 91.4%, $p_{log\text{-}rank} = 0.019$). Because CKD patients have a relatively high mortality rate, determination of aPWV may be a useful tool for individual risk stratification among CKD patients with no overt CVD.

8.4 Conclusion

For CKD patients with no clinical or conventional echocardiographic evidence of significant CVD, the variables of GLS and aPWV independently predict the occurrence of MACE. If our findings are validated in larger prospective studies, GLS and aPWV may become a useful addition to the current risk stratification tools. The aim is to prevent future CV events in this high-risk patient cohort.

CHAPTER 9

THESIS SUMMARY AND CONCLUSIONS

This thesis has explored the potential benefit of using both STE and aPWV as simple non-invasive markers of myocardial and vascular disease. These markers are particularly relevant to CKD patients, a population at increased risk of CVD.

We hypothesise that early subclinical abnormalities in cardiac structure and function are associated with kidney function decline and that these associations correlate with the presence of arterial stiffness. Furthermore, we hypothesise that indices of subclinical myocardial and vascular disease can provide useful prognostic information about our cohort.

Four manuscripts have been accepted by peer-reviewed journals, three written by myself as first author and one as second author. Our data has been presented at three international conferences. Although observational research is unable to delineate cause-and-effect associations, this thesis provides a much-needed platform from which to study the effects of early myocardial and vascular disease in asymptomatic CKD patients, and their prognostic value.

9.1 Synthesis

Hypothesis 1: **Chronic kidney disease patients, who are asymptomatic with regard to CVD, may present early myocardial dysfunction before disease becomes clinically relevant.**

Detection of myocardial dysfunction in the early, subclinical stage may have significant diagnostic and therapeutic implications. Speckle tracking echocardiography has been used for this purpose in several pathologies; however, its use in CKD patients without previous CVD is largely unknown.

This study found that, despite normal LVEF as assessed by conventional echocardiography, STE detected a reduction in LV global longitudinal systolic function (GLS/GLSRs) among patients compared with controls, as well as increased LV twist and twist rate. The test parameters were significantly associated with eGFR. Speckle tracking echocardiography also confirmed a high prevalence of DD in CKD patients, and further revealed that DD becomes more extensive as the disease progresses. The E/e' average ratio was significantly, independently, and inversely correlated with eGFR, suggesting that filling pressures increase in later CKD stages. In segmental STE analysis, the number of LV segments with DD was inversely and independently associated with eGFR, suggestive of worsening and more extensive DD in patients at advanced stages of CKD. Finally, compared with the HT and/or DM with normal renal function risk factor control group, the CKD group showed significantly reduced GLS and increased LV twist.

Hypothesis 2: **Arterial stiffness is associated with subclinical myocardial disease.**

Previous authors have already hypothesised that the abnormalities of arterial and LV geometry may begin in early-stage CKD. In a population-based study of 742 individuals, mildly reduced eGFR was significantly associated with greater LV mass, in a process that appears dependent upon increased arterial stiffness. However, the relation between arterial stiffness and systolic function in CKD patients remains largely unknown. This knowledge gap relates to the fact that conventional measures of systolic function are not sufficiently sensitive to detect subclinical dysfunction.

We examined the possible correlation between subclinical myocardial abnormalities and the presence of arterial stiffness. We found an inverse independent relationship between eGFR and arterial stiffness in our cohort. Furthermore, of all the STE parameters, only LV twist was significantly and independently associated with the presence of arterial stiffness.

Hypothesis 3: **Subclinical markers of myocardial and vascular disease can provide important prognostic value in predicting adverse outcomes in CKD patients (all stages), who have no overt CVD and have normal LVEF as assessed by conventional echocardiography.**

Previous studies identified conventional echocardiographic markers such as LVH, DD, and LV systolic dysfunction as strong predictors of outcome (all cause mortality and MACE) in CKD patients. However, most of these studies have enrolled participants who have major known structural and functional cardiac abnormalities.

We hypothesised that STE and aPWV can provide important subclinical prognostic value in a cohort without significant CVD.

The primary endpoint was a composite of MACE. Over a median follow-up period of 49 months, 26 patients (24.5%) reached the primary endpoint. On a multivariable Cox proportional hazards model, GLS and aPWV were significant independent predictors of MACE. Moreover, patients with more impaired GLS and higher aPWV had reduced MACE-free rates.

9.2 General discussion

Traditional risk factors are important predictors of adverse CV events in the general population[178]. However, calculation of the Framingham risk score underestimates cardiac events in patients with CKD[179,180]. Identification of sensitive non-invasive biomarkers for CV involvement, which can possibly be used as predictors for adverse outcomes, would be useful in patients with CKD. The aim of this study was specifically to detect early and subtle cardiac and vascular involvement using newer echocardiographic and vascular imaging parameters, which might be more sensitive than traditional parameters.

A growing body of evidence suggests that assessment of LV deformation by STE provides incremental information in clinical settings[112,113,165,171,181,182]. Resolving the multidirectional components of LV deformation can be useful for detecting subclinical states that are likely to progress into either systolic or diastolic heart

failure. Despite this potential, few studies have explored myocardial mechanics in CKD.

In general, longitudinal LV mechanics, which are predominantly governed by the subendocardial region, are the most vulnerable component of LV mechanics. They are therefore also the most sensitive to the presence of early myocardial disease[183]. The midmyocardial and epicardial function may remain relatively unaffected in early stages of myocardial disease[183], and therefore circumferential strain and LV twist remain normal or show exaggerated compensation to preserve LV systolic performance. By contrast, an acute transmural insult (e.g. acute MI) or progression of disease (e.g. severe aortic stenosis) results in concomitant midmyocardial and subepicardial dysfunction, leading to a reduction in LV circumferential and twist mechanics and a reduction in LVEF. Assessment of myocardial mechanics, therefore, can be tailored per the clinical goals.

With the use of STE in a CKD cohort with normal LVEF, we observed a reduction of subclinical longitudinal function (GLS and GLSR) and an increase in LV twist as one moves from early to advanced stages of CKD. Reduced longitudinal function is compensated for by increased LV twist and twist rate. This finding suggests a pattern of subendocardial injury, the effects of which are compensated for by the epicardial myocardial fibres (increased LV twist). The absence of regionality and normal LVEF support the hypothesis that microvascular ischaemia, which is frequent in the CKD population, may account for the GLS and LV twist patterns observed in our cohort. In addition, our results show that a GLS of -17.7% or less was associated with reduced estimated MACE-free survival rates, suggesting that GLS could serve as a biomarker

of outcome in CKD patients. We have demonstrated that alterations in LV strain are more sensitive than current imaging modalities and may eventually precede changes observed in the LVEF in patients with CKD. However, given its multifactorial complexity, the pathogenesis of subclinical myocardial dysfunction (in this instance measured by LV strain) in this population is a complex matter to explain. Pressure overload in CKD can be the result of HT, and alterations in LV compliance can be the result of LV hypertrophy (diastolic dysfunction) and arterial stiffness. Chronic kidney disease also causes fluid overload, which in turn may lead to LA dilatation—which leads to LV diastolic dysfunction. Most patients with CKD have associated DM, a condition known to cause reduced LV strain.

Previous data have shown that cofounding factors such as HT or DM, which are present in CKD patients, can affect myocardial deformation. In the Coronary Artery Risk Development in Young Adults study[50], among 2,479 patients, a high level of cumulative exposure to SBP and DBP was not associated with the reduction of LVEF, but it was associated with lower GLS as shown by STE. In that study, the average entry age was 25 years and cumulative exposure occurred over 25 years. Patients with early diabetic cardiomyopathy often have evidence of global DD but preserved systolic function, as reflected by a normal LVEF[51]. More recently, a landmark study by Ng et al.[6] showed for the first time that GLS was impaired in patients with uncomplicated Type 2 DM, suggesting the presence of subclinical dysfunction before the reduction of LVEF.

For this reason, in our study we also sought to identify echocardiographic parameters that are sensitive to detect myocardial involvement in CKD patients, and which could

be a discriminator for the effects of renal disease beyond those of associated risk factors such as HT and/or DM. We showed, for the first time, a significant reduction of GLS and an increase in LV twist in CKD patients, compared with a risk factor–matched control group with HT and/or DM and normal renal function. We hypothesise that this finding might be due to the presence of CKD non-traditional risk factors, which play a role in promoting CVD risk and are related to the severity of CKD.[21,22] In other words, such risk factors—which include inflammation, oxidative stress, elevated lipoprotein levels, and early activation of the renin-angiotensin system—are implicated in the relationship between degree of risk factor and severity of CKD[33,34].

Applanation tonometry allows non-invasive assessment of quantitative markers of arterial stiffness, such as aortic PWV. These markers are established as strong independent predictors of CV morbidity and mortality in patients with CKD[75,147,184]. Previous studies have established an association between aPWV and CKD progression[185]. With the use of atrial tonometry, the main purpose of this study was to associate the presence of arterial stiffness with subclinical LV dysfunction, to better understand arterial–ventricular interaction from a subclinical perspective.

The two main findings of this study are:

i) aPWV is independently associated with LV twist

ii) aPWV is an independent predictor of MACE in asymptomatic CKD patients with no previous history of CVD.

With increased aPWV, the reflected waves return earlier to the heart, affecting the central arteries during systole rather than diastole, thus amplifying aortic and ventricular systolic pressures. As a consequence, myocardial pressure load and oxygen consumption increase, leading to subendocardial injury—which in turn leads to 'overcompensation' of LV twist. Our findings suggest a close interaction between arterial and ventricular mechanics long before CVD becomes clinically relevant, and support the long-standing hypothesis that arterial stiffness, even in its subclinical state, may impair LV function.

Our study revealed that aPWV of 10.2m/s or more was not only associated with reduced estimated MACE-free survival rates at 49 months follow-up, but also had good discriminative power (in terms of sensitivity and specificity) to predict the primary endpoint we identified. We extend the previous data of Chen et al., whose study with Asian CKD patients (stage 3–5) showed that brachial-ankle PWV predicts a mixed endpoint, including renal aspects and mortality[186]. Our work also complements that of Baumann et al., which provided direct evidence that among European patients with CKD stage 2–4, increased aortic stiffness as determined by aPWV is a strong independent predictor of all-cause mortality[147]. Because CKD patients have an increased mortality rate when compared with general population, determination of aPWV may be a useful tool for individual risk stratification.

9.3 Limitations

This entire body of work is a series of observational studies. In principle, therefore, our approach might be open to criticism for not employing the gold standard of randomised control trials. Observational studies can be affected by unidentified variables that act as confounders and lead to unrepresentative results. Such unidentified variables are indeed common in CKD. However, we designed the study to take into account as many possible cofounders as we could conceive of, and, most importantly, we performed all the necessary adjustments using regression and multivariate techniques. This statistical approach allowed us to reduce confounding effects, generate reliable results, and improve the internal validity of the study. Larger studies with a longitudinal design and multi-centre samples are needed to assess the external validity of our findings. Another limitation of cross-sectional studies is that they may prove an association but they cannot unravel causality.

The work focused heavily on relatively new non-invasive measurements using STE and aPWV technology. One might therefore question the relevance of the findings. Speckle tracking echocardiography is a novel imaging technique that has the advantages of ease of application and analysis, along with good data reproducibility. Strain measurements using STE have been validated against gold standard cMRI and sonomicrometry. However, STE has some intrinsic limitations, which we took into account in this study. First, the local frame-by-frame tracking is based on the search for maximum likelihood between two local speckle patterns in two consecutive frames (refer to **Chapter 3.4**). All kinds of ultrasonic noise reduce the tracking

quality. Good image quality enhances the clarity of the speckle patterns and improves the accuracy and robustness of their detection. The acquisition of standardised image planes in optimised quality is therefore essential for optimal analysis, as well as for reducing inter- and intra-observer variability of tracking data. In the present study, tracking quality was evaluated visually, and regions of interest that showed poor tracking quality were either manually moved to areas with better speckle quality or deleted.

Second, STE is based on frame-by-frame tracking of echo-dense speckles within the myocardium and subsequent measurement of regional myocardial deformation. The optimal acquisition rate (i.e. number of frames per second) seems to have a notable effect on the accuracy of strain measurements. Previous literature has reported that a frame rate of 40Hz–80HZ allows correct STE measurements and analysis[129]. Accordingly, we followed this approach in our study. For the assessment of LV twist, I found an important limitation in some patients. True apical rotation seemed to be underestimated to a variable extent, owing to inability to visualise the true LV apex. Currently the anatomical landmarks are too loosely defined, and particular attention should be paid to recording the correct cross-section at the LV apical level[130]. For this study, and as described previously[130], the basal plane was defined as that which allows visualisation of the mitral valve with the cross-sectional plane as circular as possible, defined as the long-axis position in which the LV and aorta are most in line with the mitral valve tips in the middle of the sector. The apical plane was acquired distally to the papillary muscles. When obtaining the latter plane, care was taken to ensure the transducer was placed one or two intercostal spaces inferiorly.

Lastly, recent reports have shown significant discrepancies between vendors when measuring GLS using STE[187,188]. Our data for the control group (GLS -20.6%) are similar to data presented in the Japanese Ultrasound Speckle Tracking of the Left Ventricle (JUSTICE) study (GLS -21.3%)[189]. Yet our figures showed larger discrepancies compared with data presented by the HUNT study (GLS -18.7%)[190]. We used the Vivid 7 and EchoPAC GE systems (Vendor 1) whereas the HUNT study used technology from a different vendor. These discrepancies emphasise the importance of ongoing efforts to standardise speckle tracking methodologies across different vendors.

Aortic PWV is increasingly used in population studies and is recommended by the European Society of Hypertension as an aid in the management of HT. It is highly reproducible and has been validated based on clinical and epidemiological studies with different populations, including CKD patients, and whose reference values have been published[115-117]. The main limitation I encountered using the carotid–femoral approach, is that it requires a certain degree of operator skill to accurately acquire the pulses. The method is also somewhat time-consuming because acquiring the two signals sequentially cannot be done in an instant, and is intrusive in that it requires palpation of the femoral pulse near the groin.

All the patients recruited for this study had a normal echocardiogram, normal LV regionality, and no history of CVD (based on medical and General Practitioner notes). However, the presence of significant coronary epicardial artery disease was not ruled out in all patients. All patients in our cohort were clinically asymptomatic, with normal LVEF, and had no regional wall motion abnormalities as assessed by

conventional echocardiography. Therefore, they did not fulfill the criteria to be subjected to any invasive (coronary angiography) or functional testing. However, within our cohort was a subset of 20 pre-dialysis patients who underwent elective invasive angiography as part of their pre-transplant assessment after being recruited to our study. Most of this subgroup (n=19) had no significant disease in their epicardial arteries. In addition, 12 other patients in our sample, who had CKD stage 4–5, received a dobutamine stress echocardiogram as part of their pre-transplant assessment. Eleven of these patients had a negative test for ischaemia and one patient had blunted chronothropic response and thus did not achieve 85% of maximal predicted heart rate, despite the use of atropine. From a total of 32 patients that underwent functional or invasive testing to exclude the presence of significant coronary or myocardial disease, 30 patients (94%) had no significant epicardial disease or myocardial ischaemia. This fact supports the hypothesis that microvascular ischaemia may account for the GLS patterns observed in the current CKD population.

Finally, the biggest limitation in terms of recruitment was linked to the complexity of CKD and its multifactorial pathogenesis. I found it very challenging, if not impossible, to recruit CKD patients who had no associated risk factors or co-morbidities. Accordingly, the potential for confounding variables was considered in the design and implementation of this study. We took into consideration that several factors might associate with the analyses of both myocardial and vascular dysfunction among CKD patients. Confounding can be accounted for during analysis, assuming that the relevant confounding factors are measured[191]. From a practical

perspective, we took the following steps:

i. ***Measure and report all potential confounders.*** Co-morbidities (HT, DB, dyslipidaemia, or vasculitis), medication, and any factor that might affect our analysis were measured and reported for patients in each study group.

ii. ***Multivariate analyses.*** After initially examining the relationship between the exposure and the outcome (giving a 'crude' or 'unadjusted' result), confounders were then added to the model to provide an effect that is 'adjusted' for these known confounders. With the use of regression models, we were able to assess the independent contribution of eGFR on both STE and aPWV measurements. When the confounders were categorical or continuous, we used multiple logistical or linear regression. If the outcome was associated with time, we used the Cox proportional hazards model to 'adjust' the potential confounder variables in our model.

iii. ***Risk-factor control group.*** One advantage of a cohort study is that participants in cohorts can be matched, which limits the influence of cofounding variables. Because patients with CKD commonly have a high prevalence of HT and/or DM, we recruited an HT and/or DM risk factor–matched control group with normal renal function. By doing so, we limited the influence of both these risk factors in our analysis. Future research from our working group includes recruiting larger non-CKD hypertensive and non-CKD diabetic control groups, which will improve the internal validity of our findings.

9.4 Conclusions

In summary, CKD is a very complex pathology. Owing to its multifactorial nature, the pathogenesis of CV remodelling can vary. In a clinical scenario, most CKD patients possess concomitantly traditional and non-traditional risk factors for CVD; hence, determining the exact mechanism beyond both cardiac and arterial remodelling is a challenge. In current literature many mechanisms have been proposed and we have explored them in this thesis. However, irrespective of the pathophysiology, our main objective was to establish novel non-invasive CV imaging markers in order to identify an asymptomatic high-risk cohort so that in the future these markers could be used as risk stratification tools, and could thereby improve CKD patient care.

In our asymptomatic CKD cohort, we demonstrated that subclinical markers of myocardial disease are associated with worsening eGFR. Furthermore, some of these markers were associated with the occurrence of MACE. Finally, we established a significant, independent association between arterial stiffness and LV twist (a marker of subclinical systolic dysfunction).

These findings need to be confirmed by larger studies—especially blinded, multi-centre, randomised clinical trials. Nonetheless, our work has created a platform from which these novel markers can be further analysed.

CHAPTER 10

FUTURE DIRECTIONS

10.1 Future work

Future planned work from our working group, which is already in progress, includes:

i) Conducting longitudinal follow-up, calling back all the patients for second and third visits. This process will allow us to determine if the deterioration of STE and aPWV can be of prognostic importance.

ii) Recruiting additional participants in the patient group.

iii) Recruiting additional control groups, comprising non-CKD hypertensive and diabetic individuals.

iv) Including three-dimensional STE (3DSTE) in our protocol.

10.2 Speckle tracking echocardiography in clinical practice

Several studies have demonstrated the clinical applicability of STE in a variety of clinical contexts[6,100,192,193]. One of the main limitations of STE in clinical practice relates to image quality. However, in a recent study with a cohort of consecutive patients in daily practice, GLS was reliably obtained in around 90% of the segments, whereas circumferential and radial strains were obtained in only half of the patients[194]. Moreover, the reproducibility of GLS was good in previous reports, with variation coefficients of about 7%, but reproducibility was much lower for

circumferential and radial strain[182]. These differences can be explained by the fact that in systole, the LV shortens from base to apex in its longitudinal direction, thus complicating the fixed short-axis tracking required for circumferential and radial strains. Global longitudinal strain is also a superior predictor of major adverse cardiac events and all-cause mortality when compared with LVEF, in the general population with and without heart failure[100,110].

Vendors have therefore developed intuitive toolboxes to allow rapid quantification of GLS. General Electric (GE) has developed a software tool that automates 2DSTE to measure real-time GLS. The method is termed 'automated function imaging' (AFI) and has reached a level of robustness that allows it to be applied in a daily clinical setting. The AFI has proven to be very useful as an add-on or substitute for ejection fraction[195]. Despite these findings, some recent reports have shown significant differences between vendors when assessing GLS using 2DSTE in normal controls and patients with pathology[187,188]. Post-processing and vendor-specific STE algorithms appear to be the most important determinants in inter-vendor variation, whereas imaging acquisition has only a limited effect[188]. To address this discrepancy in results between vendors, the American and European Societies of Echocardiography have set up an expert group of interested researchers and industry members, to achieve agreement concerning details of what is measured by 2DSTE[128,129]. This group will hopefully standardise GLS values from different vendors in the near future. One alternative would be to use vendor-independent software on images recorded in the Digital Imaging and Communications in Medicine (DICOM) format. This option would reduce vendor variability. The main limitation

would be the frame rate (30 frames/s) in the DICOM format, potentially leading to under-sampling and speckle decorrelation. Vendor-specific software, which uses raw data with the native frame rate, is not affected by this limitation[196].

Another important limitation of GLS, in daily practice, is that data on GLS has not been applied in large clinical trials. Curiously, parameters such as LVEF have shown modest intra-observer reproducibility[197,198] as an endpoint for evaluating the effects of drugs or devices on the LV. However, indications for cardiac resynchronisation therapy and/or implantable defibrillators still rely on LVEF because of the inclusion criteria in large trials. Owing to its superiority in detecting early myocardial injury, GLS might soon be used in large clinical trials. This would extend its use even further across echocardiography laboratories worldwide. Further studies regarding GCS and LV twist are needed in order to validate these parameters in clinical practice.

10.3 Three-dimensional STE

Speckle tracking strain can be obtained by adopting 3D echocardiography. In theory, 3D strain overcomes the inherent limitations of 2D strain (e.g. out-of-plane motion of the speckle), making it more appealing for assessing radial and circumferential strain. Recently developed 3D area strain integrates all components of strain (radial, circumferential, and longitudinal) and has been used in a range of settings[199,200]. ***Figure 28*** shows an example of rotation/twist mechanics using 3DSTE. Furthermore, 3DSTE has been shown to be reproducible and correlates better with LVEF than does 2D strain data[201]. However, limitations of 3DSTE in clinical practice include: i) it has

been under-researched, compared with 2DSTE; ii) it has poor feasibility (around 75%); and iii) patients have to hold their breath for six consecutive cycles to obtain a volume rate greater than 40 frames/s. Nonetheless, 3DSTE is most likely to become the future of speckle tracking, once smaller probes—which produce higher frame rates within one cycle—are developed.

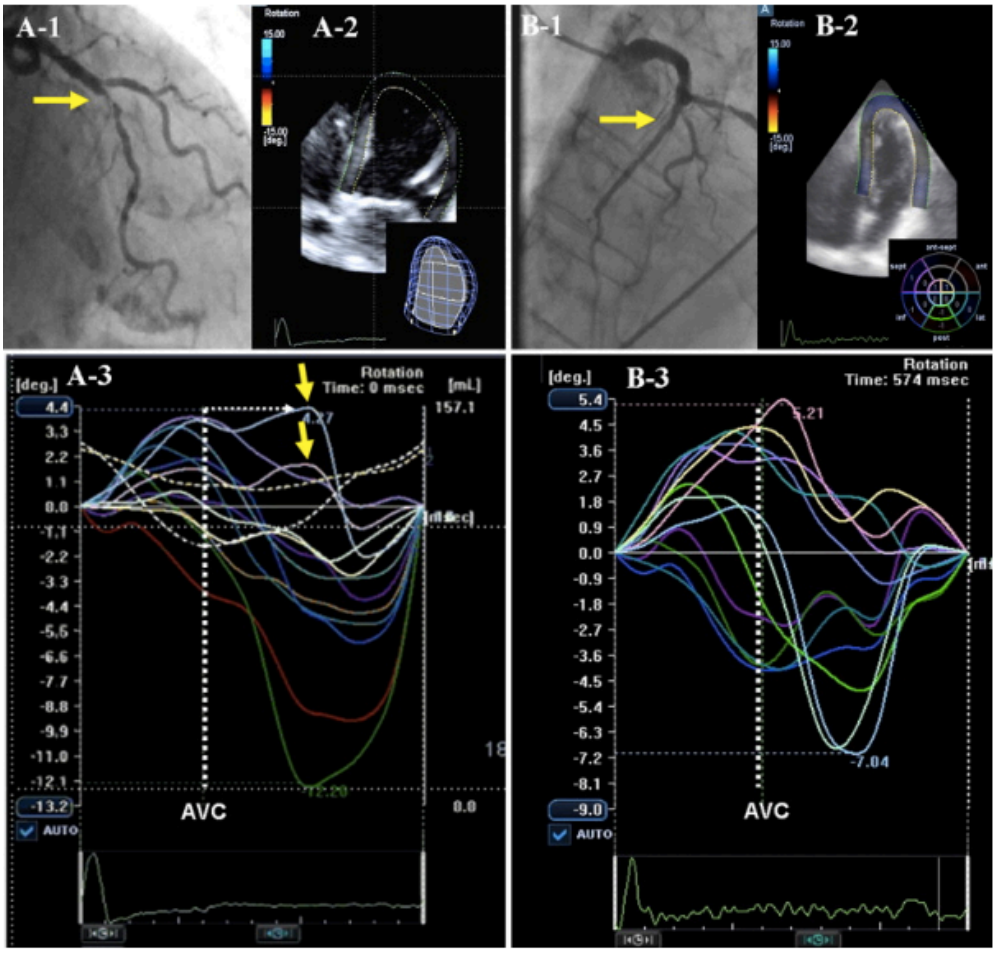


Figure 29. *LV rotational mechanics using 3DSTE. LV dysfunction (**A1–A3**) before revascularisation of a lesion in left anterior descending coronary artery (**A1**) (arrow), with a reduced ejection fraction (28%) as calculated by automated 3D speckle tracking. LV rotation in apical segments is attenuated (**A3**) (arrows), with delayed onset of untwisting in comparison with the timing of aortic valve closure (AVC). Following revascularisation (**B1**) (arrow), LV segmental rotation has marginally increased, with marked improvement in the timing of untwisting (**B3**).*

10.4 Pulse wave velocity in clinical practice

In 2007, the European Society of Hypertension and European Society of Cardiology guidelines for the management of HT included, for the first time, increased carotid-femoral aPWV as subclinical target-organ damage. The guidelines recommended the aggressive management of patients who have high PWV[202]. Since then, several studies have provided information on the predictive value of aPWV in various conditions, and reference values have been created[115,117,148,202-205].

From the evidence presented in this thesis, one would expect that assessment of arterial stiffness would be used in daily clinical practice to improve early detection of CVD. However, aPWV is not currently used in clinical practice. Currently, most of the literature on aPWV assessment mentions the SphygmoCor MM3 (AtCor Medical, Sydney). Although many studies using this system have provided evidence that aPWV is predictive of cardiovascular events and all-cause mortality in participants free of overt CVD, this system can be rather time consuming, labour intensive, and dependent on skilled operators. It is necessary to obtain an arterial waveform at the radial, carotid, and femoral arteries. Recently, a new system called the SphygmoCor XCEL (AtCor Medical) has been developed, making measurement of the carotid-femoral PWV more convenient. The advantage of this new device, over the SphygmoCor MM3, is its use of a partially inflated brachial cuff to measure the augmentation index, and a partially inflated femoral cuff—together with carotid applanation tonometry—to measure aPWV[206]. Another advantage of the XCEL device is its simultaneous recording of the carotid and femoral pulse waveforms. By

contrast, the MM3 device sequentially uses the R wave of the ECG signal to determine the time delay when recording the carotid and femoral artery waveforms.

Very few authors have published reports on studies in which the SphygmoCor XCEL has been used. However, according to the available literature there appears to be strong agreement between the two systems, and intra-test and day-to-day reliability were high. Nonetheless, more work is needed with regard to this new system. The studies available appear to lack statistical power and have used only normotensive samples. Comparisons between the two systems should be performed on hypertensive patients with a wide BP range, as well as on elderly patients with systolic HT.

From the large body of evidence presented, we believe that aPWV convincingly fulfils the criteria of a biomarker. It has a solid pathophysiological background, it can be assessed easily and replicated in clinical practice, it confers an incremental prediction in CV risk, and its modification affects prognosis. As the techniques become simpler, less costly, and more widely available, it is conceivable that very soon the measurement of arterial stiffness will become an important part of the routine assessment of patients. This could happen both in the primary care setting and in hospital practice.

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APPENDIX I

REPRODUCIBILITY MEASURMENTS FOR CONVENTIONAL ECHOCARDIOGRAPHY, STE AND ARTERIAL STIFFNESS DATA

CONVENTIONAL AND STE DATA

Intra – variability

N=20	ICC	P	Coefficient of variation
LVEF	0.90	<0.001	0.135
Posterior wall thickness	0.94	<0.001	0.098
Intraventricular septum	0.96	0.001	0.086
E/E' TDI	0.89	0.001	0.28
GLS	0.79	<0.001	0.137
GLSR	0.70	<0.001	0.125
GCS	0.85	<0.001	0.189
LV twist	0.89	<0.001	0.201

***Table 16**. Intra- variability data (n=20) is expressed as intra correlation coefficient (ICC), p value and coefficient of variation (standart deviation÷mean) from one experienced operator.*

Inter – variability

N=20	ICC	P	Coefficient of variation	Mean
LVEF	0.83	0.01	0.121	59.6
Posterior wall thickness	0.86	0.001	0.178	1.1
Intraventricular septum	0.79	0.001	0.211	1.2
E/E' TDI	0.80	0.01	0.370	11.7
GLS	0.82	<0.001	0.178	-18.5
GLSR	0.88	0.001	0.231	-1.46
GCS	0.71	<0.001	0.137	-24.3
LV twist	0.79	<0.001	0.155	17.4

***Table 17**. Inter-variability data (N=20) is expressed as intra correlation coefficient (ICC), p value, coefficient of variation (standart deviation÷mean) and mean, between two experienced operators (one whom conducted all the measurements).*

Arterial stiffness data

	PWV 1	PWV2	ICC	P
Intraobserver (n=20)	9.7±2.8	9.6±3.1	0.94	<0.001
Interobserver (n=20)	9.7±2.8	9.35±2.9	0.89	<0.001
ICC. Intraclass correlation; PWV, Pulse wave velocity (m/s); PVW1, first measured; PWV2, second measured				

Table 18. *Strength of agreement for intra-and inter-observer measurements of PWV . Data is expreesed as first and second pulse wave velocity measurements, intraclass correlation coefficient and its significance.*

APPENDIX II

PATIENT INFORMATION SHEET

Study Title: Global and regional left ventricular mechanics in chronic kidney disease assessed by two-dimensional speckle tracking echocardiography: correlation with aortic stiffness

Chief Investigator: Professor Petros Nihoyannopoulos

You are being invited to take part in a research study under the supervision of Professor of Cardiology Petros Nihoyannopoulos. Before you decide on whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Carefully decide whether or not you wish to take part.

Why have I been chosen?

You are invited to take part because you suffer from chronic kidney disease.

Why are we doing this study?

End-stage chronic kidney disease (CKD) is associated with a variety of alterations in cardiac structure and function, mainly cardiac dilatation (increased dimensions) and hypertrophy (increased thickness) that affect the ability of the heart to contract and relax in order to pump the blood throughout the body. Also large artery stiffness is increased in patients with end-stage renal disease as a result of calcium deposition at the artery walls.While these findings are well established and have an impact for the overall survival of patients with end-stage renal disease, few data are available for cardiac and arterial abnormalities in early stages CKD although preventive treatment may be most effective in this stage. This may be in part a result of the use of conventional diagnostic techniques that proved insensitive to detect abnormalities in an early stage.

So we will use new advanced diagnostic technology, to analyze the pictures taken from your cardiac ultrasound scan, called speckle tracking echocardiography and try to detect cardiac abnormalities in the early stage. With this we will be able to analyse the condition of your heart in greater detail. Also we will measure in a non invasive and painless way how stiff your arteries are and correlate the findings with the condition of your heart. Our hope is that this will provide useful scientific information and in the future will improve the management and cardiac monitoring of the renal patients in

general, if the use of these technologies proves to give valuable new data about the cardiovascular condition of patients with various stages of CKD.

Do I have to take part?

It is up to you to decide whether or not to take part. You will have time to think about it, at least for 24 hours after you were given this information sheet. After that, you will be contacted by a member of the nephrology clinical care team to find out if you would like to take part. If you have decided to participate please contact Mr Samir Sulemane (Mobile 07794349599) at the Echocardiography department (Tel: 0208 383 2333 / fax: 0208 383 4392) or via email: smrsulemane@gmail.com , who will arrange an appointment

for you to come in for your scan. The appointment will be arranged so that you come in on the same day that you will be attending the Nephrology outpatient's clinic. This means that there will be no additional visits required. Before you participate you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What is involved in this study?

The whole study lasts about 60 minutes (including 10 minutes for reading and signing the consent form) and will take place at the Echocardiography Department of Hammersmith Hospital. As part of the trial, you will have a echocardiography scan where excellent quality pictures of your heart will be taken, stored and then later analyzed by us using the new speckle tracking technology. Immediately afterwards; we will perform the arterial stiffness measurement. Your normal care will be unaffected; your ***treatment will be identical to that of someone with your condition who is not involved in the trial***.

What does the Two Dimensional Echocardiography scan involve?

The specific exam will only take 30 minutes and is the standard echocardiographic scan that you may have had before. This will be performed stored and interpreted as usual. After the exam, we will take some of the high quality pictures of the heart and analyse them with special new software called spackle tracking echocardiography. The test is non-invasive and totally harmless requiring no needles or agents.

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What does the arterial stiffness measurement involve?

Immediately after the echocardiography scan and before you get up from the echocardiography exam bed we will measure how stiff your arteries are. We will firstly measure your blood pressure. Then with a small tonometer (scanner

that senses small pressure changes and has the size of a pen) we will find and scan your pulse in 3 places; your hand (radial artery), your neck (carotid artery) and thigh (femoral artery). For scanning the pulse at the thigh you may have to remove any clothing to allow access to the femoral pulse at the base of your thigh. Then the device we will use called Sphygmocor analyses the data from the pulses and gives an estimation of your artery stiffness. The test is non-invasive and totally harmless requiring no needles or agents and will last about 20 minutes.

Will anything else be done?

We would like to compare the findings from the scans with the results of routine tests that you will have as part of your standard clinical care. As this requires looking at your hospital notes, we will require your consent for this.

What else do I have to do?

In order for us to obtain accurate measurements of arterial stiffness we request that you do not smoke, drink coffee or alcohol six hours prior to the test.

What are the side effects of any treatment received when taking part?

There is no known risk in having an echocardiographic or artery stiffness

scan.

What are the possible disadvantages and risks of taking part?

There are no disadvantages and risks of taking part

What if I want to become pregnant?

There is no risk from having an echocardiographic or artery stiffness exam if you are or want to become pregnant.

What are the possible benefits of taking part?

You should not expect any direct benefits from taking part. However, your help and the information we get from this study may improve the way in which cardiovascular disease is examined and monitored in renal disease patients. We think that these two methods (echocardiography with speckle tracking analysis and arterial stiffness measurement) have the potential to do this in a safe and accurate manner.

Will I get compensated for my travel expenses?

You will not be reimbursed for your travel expenses as the research procedures will take place on the day you will be coming in for a routine visit (i.e. there are no additional visits)

What if new information becomes available?

It is unlikely that new information will become available during the study that will affect your involvement. However, if this does occur we will let you know and discuss with you whether you wish to continue in the study.

What will the investigators do if an abnormality is found during the scans?

If an abnormality is found during the exams, you will be informed as soon as possible. We will then stop your participation in the project and refer you to a specialist to be assessed and treated as appropriate. Also your attending physicians will be informed immediately for the findings. We will also notify your GP after consulting with you.

What happens when the research study is completed?

Once the research study is completed, your routine echo will be analyzed, reported and the results along with your arterial stiffness results will be given to your attending physicians.

What if something goes wrong?

Imperial College London Healthcare NHS Trust's research is covered by the standard NHS Hospital Indemnity for negligent or wrongful harm and this is provided under clinical negligence scheme for Trusts clinical risk management NHS Litigation Authority (NHSLA) for NHS Trusts in England

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Professor Petros Nihoyannapoulos Tel: 0208 383 3948). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

Will my taking part in this study be kept confidential?

All information collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it. We will also inform your GP of your involvement in the trial. Please let us know if you do not wish us to inform them.

What will happen to the results of the research study?

If you wish, the medical journal in which the results are published can also be forwarded to you. If the research results are positive, you will be able to see

how the study has improved the way in which people with CKD are assessed and managed. In addition, we welcome feedback about the study and the way in which it was conducted.

Who is organising the research?

The research is organised by Imperial College Healthcare NHS Trust with the collaboration of 2 departments: The Echocardiography Department and the Nephrology Department.

Who has reviewed the study?

The local Research Ethics Committee, have reviewed the study and agree with the protocols we have drawn up. This study has ethical approval from East London REC 2 Research Ethics Committee.

Who can I contact for further information?

You can contact Mr Samir Sulemane (Mobile 07794349599) at the Echocardiography department, (Tel: 0208 383 2333 / fax: 0208 383 4392) or via email: s.sulemane11@imperial.ac.uk

Thank you for your time taken in considering this study.

VOLUNTEER INFORMATION SHEET

Study Title: Global and regional left ventricular mechanics in chronic kidney disease assessed by two-dimensional speckle tracking echocardiography: correlation with aortic stiffness

Chief Investigator: Professor Petros Nihoyannopoulos

You are being invited to take part in a research study under the supervision of Professor of Cardiology Petros Nihoyannopoulos. Before you decide on whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Carefully decide whether or not you wish to take part.

Why have I been chosen?

You are invited to take part as a control volunteer.

Why are we doing this study?

End-stage chronic kidney disease (CKD) is associated with a variety of alterations in cardiac structure and function, mainly cardiac dilatation (increased dimensions) and hypertrophy (increased thickness) that affect the ability of the heart to contract and relax in order to pump the blood throughout the body. Also large artery stiffness is increased in patients with end-stage renal disease as a result of calcium deposition at the artery walls. While these findings are well established and have an impact for the overall survival of patients with end-stage renal disease, few data are available for cardiac and arterial abnormalities in early stages CKD although preventive treatment may be most effective in this stage. This may be in part a result of the use of conventional diagnostic techniques that proved insensitive to detect abnormalities in an early stage.

So we will use new advanced diagnostic technology, to analyze the pictures taken from your cardiac ultrasound scan, called speckle tracking echocardiography and try to detect cardiac abnormalities in the early stage. With this we will be able to analyse the condition of your heart in greater detail. Also we will measure in a non invasive and painless way how stiff your arteries are and correlate the findings with the condition of your heart. Our hope is that this will provide useful scientific information and in the future will improve the management and cardiac monitoring of the renal patients in general, if the use of these technologies proves to give valuable new data about the cardiovascular condition of patients with various stages of CKD.

Do I have to take part?

It is up to you to decide whether or not to take part. You will have time to think about it, at least for 24 hours after you were given this information sheet. After that, you will be contacted by a member of the nephrology clinical care team to find out if you would like to take part. If you have decided to participate please contact Mr Samir Sulemane (Mobile 07794349599) at the Echocardiography department (Tel: 0208 383 2333 / fax: 0208 383 4392) or via email: s.sulemane11@imperial.ac.uk, who will arrange an appointment

for you to come in for your scan. Before you participate you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

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What else do I have to do?

In order for us to obtain accurate measurements of arterial stiffness we request that you do not smoke, drink coffee or alcohol six hours prior to the test.

What are the side effects of any treatment received when taking part?

There is no known risk in having an echocardiographic or artery stiffness scan.

What are the possible disadvantages and risks of taking part?

There are no disadvantages and risks of taking part

What if I am or become pregnant?

There is no risk from having an echocardiographic or artery stiffness exam if you are or want to become pregnant.

What are the possible benefits of taking part?

You should not expect any direct benefits from taking part. However, your help and the information we get from this study may improve the way in which

cardiovascular disease is examined and monitored in renal disease patients. We think that these two methods (echocardiography with speckle tracking analysis and arterial stiffness measurement) have the potential to do this in a safe and accurate manner.

Will I get compensated for my travel expenses?

You will be reimbursed for your travel expenses if necessary.

What if new information becomes available?

It is unlikely that new information will become available during the study that will affect your involvement. However, if this does occur we will let you know and discuss with you whether you wish to continue in the study.

What will the investigators do if an abnormality is found during the scans?

If an abnormality is found during the exams, you will be informed as soon as possible. We will then stop your participation in the project and refer you to a specialist to be assessed and treated as appropriate. Also your attending physicians will be informed immediately for the findings. We will also notify your GP after consulting with you.

What happens when the research study is completed?

Once the research study is completed, your routine echo will be analyzed, reported and the results along with your arterial stiffness results will be given to your attending physicians.

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If you wish, the medical journal in which the results are published can also be forwarded to you. If the research results are positive, you will be able to see how the study has improved the way in which people with CKD are assessed and managed. In addition, we welcome feedback about the study and the way in which it was conducted.

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Thank you for your time taken in considering this study.

CONSENT FORM

Title of Project: Global and regional left ventricular mechanics in chronic kidney disease assessed by two-dimensional speckle tracking echocardiography: correlation with aortic stiffness

Chief Investigator: Professor Petros Nihoyannopoulos

Please initial box

1. I confirm that I have read and understood the information sheet dated 10/08/2010. (Version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and they have been answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by responsible individuals from Imperial College Healthcare NHS Trust, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records that are relevant to this research. ☐

4. I understand that the study will take place in Hammersmith Hospital and particularly in the Echocardiography Department ☐

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5. I agree to my GP being informed of my participation in the study. ☐

6. In the event that any abnormalities are found during the scanning I give permission for my GP to be informed and for me to be referred onto an appropriate specialist to be assessed. ☐

7. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

When completed, one copy for patient; one copy for researcher site file; 1 (the original) to be kept in patients' medical notes

PUBLICATIONS ARISING FROM THIS THESIS

REVIEW

WILEY Echocardiography

Echocardiographic assessment in patients with chronic kidney disease: Current update

Samir Sulemane MSc[1] | Vasileios F. Panoulas MD, MRCP, PhD, FESC[1,2] | Petros Nihoyannopoulos MD, FRCP, FESC, FACC, FAHA[1]

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Patients with chronic kidney disease (CKD) carry a high cardiovascular risk. An abundance of evidence has emerged in recent years establishing minor reductions in estimated glomerular filtration rate as an independent risk factor for cardiovascular mortality. Additionally, cardiac changes, such as left ventricular hypertrophy and impaired left ventricular systolic function, have been associated with an unfavorable prognosis. Despite the significant prevalence of underlying cardiac abnormalities, symptoms may not manifest in many patients with CKD. A range of available and emerging echocardiographic modalities may assist with diagnosing heart disease in CKD. Furthermore, some of these emerging techniques can give an important insight into the pathophysiology of subclinical dysfunction in CKD. This review discusses how current and emerging echocardiographic modalities such as speckle tracking echocardiography and 3D echocardiography might help cardiologists in providing important information to help with diagnosis and risk stratification of cardiac-related morbidity and mortality in patients with renal disease, as well applicability of these tools to current clinical practice.

KEYWORDS
chronic kidney disease, deformation imaging, echocardiography, stress echocardiography, three-dimensional echocardiography

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Early detection of subclinical left ventricular myocardial dysfunction in patients with chronic kidney disease

Vasileios F. Panoulas[1,2]*, Samir Sulemane[2], Klio Konstantinou[1], Athanasios Bratsas[1], Sarah J. Elliott[2], David Dawson[1], Andrew H. Frankel[1], and Petros Nihoyannopoulos[1,2]

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Aims To identify subclinical left ventricular (LV) myocardial dysfunction using speckle tracking echocardiography (STE) in patients with chronic kidney disease (CKD), preserved LV ejection fraction (LVEF), and no cardiovascular history or symptoms.

Methods and results Cross-sectional comparisons of conventional and STE parameters were performed between controls and patients with different stages of CKD. CKD patients were followed up for major adverse cardiovascular events (MACEs). We recruited 106 CKD patients and 38 controls. Mean age was 54.4 ± 15.1 and 36.9 ± 11.5 years, respectively ($P < 0.001$), with 49.1 vs. 52.6% being female ($P = 0.705$). There were 29 (27.4%) patients with CKD stages 1/2, 38 (35.8%) with stage 3, and 39 (36.8%) with stages 4/5. Global longitudinal strain (GLS) was more impaired when moving from controls to CKD stages 4/5 (−20.67 ± 3.06, −20.39 ± 2.29, −18.33 ± 3.81, −18.01 ± 2.64, controls vs. CKD stages 1/2, vs. CKD stage 3, vs. CKD stages 4/5, respectively, $P_{adjusted} = 0.016$), whereas LV twist (16.2 ± 4.8, 18.51 ± 4.36, 19.91 ± 5.35, 24.6 ± 5.35, $P_{adjusted} < 0.001$) and LV twist rate (101.7 ± 30.3, 110.4 ± 30.1, 121 ± 31.4, 154.8 ± 36.7, $P_{adjusted} < 0.001$) increased. Risk factor-adjusted GLS (standardized beta $\beta = -0.245$, $P = 0.025$), strain rate (SR) [global longitudinal strain rates (GLSRs); $\beta = -0.236$, $P = 0.019$], and early diastolic longitudinal strain rate (GLSRe; $\beta = 0.247$, $P = 0.019$) were significantly associated with estimated glomerular filtration rate (eGFR), whereas LV twist ($\beta = -0.432$, $P < 0.001$), LV twist rate ($\beta = -0.433$, $P < 0.001$), and number of segments with diastolic dysfunction ($\beta = -340$, $P < 0.001$) were inversely and independently associated with eGFR. Impaired GLS (more than −16%) was observed in almost a quarter of CKD patients and associated with a reduced estimated MACE-free survival at 12-month follow-up (88.5 vs 93.7%, $P_{log rank} = 0.038$).

Conclusion In CKD patients with no cardiovascular symptoms or history and preserved LVEF, STE can identify subclinical abnormalities of both systolic (decreased GLS and GLSR, increased LV twist, and twist rate) and diastolic (decreased GLSRe and increased number of segments with diastolic dysfunction) LV function.

Keywords Chronic kidney disease • Speckle tracking echocardiography • Strain • Strain rate • Twist

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Cardiovascular Ultrasound

RESEARCH Open Access

Left ventricular twist mechanics and its relation with aortic stiffness in chronic kidney disease patients without overt cardiovascular disease

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Abstract

Background: Recent studies hypothesized left ventricular (LV) twist as a potential biomarker for evaluation of sub clinical myocardial disease, however its relationship with aortic stiffness has yet to be investigated. Chronic kidney disease (CKD) has been identified as a risk factor for both myocardial and arterial disease. As such we sought to explore the relationship between aortic stiffness and LV twist in CKD patients without known cardiovascular disease (CVD).

Methods: In this prospective, observational study we enrolled 106 CKD patients (Stages 1 to 5) with normal LVEF as assessed by conventional echocardiography. Aortic stiffness was measured using aortic pulse wave velocity (aPWV). We defined increased aPWV as ≥10 m/s. LV Twist was measured using two-dimensional speckle tracking echocardiography.

Results: Patients with increased aPWV had higher LV twist ($p = 0.002$) but similar LVEF ($p = 0.486$). Aortic PWV correlated crudely with age ($p < 0.001$), the presence of diabetes ($p < 0.001$), hypertension ($p < 0.001$), eGFR ($p < 0.001$), LVMI ($p = 0.01$), e/e' ($p < 0.001$) and LV twist ($p = 0.003$). In multivariable analyses after adjusting for age, gender, cardiovascular risk factors and hypertensive medication, aPWV was independently associated with LV twist ($\beta = 0.163$, $p = 0.025$).

Conclusions: Aortic stiffness independently associates with LV Twist in asymptomatic CKD patients. These findings suggest a close interaction between LV twist mechanics and arterial remodeling even before CVD becomes clinically relevant.

Keywords: Chronic kidney disease, LV twist, Speckle tracking echocardiography, Arterial stiffness, Aortic pulse wave velocity

Int J Cardiovasc Imaging
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ORIGINAL PAPER

Subclinical markers of cardiovascular disease predict adverse outcomes in chronic kidney disease patients with normal left ventricular ejection fraction

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Abstract Emerging cardiovascular biomarkers, such as speckle tracking echocardiography (STE) and aortic pulse wave velocity (aPWV), have recently demonstrated the presence of subclinical left ventricular dysfunction and arterial stiffening in patients with chronic kidney disease (CKD) and no previous cardiovascular history. However, limited information exists on the prognostic impact of these biomarkers. We aimed to investigate whether STE and aPWV predict major adverse cardiac events (MACE) in this patient population. In this cohort study we prospectively analysed 106 CKD patients with no overt cardiovascular disease (CVD) and normal left ventricular ejection fraction. Cardiac deformation was measured using STE while aPWV was measured using arterial tonometry. The primary end-point was the composite of all-cause mortality, acute coronary syndrome, stable angina requiring revascularization (either using percutaneous coronary intervention or coronary artery bypass surgery), hospitalization for heart failure and stroke. Over a median follow up period of 49 months (interquartile range 11–63 months), 26 patients (24.5%) reached the primary endpoint. In a multivariable Cox hazards model, global longitudinal strain (GLS) (HR 1.12, 95% CI 1.02–1.29, p = 0.041) and aPWV (HR 1.31, 95% CI 1.05–1.41, p = 0.021) were significant, independent predictors of MACE. GLS and aPWV independently predict MACE in CKD patients with normal EF and no clinically overt CVD.

Keywords Chronic kidney disease · Speckle tracking echocardiography · Aortic pulse wave velocity · Cardiovascular outcomes

Introduction

Chronic kidney disease (CKD) patients are a group at significantly increased risk of cardiovascular (CV) events, as a result of prolonged exposure to an ever-growing array of traditional and non-traditional risk factors [1]. Most risk factors exert their influence on the myocardium and arterial wall itself, causing collateral damage to organs when circulatory function is altered sufficiently to result in clinical disease [2]. The process of circulatory damage occurs over many years, and although outcomes [such as stroke or myocardial infarction (MI)] occur at identifiable discrete times, it is much more difficult to cross-sectionally assess the influence of established biomarkers of CV risk, such as glucose or cholesterol, without some surrogate for cardiovascular outcomes [3]. In the last few years a number of surrogate markers of subclinical myocardial and arterial

PUBLISHED ABSTRACTS

Abstract: P5420

Subclinical abnormalities of two-dimensional speckle tracking echocardiography associate with early peripheral vascular disease in patients with chronic kidney disease

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Topic(s):
Echo-ventricular function and myocardial diseases

Citation:
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Background: Very few studies have investigated the relationship between arterial stiffness and subclinical left ventricular (LV) dysfunction and none used two dimensional speckle tracking echocardiography (2DSTE).

Objectives: We sought to investigate the association between subclinical myocardial abnormalities assessed by 2DSTE and arterial stiffness in chronic kidney disease (CKD) patients with normal LV ejection fraction (LVEF) and no previous history of cardiovascular disease.

Methods: In this cross-sectional study we have enrolled 106 CKD patients. Pressure waveforms were recorded on radial, carotid and femoral arteries using applanation tonometry to estimate augmentation index (AIx) and aortic pulse wave velocity (aPWV). Conventional echocardiography and 2DSTE were used to determine LVEF, LV mass index (LVMI), global longitudinal and circumferential strain (GLS, GCS), number of segments with diastolic dysfunction (DD) and LV twist.

Results: Aortic PWV correlated crudely with the presence of diabetes ($r=0.58$, $p<0.001$), hypertension ($r=0.32$, $p<0.001$), LVMI ($r=0.29$, $p=0.01$), GLS ($r=0.300$, $p<0.003$), number of segments with DD ($r=0.416$, $p<0.001$), LV twist ($r=0.288$, $p<0.003$) while AIx was only associated with hypertension ($r=0.19$, $p=0.046$) and number of segments with DD ($r=0.317$, $p<0.001$). When adjusting for age, gender and cardiovascular risk factors aPWV was independently associated with LV twist ($\beta=0.160$, $p=0.017$) and number of segments with DD ($\beta=0.221$, $p=0.011$). Stepwise, multiple regression models established that LV twist improved the prediction of arterial stiffness over and above age, gender and cardiovascular risk factors alone ($R2=0.17$, $p=0.01$)

Conclusions: Subclinical LV dysfunction, identified with 2DSTE is strongly associated with aPWV in CKD patients. This is the first study to provide a link between subclinical LV dysfunction and early peripheral arterial disease in CKD patients.

Abstract: P1235

Can myocardial deformation imaging provide insights in early detection of chronic kidney disease?

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Topic(s):
Clinical applications

Citation:
Eur Heart J Cardiovasc Imaging Abstracts Supplement (2013) 14 (Supplement 2), ii233

Background: Chronic Kidney Disease (CKD) associates with adverse cardiovascular outcomes. This relationship is not restricted to end-stage disease but also to milder stages of CKD. However in literature there is inconclusive evidence of presence of subclinical abnormalities in such patients. With the use of conventional and advanced echocardiography we aim to look at the effect of CKD on left ventricular (LV) systolic and diastolic myocardial deformation parameters and LV twist.

Methods: One hundred and fifteen consecutive CKD patients (Stages 1 -5) and 28 controls were assessed using conventional and 2D speckle tracking echocardiography(2DSTE) (Vivid 7-GE). We classified our patients with evidence of kidney damage lasting for >3 months into 3 CKD groups A, B and C, based on estimated glomerular filtration rate (eGFR) level (mL/min/1.73 m2) of 90–60, 59–30 and <15-29, respectively. LV Ejection Fraction (LVEF), LV mass (LVM), Global Longitudinal (GLS) and Global Circumferential (GCS) strain, early (GSRe) and late (GSRa) diastolic longitudinal strain rate, LV twist and twist rate were measured.

Results: Chronic Kidney Disease patients were older than controls (54.7±20.2 vs. 41.6±12.0,p<0.001). LVEF was similar between CKD and controls (63.5±6.1vs.63.2±5.3, p=0.802). LVM is normal in all CKD groups(61.4±

18.7 vs. 70.8±19.2 vs 75.9±17.9, p<0.03). GLS was significantly reduced in CKD patients when compared to controls (-18.9±3.4 vs. 20.7±3.1 p=0.02) whereas GCS was lower, but not significantly so(-24.4±4.7 vs. -25.4±4.1, p=0.283). When adjusting for age and gender there was no significant difference in GLS between CKD and controls (beta 0.635, p=0.316). LV twist (21.4±6.7 vs. 16.2±4.9, p<0.001) and twist rate (125.6±36.0 vs. 98.9±26.3, p<0.001) were significantly higher amongst CKD patients, even after age adjustment. CKD patients had more segments with diastolic dysfunction compared to controls [7.0 (4.0 to 11.0) vs. 1.5 (0 to 4.5), p<0.001]. eGFR correlated with GLS (r=0.269, p=0.028), LV twist (r=-0.275, p=0.03), LV twist rate (r=-0.514, p<0.001) and RWT (r=-0.265, p=0.05). In a linear regression model with dependent variable eGFR, LV twist (beta -1.75, p=0.003), LV twist rate (beta -0.17, p=0.010) were the strongest independent significant predictors.

Conclusion: Conventional measures of systolic function are preserved in CKD whereas worsening eGFR is associated with a deterioration of subclinical LV function quantified by 2DSTE. These markers could be useful in early detection of LV dysfunction in CKD leading to early optimal management and improved survival.

Abstract: P662

Arterial stiffness and its relation to left ventricular mechanics in patients with chronic kidney disease and normal ejection fraction

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Topic(s):
Miscellaneous

Citation:
Eur Heart J Cardiovasc Imaging Abstracts Supplement (2013) 14 (Supplement 2), ii125

Objectives: Increased arterial stiffness and wave reflection have been reported amongst Chronic Kidney Disease (CKD) patients. Concomitantly, abnormal LV geometry (LV hypertrophy or LV dysfunction) is also present in a high proportion of CKD patients. However, the relation between these two pathophysiological factors remains unclear. The purpose of this study was to use advanced echocardiography and applanation tonometry to explore ventricular-arterial mechanics in CKD patients.

Methods: One hundred and fifteen CKD patients (Stages 1 -5) and 28 healthy controls underwent conventional and 2D speckle tracking echocardiography (EchoPAC-GE) to explore markers of sub clinical LV dysfunction. Pulse wave velocity (PWV) was evaluated as a marker of arterial stiffness using applanation tonometry. Patients with (i) left ventricular systolic dysfunction, (ii) moderate to severe valvular disease, (iii) heart rhythm other than sinus or (iv) poor echodiagnostic acoustic windows were excluded (n=14).

Results: CKD patients were older than controls (54.7 ±20.2 vs. 41.6 ±12.0, p<0.001). When compared with controls, CKD patients had similar LV ejection Fraction (63.5±6.1 vs. 63.2±5.3, p=0.802), higher PWV (9.6±2.1 vs. 6.8±1.1, p<0.001), lower Global Longitudinal Strain (GLS) (18.9±3.4 vs. 20.7±3.1 p=0.02), higher twist (21.2±4 vs 16.9±4.8 p<0.001) and more LV segments with diastolic dysfunction (DD) [7.0 (4.0 to 11.0) vs. 1.5 (0 to 4.5), p<0.001). Excluding GLS (beta 0.635, p=0.316) all other parameters remained significant after age and gender adjustment. PWV correlated with GLS(r=0.312,p<0.001), LV Twist(r=0.417, p<0.001), and number of segments with DD(r=0.481, p<0.001)). When adjusting for cardiovascular risk factors (Diabetes and Hypertension) a linear regression model established that PWV is an independent significant predictor for LV twist (beta 0.369, p<0.001).

Conclusion: In CKD patients with normal LVEF, parameters of arterial stiffness are associated with myocardial deformation indices of LV systolic and diastolic function. In addition, PWV is an independent predictor of LV twist, suggesting that the severity of arterial stiffness directly correlates with LV twist mechanics. Further outcomes studies are necessary to identify these associations.

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Imaging

SUBCLINICAL ABNORMALITIES OF LEFT VENTRICULAR MYOCARDIAL DEFORMATION IN PATIENTS WITH CHRONIC KIDNEY DISEASE AND NORMAL LEFT VENTRICULAR EJECTION FRACTION

Poster Contributions
Poster Sessions, Expo North
Monday, March 11, 2013, 9:45 a.m.-10:30 a.m.

Session Title: Imaging: Cardiac Abnormalities in Non-Cardiac Disorders, Hand Held Echocardiography, Cardiac Masses
Abstract Category: 18. Imaging: Echo
Presentation Number: 1311-323

Authors: *Samir Sulemane, Vasileios F. Panoulas, Athanasios Bratsas, Andrew Frankel, Edwina Brown, Petros Nihoyannopoulos, Imperial College Healthcare, NHS Trust, London, United Kingdom*

Background: Left ventricular ejection systolic function (LVEF) has been shown to be a good predictor of future cardiovascular outcomes in renal patients. Chronic kindey disease (CKD) patients with preserved LVEF also have excess cardiovascular mortality. To date, there is sparse, inconclusive evidence of presence of subclinical echocardiographic abnormalities in such patients. Our aim was to identify early markers of cardiovascular disease (CVD) in CKD patients using speckle tracking echocardiography .

Methods: We recruited 95 CKD patients (all stages) with no CVD and 33 healthy controls. All participants underwent conventional and 2D myocardial deformation echocardiography. Participants with LV systolic impairment or valvular disease were excluded. Segmental diastolic dysfunction (DD) was defined as SRe/SRa<1.1.

Results: CKD patients were older than controls (53.5 ±20.2 vs. 36.7±12.0, p<0.001). There were 46.7% females in the CKD group vs. 54.5% in the control group (p=0.409). LVEF was similar between CKD and controls (63.5±6.1 vs. 63.2±5.3, p=0.802). In the CKD group the peak global longitudinal strain (GLS) was slightly reduced (trend) compared to controls (-19.1±3.0 vs. -20.2±2.4, p=0.067) whereas peak global circumferential strain (GCS) was similar in both groups (-24.4±4.7 vs. -25.4±4.1, p=0.283). When adjusting for age and gender there was no significant difference in GLS between CKD and controls (beta 0.635, p=0.316). Peak left ventricular twist (21.4±6.7 vs. 16.2±4.9, p<0.001) and twist rate (125.6±36.0 vs. 98.9±26.3, p<0.001) were significantly higher amongst CKD patients, even after age adjustment. CKD patients had more segments with DD compared to controls [7.0 (4.0 to 11.0) vs. 1.5 (0 to 4.5), p<0.001], an association that remains significant after adjustment for age. Worsening eGFR correlated with increased twist (-0.439, p<0.001), twist rate (-0.453, p<0.001) and higher number of segments with DD (rho=-0.304, p=0.005) but not with GLS or GCS.

Conclusions: In CKD patients with preserved LVEF, GLS and GCS appear intact whereas the number of segments with DD is increased. Peak twist and twist rate are increased and may represent a compensatory mechanism for worsening DD.

Abstract: P5145

Subclinical abnormalities of the arterial tree and left ventricular myocardial deformation, relaxation and twist in chronic kidney disease

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Topic(s):
Diastolic dysfunction

Citation:
European Heart Journal (2012) 33 (Abstract Supplement), 932

Purpose: Chronic kidney disease (CKD) associates with adverse cardiovascular outcomes. However, the disadvantageous effects of renal dysfunction on left ventricular systolic and diastolic function remain unclear. The objective of this study was to look at the effect of CKD on (i) left ventricular (LV) systolic and diastolic strain patterns and LV twist with the use of conventional and 2D speckle tracking echocardiography, and (ii) arterial stiffness as measured by pulse wave velocity (PWV).

Methods: Seventy four consecutive CKD patients were assessed using conventional, 2D speckle tracking echocardiography (EchoPAC-GE) and applanation tonometry (Sphygmocor). Patients with (i) LV systolic dysfunction or regional wall motion abnormalities, (ii) moderate to severe valvular disease or (iii) heart rhythm other than sinus were excluded (N=5). Global systolic strain (GS) and strain rate (GSRs), early (GSRe) and late (GSRa) diastolic longitudinal strain rate, LV twist and twist rate, mitral inflow, tissue PW –Doppler velocities and PWV were recorded.

Results: The mean age of CKD patients was 54.2±15.4 years and 34 (49.3%) were females. Mean biplane LV ejection fraction (EF) was 63.2±5.4%. Six (8.7%) patients were stage 1 CKD, 17 (24.6%) stage 2, 29 (42%) stage 3, 12 (17.4%) stage 4 and 5 (7.2%) stage 5. Estimated Glomerular Filtration Rate (eGFR) correlated significantly with PWV (r=-0.459, p<0.001), GSRe (r=0.269, p=0.028), LV twist rate (r=-0.275, p=0.026) and E/E' ratio (r–0.370, p=0.002). There was a trend of a correlation between eGFR and LV twist (r=-0.216, p=0.083), isovolumic relaxation time (IVRT) (r=-0.232, p=0.055) and E/A ratio (r=0.217, p=0.073). There was no correlation between eGFR and GS, GSRs or biplane ejection fraction. When including the echocardiographic parameters in a linear regression model with dependent variable eGFR, E/E' (beta -1.75, p=0.007) and LV twist rate (beta -0.17, p=0.021) were independent significant predictors. Amongst CKD stages 1,2 patients there were 6 (33.3%) with diastolic dysfunction, in stage 3 the figure increased to 17 (75%) and in stages 4,5 further to 14 (82.4%) (Chi-Square 13.7, p=0.033).

Conclusions: Arterial stiffness and LV relaxation demonstrate progressive deterioration with worsening eGFR whereas LV filling pressure and twist rate appear to increase, compensating for the impaired diastolic filling. Outcome studies may be required to identify the association of these early markers of cardiovascular disease in CKD patients.

ORAL PRESENTATIONS

Invited lecturer, Myocardial function seminars*, Imperial College London, Hammersmith Campus*, September 2013

Invited lecturer, 47th Renal Research Forum, *Imperial College Kidney and Transplant Institute*, April 2013

GRANTS

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