Cardiac Risk Assessment Using 2D and 3D Transthoracic Echocardiography in Patients Undergoing Haemodialysis

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy (phD) in the Faculty of Medical and Human Sciences

Diana Yuan Yng Chiu
School of Medicine
Institute of Population Health
2016
List of contents

List of tables..................................................................................................................................................6
List of figures...................................................................................................................................................8
List of abbreviations.......................................................................................................................................10
Abstract........................................................................................................................................................13
Declaration....................................................................................................................................................14
Copyright statement.......................................................................................................................................14
Thesis format..................................................................................................................................................15
Acknowledgements.......................................................................................................................................16
The author....................................................................................................................................................17
Contribution of the author to the research.................................................................................................18

Chapter 1- Echocardiography in Haemodialysis Patients: Uses and Challenges........................................19
  1.1 Preface ..................................................................................................................................................19
  1.2 Introduction ........................................................................................................................................20
  1.3 Left Ventricular Mass and Volume .....................................................................................................20
  1.4 Left Ventricular Systolic Function ......................................................................................................30
  1.5 Sub-clinical Systolic Dysfunction with Normal Ejection Fraction .................................................32
  1.6 Diastolic Dysfunction ..........................................................................................................................33
  1.7 Other Echocardiographic Considerations in Patients Undergoing Haemodialysis ..........................36
  1.8 Conclusions .......................................................................................................................................38
  1.9 References ..........................................................................................................................................39

Chapter 2- Hypothesis and Objectives.........................................................................................................46
  2.1 Hypothesis ..........................................................................................................................................46
  2.2 Objectives ..........................................................................................................................................47

Chapter 3- Methods .....................................................................................................................................48
  3.1 Preface ..............................................................................................................................................48
Chapter 4 - Non-Recruitment To and Selection Bias in Studies using Echocardiography in Haemodialysis Patients

4.1 Preface .................................................................71
4.2 Abstract ...............................................................71
4.3 Introduction ..........................................................72
4.4 Methods ....................................................................73
4.5 Results .......................................................................75
4.6 Discussion ...............................................................83
4.7 References ..............................................................86

Chapter 5 - Novel Approach to Cardiovascular Outcome Prediction in Haemodialysis Patients

5.1 Preface ....................................................................88
5.2 Abstract ....................................................................89
5.3 Introduction ..............................................................89
Chapter 6- Cardiovascular Risk Assessment in Haemodialysis Patients with Preserved Left Ventricular Ejection Fraction and Left Ventricular Hypertrophy .................................................110
6.1 Preface ........................................................................................................110
6.2 Abstract .......................................................................................................110
6.3 Introduction ................................................................................................111
6.4 Methods ......................................................................................................112
6.5 Results .........................................................................................................115
6.6 Discussion ....................................................................................................122
6.7 References ...................................................................................................125

Chapter 7- Speckle Tracking Determination of Tissue Motion Annular Displacement: Comparison with Strain and Ejection Fraction, and Association with Outcomes in Haemodialysis Patients ............128
7.1 Preface ........................................................................................................128
7.2 Abstract .......................................................................................................128
7.3 Introduction ................................................................................................129
7.4 Methods ......................................................................................................131
7.5 Results .........................................................................................................133
7.6 Discussion ....................................................................................................139
7.7 References ...................................................................................................141

Chapter 8- Comparison of Two-dimensional and Three-dimensional Echocardiographic Parameters and Association with Mortality in Haemodialysis Patients .........................................................143
8.1 Preface ........................................................................................................143
8.2 Abstract .......................................................................................................144
8.3 Introduction ................................................................................................145
8.4 Methods ......................................................................................................146
# Table of Contents

## Chapter 9 - 3D Echocardiographic Left Ventricular Dyssynchrony Indices in End Stage Kidney Disease: Associations and Outcomes

9.1 Preface .................................................................................................................... 161
9.2 Abstract .................................................................................................................. 162
9.3 Introduction ........................................................................................................... 162
9.4 Methods ................................................................................................................ 164
9.5 Results .................................................................................................................... 167
9.6 Discussion ............................................................................................................. 172
9.7 References ............................................................................................................. 175

## Chapter 10 - Conclusion - Evaluation of Findings, Discussion and Plans for Future Studies

10.1 Preface .................................................................................................................. 177
10.2 Introduction .......................................................................................................... 177
10.3 Overall Summary ................................................................................................. 182
10.4 Future Studies ..................................................................................................... 182
10.5 References .......................................................................................................... 185

Appendix 1 Publications and abstracts arising from PhD........................................ 187

**Word count (including footnotes and endnotes)**...........57,327
List of tables

Table 1-1. Some typical normal and abnormal values for two-dimensional echocardiographic parameters ............................................................................................................. 22
Table 1-2. Eccentric and concentric hypertrophy in dialysis patients. ................. 23
Table 1-3. Comparison of 2D echocardiographic methods to calculate LV volume. 27
Table 1-4. Comparison of 2D transthoracic echocardiography, 3D transthoracic echocardiography and cardiac magnetic resonance imaging ................................................. 29
Table 1-5. Echocardiographic parameters that may indicate diastolic dysfunction...35
Table 1-6. Causes of an enlarged left atrium in haemodialysis patients .................. 36
Table 3-1. Biochemical and haematological tests performed ................................ 66
Table 3-2. Blood assays performed........................................................................ 66
Table 4-1. Baseline clinical demographics, co-morbidities and laboratory results of patients who were recruited, refused consent and excluded from the study......... 77
Table 4-2. Residential postcode related deprivation scores and distance from Salford Royal Hospital in patients recruited, declined consent and excluded patients. ....... 78
Table 4-3. Mean blood and dialysis prescription results 3 months prior to consent.. 79
Table 4-4. Baseline characteristics of patients who were excluded and reasons for exclusion. ...................................................................................................................... 80
Table 4-5. Univariate analysis for all-cause mortality in the whole group. ............ 81
Table 4-6. Final Cox regression model for all-cause mortality. ............................ 81
Table 5-1. Baseline demographics and clinical characteristics of patients with division according to survival status ................................................................. 96
Table 5-2. Echocardiographic and pulse wave velocity results for whole cohort, and for patients who died versus survivors .................................................... 97
Table 5-3. Multivariable linear regression model for factors significantly associated with global longitudinal strain (GLS) and pulse wave velocity (PWV). .......... 98
Table 5-4. Outcomes of patients separated into a cut-off of abnormal global longitudinal strain (GLS) ≥ -15%. ................................................................. 101
Table 5-5. Univariate analysis in relation to outcome ........................................... 102
Table 5-6. Final multivariable Cox regression model illustrating the significance of each included variable .................................................................................. 103
Table 5. Multivariable model including pulse wave velocity and global longitudinal strain in the same model as Table 5-6.

Table 6-1. Clinical, laboratory, echocardiographic and Vicorder™ characteristics of the study cohort between patients with and without left ventricular hypertrophy.

Table 6-2. Baseline, laboratory, echocardiographic and Vicorder™ characteristics comparison between patients with concentric versus eccentric left ventricular hypertrophy (LVH).

Table 6-3. Baseline characteristic comparisons between patients with and without preserved LVEF.

Table 6-4. Final cox regression model with all adjusted factors for all-cause mortality in a cohort of haemodialysis patients with LVH and preserved left ventricular ejection fraction groups respectively.

Table 7-1. Baseline characteristics, medications, dialysis prescriptions and medications of study cohort (N=198).

Table 7-2. Echocardiographic and speckle tracking analysis parameters for the study population (N=198).

Table 7-3. The final cox regression analysis models for all-cause mortality, cardiac death and major cardiac events.

Table 8-1. Baseline demographics of patients with and without adequate RT3DE.

Table 8-2. Inter-observer variability for 20 randomly selected patients by 2 independent interpreters (DC and JH) for 2DE and RT3DE determined parameters. Bias and limits of agreement as determined by Bland-Altman Analysis.

Table 8-3. Comparison of 2DE and RT3DE measured parameters (N=69) by linear regression analysis.

Table 8-4. Mean values for LV volumes, ejection fraction and mass as determined by 2DE and RT3DE. Comparison of means by student paired t test.

Table 9-1. Clinical and echocardiographic parameters for the study group and comparison between patients above and below Tmsv-16 SD of 3%.

Table 9-2. Outcomes of patients separated into above and below Tmsv-16 SD of 3%.
List of figures

Figure 1-1. Classification of LV geometry.................................................................21
Figure 1-2. Two-dimensional echocardiography images when measuring left
ventricular (LV) diameters in M-mode. .................................................................24
Figure 1-3. Schematic representation of left ventricle pre- and post-dialysis in end-
diastole. .......................................................................................................................25
Figure 1-4. Three-dimensional transthoracic echocardiographic image of the left
ventricle, direct measurements may be taken without geometric assumptions......28
Figure 1-5. Schematic representation of the 3 components of left ventricular
contraction......................................................................................................................32
Figure 3-1. Biplane's Simpson's method of discs in deriving left ventricular volumes.
.................................................................................................................................56
Figure 3-2. Using biplane's Simpson's method of discs, the volume of the left atrium
was determined..........................................................................................................57
Figure 3-3. Speckle tracking echocardiography assessment in the determination of
global longitudinal strain.........................................................................................59
Figure 3-4. TMAD measurements. .............................................................................60
Figure 3-5. An example of tracing of endocardial border of left ventricle using three-
dimensional transthoracic echocardiography.......................................................62
Figure 3-6. Parametric imaging of the same patient as in the previous figures........63
Figure 3-7. Performing Vicorder™ measurements to ascertain pulse wave velocity.
.................................................................................................................................65
Figure 4-1. Flow diagram illustrating the numbers of patients who were screened,
excluded, declined consent and recruited; with associated reasons......................76
Figure 4-2. Adjusted cumulative survival plot for the groups (recruited, declined
consent and excluded patients)................................................................................82
Figure 5-1. Summary of recruitment, with inclusion and exclusion criteria............94
Figure 5-2. Unadjusted Kaplan-Meier survival plots for global longitudinal strain
(GLS) and pulse wave velocity (PWV) in prediction of all-cause mortality...........100
Figure 6-1. Inclusion, exclusion criteria and final number of patients available for
analysis.........................................................................................................................116
Figure 7-1. Bland-altman plots for intra- (a) and inter- (b) observer variability for TMAD measurements. .......................................................... 136
Figure 7-2. Correlation between tissue motion annular displacement (TMAD) and global longitudinal strain (GLS) and left ventricular ejection fraction (LVEF). ..... 137
Figure 8-1. Inclusion and exclusion criteria for the study, in addition to the number of patients with adequate 2DE and RT3DE. .......................................................... 149
Figure 8-2. Bland-Altman plots for inter-interpreter agreement.......................... 153
Figure 8-3. Box and whisker plot comparing measurements of left ventricular volume, ejection fraction and mass between 2DE and RT3DE...................................... 154
Figure 9-1. Flow-diagram of inclusion and exclusion criteria for the study......... 168
Figure 9-2. Adjusted cumulative survival plot for time to admission for heart failure in patients with and without LV mechanical dyssynchrony. ......................... 171
List of abbreviations

2D 2-dimensional
2DE 2-dimensional echocardiography
3D 3-dimensional
A Atrial mitral flow velocity
ACEI Angiotensin converting enzyme inhibitor
ARB Angiotensin II receptor blocker
BP Blood pressure
BMI Body mass index
BSA Body surface area
CAD Coronary artery disease
CCB Calcium channel blocker
CCF History of heart failure
CI Confidence interval
CKD Chronic kidney disease
CKD-MBD Chronic kidney disease mineral and bone disorder
CMRI Cardiac magnetic resonance imaging
CRISIS Chronic Renal Insufficiency Standards Implementations Study
CRP C-reactive protein
CRT Cardiac resynchronisation therapy
CSA Cross sectional area
DM Diabetes mellitus
E Mitral E-wave deceleration time
e’ Early mitral annulus velocity
E/A Early mitral flow velocity/atrial mitral flow velocity
E/e’ Early mitral flow velocity/early mitral annulus velocity
E/SRIVR Mitral early diastolic velocity/diastolic strain rate during the iso-volumetric relaxation
ECG Electrocardiogram
ECHO Echocardiography
EDTA Ethylenediaminetetraacetic acid
EDV End diastolic volume
EF  Ejection fraction
eGFR  Estimated glomerular filtration rate
ESKD  End stage kidney disease
GLS  Global longitudinal strain
HBA1c  Glycated haemoglobin (A1c)
HD  Haemodialysis
HR  Hazard ratio
HTML  Hypertext markup language
IVC  Inferior vena cava
IVSTD  Interventricular septal thickness in end diastole
LAV  Left atrial volume
LAVi  Left atrial volume indexed to height^{2.7}
LBBB  Left bundle branch block
Li-Heparin  Lithium-heparin
LOA  Limits of agreement
LV  Left ventricular
LVEF  Left ventricular ejection fraction
LVEDV  Left ventricular end diastolic volume
LVESV  Left ventricular end systolic volume
LVH  Left ventricular hypertrophy
LVIDD  Left ventricular internal dimensions in end diastole
LVIDS  Left ventricular internal diameter in systole
LVMI  Left ventricular mass index
LVMI/HT^{2.7}  LV mass was indexed to height^{2.7}
MACE  Major adverse cardiac event
MV  Mitral valve
M/V  Mass-end diastolic volume
NHS  National health service
PDF  Portable Document Format
PTH  Parathyroid hormone
PVD  Peripheral vascular disease
PWTD  Posterior wall thickness at end diastole
PWV  Pulse wave velocity
OR  Odds ratio
RR  Relative risk
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT3DE</td>
<td>Real time three-dimensional echocardiography</td>
</tr>
<tr>
<td>RWT</td>
<td>Relative wall thickness</td>
</tr>
<tr>
<td>SCD</td>
<td>Sudden cardiac death</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social science</td>
</tr>
<tr>
<td>STE</td>
<td>Speckle tracking echocardiography</td>
</tr>
<tr>
<td>TDI</td>
<td>Tissue doppler imaging</td>
</tr>
<tr>
<td>TMAD</td>
<td>Tissue motion annular displacement</td>
</tr>
<tr>
<td>TmsV</td>
<td>Time interval to the minimum systolic volume</td>
</tr>
<tr>
<td>UF</td>
<td>Ultra-filtration</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>URR</td>
<td>Urea reduction ratio</td>
</tr>
<tr>
<td>USRDS</td>
<td>United States Renal Data System</td>
</tr>
<tr>
<td>VL</td>
<td>Long axis length</td>
</tr>
</tbody>
</table>
Abstract
Cardiac Risk Assessment using 2D and 3D Transthoracic Echocardiography in Patients Undergoing Haemodialysis
Diana Yuan Yng Chiu, For the degree of Doctor of Philosophy at the University of Manchester, March 2016
Haemodialysis (HD) patients have a high mortality risk and most have echocardiographic evidence of abnormal cardiac structure or function. Markers, such as left ventricular hypertrophy (LVH), show association with adverse outcome in the general population and can aid in clinical decision making. The aim of this research was to explore the prognostic utility of established and novel two-dimensional (2DE) and three-dimensional transthoracic echocardiographic (RT3DE) techniques in HD patients.

Adult maintenance HD patients from a single tertiary nephrology centre including satellite dialysis units were enrolled. Exclusion criteria were if patients were clinically unstable, unable to consent, or if required ambulance transportation for echocardiography visits. Consent patients underwent 2DE with speckle tracking (STE), RT3DE and VicorderTm measurements of pulse wave velocity (PWV) on a non-dialysis day, after the short interdialytic break. Clinical phenotype data, 3-month averaged blood results and dialysis prescriptions were obtained from the hospital electronic patient records. All patients screened were followed-up until death, renal transplantation, moving out of the region, or 16th November 2015. Regression analysis was used to assess the cross-sectional relationship between echocardiographic parameters. Relationship of echocardiographic parameters with outcome was assessed by Cox regression analysis.

The first study explored whether patients recruited had similar characteristics and survival compared with patients who declined consent or who were excluded from the study. Patients who declined consent had an adjusted hazard ratio (HR) for all-cause mortality compared with recruited patients of 1.70, 95% confidence interval (CI) 1.10-2.52, and excluded patients had an adjusted HR of 1.30, 95% CI 0.75-2.25. Recruited patients may be a 'fitter' population and this needs to be considered when interpreting results.

The second study reports that when global longitudinal strain (GLS) is combined in a multivariable model with PWV; PWV is superior to GLS in its association with mortality (adjusted HR 1.23, 95% CI 1.03-1.47 versus HR 1.00, 95% CI 0.86-1.17). When this analysis was repeated in a sub-group of patients with LVH, neither GLS nor PWV were associated with mortality, whilst both were prognostically significant in a preserved LVEF sub-group (PWV: HR 1.23, 95% CI 1.04-1.4 and GLS: HR 1.16, 95% CI 1.01-1.33). Therefore GLS has different prognostic implications in different patient sub-groups.

The third study explored whether tissue motion mitral annular displacement (TMAD) measured by STE may be a more useful alternative to GLS as it measures strain but is quicker and less user-dependent. TMAD was closely correlated to GLS (r=−0.614, p<0.001), but had no prognostic power for mortality (adjusted HR 1.04,95% CI 0.91-1.19).

The correlation between 2DE and RT3DE determined LV mass and volume measurements and the prognostic significance of RT3DE measurements were assessed. Although there was good correlation between 2DE and RT3DE LV volume measurements, 2DE overestimated LV mass compared to RT3DE. RT3DE measures gave no added prognostic value, and there were added difficulties in obtaining adequate images for RT3DE (35% of patients who had adequate 2D images). Furthermore, although RT3DE determined LV mechanical dyssynchrony index was prolonged in HD patients compared with published general population controls, it failed to show any prognostic significance (HR 2.16, 95% CI 0.96-4.89) for mortality, but was associated with hospitalisation for heart failure (HR 1.03, 95% CI 1.00-1.06). These results indicate that novel measurements of sub-clinical cardiac dysfunction have the potential to aid prognostication in this high risk population. Follow-up studies exploring the longitudinal change in these parameters is ongoing.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright statement

i. The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the “Copyright”) and s/he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trade marks and other intellectual property (the “Intellectual Property”) and any reproductions of copyright works in the thesis, for example graphs and tables (“Reproductions”), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=487), in any relevant Thesis restriction declarations deposited in the University Library, The University Library’s regulations (see http://www.manchester.ac.uk/library/aboutus/regulations) and in The University’s policy on Presentation of Theses.
Thesis format

This PhD thesis is presented in the alternative format. Most chapters take the form of manuscripts that have been published or are suitable for publication in a peer-reviewed journal. The exception is the methods chapter (chapter 3) which provides overall detail of the epidemiological study to which patients were recruited for each of the experiments detailed in each subsequent results chapter. Each results chapter then provides a chapter-specific abstract, introduction and methods, as well as a preface to provide context to the overall aim of the thesis. Each introductory and results chapter has a heading that corresponds to the title of the published/submitted article. As per submitted manuscripts, after the title there will be a list of co-authors and a link to the relevant journal publisher's IPR policy allowing use of any published material in this thesis. Tables and figures for the whole manuscripts are embedded within the text. The references for each chapter are contained within that manuscript. This structure is in accordance with the University of Manchester presentation of thesis policy.

It was felt that the alternative format was appropriate for this thesis because each results chapter contains distinct, non-overlapping experiments which have each been submitted to peer review journals as independent papers. Because each chapter is a separate manuscript but all address a similar subject area there will be some unavoidable repetition, in particular in the introduction, methods, and references. However, each chapter is an independent body of work and the thesis overall is set out as a coherent body of work. The thesis will be organised into separate chapters, starting with an introduction to the background to the research.

The final concluding chapter brings the thesis together and presents plans for how the use of the results will lead to future projects. Lists of publications and abstracts that have arisen from this thesis are included in the appendix.
Acknowledgements

This research project would not have been possible if it wasn't for the kind generosity of the haemodialysis patients from Salford Royal Hospital Foundation Trust who agreed to participate in the study, therefore I would first like to thank all the patients for their participation. I hope that the results generated from this thesis will go on to benefit future patients with renal disease.

I would like to give sincere thanks and acknowledgement to my supervisors. I owe my entire clinical and academic career in nephrology to Professor Philip A Kalra who has, ever since I was a medical student, given me extensive support, encouragement and guidance for which I am eternally grateful. In addition, I would like to thank him for providing excellent supervision during my PhD project and for being so supportive. I am also immensely grateful to my co-supervisor, Dr Darren Green, who has been a major influence and role model throughout my career. He has been an exceptional supervisor; his advice and support has been invaluable. I would also like to thank my co-supervisor, Dr Smeeta Sinha, for all her help and guidance.

Special thanks goes to Dr Nik Abidin, who has been a great mentor and trainer for everything I learnt about echocardiography during this PhD project. I would also like to express my gratitude to the echocardiographic technicians who performed the echocardiograms, vascular research nurses, in particular, Laura Johnstone, and research assistants, especially Dace Dimza for helping with the logistics of the study.

I would like to take this opportunity to thank my parents who I owe my education and any success to. In addition, I would like to give acknowledgement and sincere gratitude to my husband, David Wong, for his unconditional understanding, love and support for everything I have done. Finally, I would like to dedicate this thesis to my beautiful daughter, Summer Wong, and my unborn child because they make everything in my life worthwhile.
The author

I studied medicine at the University of Manchester and since graduation, I have worked within the North West region for both basic medical and nephrology specialty training. Ever since I was a medical student, I have been inquisitive and find it exciting to try to answer questions where current knowledge does not have an answer. I got my first taste of research during a special study module when I investigated the sensitivity of myocardial perfusion imaging in comparison with coronary angiography in pre-renal transplant patients, as part of transplant work-up. I presented the findings at the Renal Association as a final year medical student and then took part in the write-up of the results; published in the International Journal of Cardiology. I loved the process of carrying out research, with presentation of the findings to a wider audience. As a result, in my own time, I continued to fuel my interests with further publications of case studies, reviews and original investigations alongside my medical training. All the research has been in nephrology, as this has been my interest from an early age.

I am grateful for the opportunity to have studied for a PhD because it has not only given me the chance to study in-depth a specific area of great interest in the nephrology field, I have learnt many new skills. In particular, I have learnt more about echocardiography, gained a deeper understanding of research methodology and improved upon my statistical analysis abilities. In addition, I have had the opportunity to carry out research outside of the thesis project, participated in clinical trials work, co-authored 3 book chapters and presented oral presentations at international conferences. I hope that the knowledge and experience that I have gained during my research will be useful, in the future, to help improve patient care.

Diana Yuan Yng Chiu, March 2016
Contribution of the author to the research

The supervisors (Dr. Darren Green, DG, Professor Philip A Kalra, PAK) conceived the original idea, applied for the research project grant and amendment for ethical approval alongside Dr. Nik Abidin (NA). The author (Dr. Diana Chiu, DC) set out the hypotheses for the thesis, refined the initial protocol and input ideas into the design of the study. The aims and design of each of the investigational chapters in this thesis was conceived and designed by DC, with support and input from her supervisors (DG, PAK) and NA.

DC was responsible for screening the dialysis patients, recruitment and consenting the majority of patients. Research nurse, Laura Johnstone, consented some of the patients. Research assistant, Dace Dimza, helped with some of the logistics of the project such as booking transport for patients. DC was present at assessments. She was trained to use the Vicorder™ system by the manufacturer. DC and the vascular research nurses carried out the pulse wave velocity measurements, electrocardiogram, and organised the centrifugation, storage and management of blood samples. Consultant cardiologist (NA) and echocardiographic technician (Janet Sewell, JS) trained DC to perform and interpret two-dimensional and three-dimensional transthoracic echocardiography. DC also attended courses on the carrying out of transthoracic echocardiography and interpretation of scans. However, DC did not utilise the echocardiographic scans she performed for this project in order to avoid the introduction of bias and maintain high validity of results; all scans were performed by an experienced echocardiographic technician (JS) or consultant cardiologist (NA).

DC was solely responsible for data collection, interpretation and all statistical analysis. She created the patient self reporting questionnaire for data capture. Dr. John Hughes, JH (another trained researcher) interpreted a sample of 20 scans for inter-observer variability.

DC carried out all the follow-up data (review of medical notes, questionnaires and contact with primary care physicians). Vaidehi Kataria, VK (another researcher) verified the causes of death and cardiac event data as another independent assessor. DC wrote all manuscripts, results and discussions, with supervision and editorial input from her supervisors (PAK, Dr. Smeeta Sinha, SS and DG) and NA.
Chapter 1

Echocardiography in Haemodialysis Patients: Uses and Challenges

Diana YY Chiu, Darren Green, Nik Abidin, Smeeta Sinha, Philip A Kalra.

Elsevier IPR policy: [http://www.elsevier.com/wps/find/authorsview.authors/rights](http://www.elsevier.com/wps/find/authorsview.authors/rights)

1.1 Preface

This chapter is a review article that provides background to this thesis with regard to the imaging methodology used. It aims to:

I. Discuss the common echocardiographic findings and the prognostic implications of cardiac changes in patients undergoing haemodialysis.

II. Highlight the strengths, limitations and potential solutions of using transthoracic echocardiography in this setting.

Cardiac structure and function are frequently abnormal in patients with end stage kidney disease, undergoing haemodialysis. Many measured cardiac parameters are predictive of poor prognosis. Two-dimensional (2D) transthoracic echocardiography is a rapid and widely used tool in clinical practice to assess any underlying abnormality. However, it is not perfect and has limitations, with some challenges specific to the haemodialysis population. This chapter highlights some of the difficulties of obtaining accurate and sensitive markers of cardiac dysfunction using 2D transthoracic echocardiography in patients undergoing haemodialysis, including an explanation for the need of novel echocardiographic techniques. This will set the scene for important considerations when setting up a study involving 2D transthoracic echocardiography: such as the importance of carrying out 2D echocardiography on a non-dialysis day when patients are at dry weight, in order for measurements like left ventricular ejection fraction to be measured more accurately. Second, it highlights the main rationale for the aims and objectives of this thesis, the exploration of novel echocardiographic techniques in providing prognostic information. The parameters which are explored include speckle tracking...
echocardiography determined global longitudinal strain, and tissue motion annular displacement of mitral valve, and three dimensional transthoracic echocardiographic measurements of left ventricular mass, volume and contractility. An analysis of each measurement's value and prognostic significance will form the subsequent experimental chapters of this thesis. This manuscript has been published in the *American Journal of Kidney Diseases* as a review article.

### 1.2 Introduction

Mortality is high in patients undergoing haemodialysis; in 2010 the incidence of all-cause mortality was 236 deaths per 1000 patient years at risk (1, p268). Almost half of all fatal events were due to cardiovascular causes (1, p249). About half of these were defined as due to sudden cardiac death (SCD); unexpected cardiac death within 1 hour of onset of symptoms (2). This high incidence, in part, reflects the frequent structural and functional cardiac abnormalities within this patient group (3,4). Early detection of these changes may be possible using echocardiography.

Echocardiography is radiation-free, non-invasive and widely available. As such, the 2012 US Renal Data System (USRDS) annual report acknowledges the importance of using echocardiography in patients with end stage kidney disease (ESKD) for diagnosis, guidance of treatment, and pre-transplantation evaluation (1, p248). Accurate interpretation of echocardiograms requires good image acquisition, which is both operator and patient anatomy dependent. Furthermore, there are nuanced in its utility specific to this patient group.

This review discusses the common echocardiographic findings and prognostic implications of cardiac changes in patients undergoing haemodialysis. It also highlights the strengths, limitations, and potential solutions involved with using echocardiography in this setting.

### 1.3 Left Ventricular Mass and Volume

The incidence of left ventricular hypertrophy (LVH) increases with deteriorating kidney function (5,6). Eventually, over 70% of patients with ESKD will have LVH at initiation of dialysis therapy (4,7). This is an adaptive remodelling response to repeated volume and/or
pressure overload. There are also non-haemodynamic factors that directly stimulate cell growth, resulting in LVH, including chronic kidney disease-mineral and bone disorder (CKD-MBD), renin-angiotensin system activation, and endothelin (8,9). Repeated cycles of myocardial stunning may occur with each dialysis session leading to fibrosis and uraemic cardiomyopathy (10).

Hypervolaemia, renal anaemia and arterio-venous fistulae contribute to volume overload (11,12), and risk of eccentric hypertrophy, in which ventricular volume increases with minimal increase in wall thickness. Pressure excess is a source of increased afterload; this may be secondary to hypertension, vascular calcifications, and increased sympathetic activation. The result is concentric hypertrophy, in which ventricular wall thickness increases but the ventricular volume remains the same or decreases.

**Figure 1-1. Classification of LV geometry.**

![Diagram of Left ventricular geometry with concentric and eccentric hypertrophy, normal, and concentric remodelling]

**Abbreviations:** LVMI=left ventricular mass index, RWT= Relative wall thickness.  
**Note:** Relative wall thickness is calculated from echocardiographic parameters by \((2 \times \text{posterior wall thickness})/\text{Left ventricular diastolic diameter or septal wall thickness} + \text{posterior wall thickness}/\text{Left ventricular diastolic diameter}\). Because left ventricular (LV) mass-end diastolic volume (M/V) is directly proportional to RWT (RWT 0.32-0.42 corresponds to M/V 1.0-1.5) therefore RWT may be replaced by M/V whereby an enlarged M/V with increased LV mass is classified as concentric hypertrophy. In concentric remodelling and hypertrophy, end diastolic volume is reduced whilst in eccentric hypertrophy, end diastolic volume is enlarged. Figure 1-1 reproduced with permission from Gerdts, European Heart Journal Supplement, 2008; 10: E23-E30 with permission of Oxford University Press (15).
Concentric hypertrophy is more common than eccentric hypertrophy in haemodialysis patients, reflecting the high prevalence of chronic hypertension in this group (Table 1-1) (13). The geometry of LVH may be classified further depending on end-diastolic volume, left ventricular mass index (LVMI) and relative wall thickness (or mass-volume ratio; Figure 1-1, Table 1-2) (14,15).

Table 1-1. Some typical normal and abnormal values for two-dimensional echocardiographic parameters.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Left atrial diameter (cm)</td>
<td>3.0-4.0</td>
</tr>
<tr>
<td>Left atrial volume (ml)</td>
<td>18-58</td>
</tr>
<tr>
<td>LV end diastolic diameter (cm)</td>
<td>4.2-5.9</td>
</tr>
<tr>
<td>LV end diastolic volume, (ml)</td>
<td>67-155</td>
</tr>
<tr>
<td>LV end systolic volume (ml)</td>
<td>22-58</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>88-224</td>
</tr>
<tr>
<td>LV mass/BSA (g/m²)</td>
<td>49-115</td>
</tr>
<tr>
<td>Relative wall thickness (cm)</td>
<td>0.22-0.42</td>
</tr>
<tr>
<td>Septal wall thickness (cm)</td>
<td>0.6-1.0</td>
</tr>
<tr>
<td>Posterior wall thickness (cm)</td>
<td>0.6-1.0</td>
</tr>
<tr>
<td>Endocardial fractional shortening (%)</td>
<td>25-43</td>
</tr>
<tr>
<td>Mid-wall fractional shortening (%)</td>
<td>14-22</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>≥55</td>
</tr>
</tbody>
</table>

Abbreviations: LV= left ventricular, BSA= body surface area.
### Table 1-2. Eccentric and concentric hypertrophy in dialysis patients.

<table>
<thead>
<tr>
<th>Authors/year</th>
<th>Study group</th>
<th>Prevalence of LVH (%)</th>
<th>Concentric hypertrophy (%) of study group</th>
<th>Eccentric hypertrophy (%) of study group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al., 2012 (17)</td>
<td>98 ESKD patients with preserved ejection fraction ≥ 50%</td>
<td>74.5%</td>
<td>49%</td>
<td>25.5%</td>
</tr>
<tr>
<td>Parfrey et al., 1996 (18)</td>
<td>432 ESKD at initiation of haemodialysis</td>
<td>66%</td>
<td>41%</td>
<td>28%</td>
</tr>
<tr>
<td>Wang et al., 2003 (19)</td>
<td>246 ESKD on peritoneal dialysis</td>
<td>95%</td>
<td>69.5%</td>
<td>25.6%</td>
</tr>
<tr>
<td>Nishimura et al., 2004 (20)</td>
<td>154 diabetic haemodialysis patients</td>
<td>46.8%</td>
<td>27.2%</td>
<td>19.4%</td>
</tr>
<tr>
<td>Zoccali et al., 2001 (21)</td>
<td>254 dialysis patients</td>
<td>70%</td>
<td>37.4%</td>
<td>32.7%</td>
</tr>
<tr>
<td>Foley et al., 1995 (22)</td>
<td>404 haemodialysis patients</td>
<td>62%</td>
<td>35%</td>
<td>27%</td>
</tr>
</tbody>
</table>

**Abbreviations:** LVH, left ventricular hypertrophy, ESKD, end stage kidney disease  
**Note:** Table generated from selected studies published in the English language within the last 20 years.

#### 1.3.1. Prognostic Implications

LVH, measured by LVMI, is predictive of mortality and cardiovascular events in dialysis patients (21,22). Pathological mechanisms include reduced endocardial perfusion, myocyte cell death, fibrosis and compensatory hypertrophy (23–26). With time, cardiac fibrosis decreases compliance of ventricles and may result in cardiac failure, with associated mortality.

A progressive increase in LVMI over time is also a predictor of cardiac death in haemodialysis patients (27). This increased LVMI to body surface area (BSA; >116g/m² in men, >104g/m² in women) (16) potentially is reversible by correcting fluid status and
metabolic control (28), with increased haemodialysis frequency (29,30) or kidney transplantation (31). Improvements in LVMI may translate to improved outcome. London et al. (32) found that controlling blood pressure and correcting anaemia achieved a mean LV mass reduction of 290 ± 80g to 264 ± 86g (P < 0.01) accompanied by a 28% risk reduction for mortality over a 5 year period. Methods to reduce LVH may be promising in the attempt to improve prognosis.

LV geometry also has an impact on prognosis. In a study of 433 patients with a normal cavity volume (≤ 90mL/m²), increased LVMI (>120g/m²) and mass-volume ratio (>2.2g/mL) were independently predictive of mortality after 2 years from initiation of dialysis (adjusted relative risks [RR], 3.29 and 2.24, respectively). High cavity volume (>120mL/m²) and reduced mass to volume ratio (<1.8g/mL) were highly predictive of mortality (adjusted RRs, 17.14 and 4.27, respectively) (22) in the same study.

1.3.2. Challenges and Limitations

Many formulas exist for calculating LV mass. A validated, commonly used equation is the Devereux formula (Figure 1-2).

**Figure 1-2. Two-dimensional echocardiography images when measuring left ventricular (LV) diameters in M-mode.**

![Two-dimensional echocardiography images when measuring left ventricular (LV) diameters in M-mode.](image)

**Abbreviations:** LV- left ventricular, LVIDD- Left ventricular internal diameter in diastole, LVIDS – Left ventricular internal diameter in systole, LVDD = left ventricular internal dimensions in end diastole, PWTD = posterior wall thickness at end diastole, IVSTD = interventricular septal thickness in end diastole. Note: Measurements are taken in the parasternal long axis view. The M-mode cursor is placed at the level of the mitral valve tips. Measurements are taken in end diastole or end systole to give the internal ventricular diameter. Using the LV internal dimension in end-diastole (LVIDD) measurement and other echocardiographic measures, LV mass may be calculated using the Devereux formula: LV mass (g): 0.8 (1.04 ([LVIDD + PWTD + IVSTD]^3 - [LVIDD]^3)) + 0.6 g.
Acquisition of these measurements and use of this formula have limitations. First, LV diameter may not be calculated reliably if epicardial and endocardial borders are not well delineated. Second, the dimensions are measured in a single plane and the formula assumes perfect cubic dimensions of the left ventricle that may not be correct, especially in patients with ESKD. Up-to 30% of dialysis patients have eccentric remodelling, asymmetric hypertrophy, and distorted LV geometry (17,33). Third, dialysis patients may be wide fluctuations in fluid volume between dialysis sessions. Intra-ventricular volume is generally higher before than after haemodialysis sessions because of ultrafiltration. When comparing measurements obtained before and after dialysis, LVMI and end-diastolic diameter can be reduced by 26.2-36.1 g/m² and 4-8.4 mm, respectively (34,35) (Figure 1-3). Hence, when interpreting LVMI in clinical studies, it is important to be aware of the timing of echocardiography in relation to the dialysis session. Many study groups perform echocardiography on a non-dialysis day and at the patient’s target weight to avoid inaccuracies of LVMI measurement.

Figure 1-3. Schematic representation of left ventricle pre- and post-dialysis in end-diastole.

Pre-Dialysis  
Post-Dialysis

Abbreviations: LVIDD, Left ventricular internal diameter in diastole  
Note: Postdialysis, after ultrafiltration, there may be a smaller left ventricular (LV) internal diameter in diastole (LVIDD) and intra-ventricular volume. This may affect the calculations of LV mass because the Devereux formula uses the dimension of LVIDD.

The Devereux formula uses linear measurements in one plane, but there are also 2-dimensional (2D) methods to calculate LV volume, and hence LV mass, in order to improve accuracy:

I. The area-length method uses the cross-sectional area of the left ventricle in end-diastole and the end-diastolic long axis length in a calculation of volume.

II. The truncated ellipsoid method uses the same measurements in a different formula, assuming that the left ventricle is partly ellipsoid in shape.
III. Biplane Simpson’s rule applies the principle of separating the left ventricle into a series of disks and the volume is the summation of these areas.

IV. The method of multiple diameters utilises the average diameter of the left ventricle in end-diastole to estimate the volume.

When end-diastolic volume is calculated by any of these methods, LV mass may be calculated using the equation: (epicardial volume/total volume-end diastolic volume) x myocardial density (1.05). Although 2D methods may be more accurate than linear measurements, each method has its limitations and strengths (Table 1-3). For example, the area-length and truncated ellipsoid methods assume that the left ventricle is a uniform ellipsoid or cylindrical shape and may over- or underestimates an asymmetrical ventricle. The biplane Simpson’s rule may be inaccurate if the LV measurement is foreshortened because the apical part of the ventricle is not seen and thus estimated to be shorter than it is.

Conventionally, LV mass is indexed to BSA. Determination of BSA may be inaccurate in patients with ESKD who have fluctuations in body fluid, muscle or fat and may have limb amputations. One suggested solution is to index LV mass to height$^{2.7}$. Zoccali et al.(21) compared LVM/Height$^{2.7}$ to LVM/BSA in a prospective study of 254 ESKD patients. Although both methods of indexing were independently predictive of cardiovascular and all cause mortality ($P < 0.001$), the height$^{2.7}$-based method produced a better fit statistical model ($P \leq 0.02$).

The geometry of LVH has implications for prognosis however there may be inaccuracies in the measurements required for classification. RWT is unaffected by changes in BSA, but differing values may be obtained in non-uniform ventricular walls (14). Interpretation of LV geometry may differ depending on timing of echocardiography. If echocardiography was performed immediately after ultra-filtration, post-dialysis, reduced end-diastolic volume and increased relative wall thickness might lead to a classification of concentric hypertrophy. However, pre-dialysis echocardiography, when a patient’s fluid volume status is maximal, might demonstrate LV dilatation with the diagnosis of eccentric hypertrophy. Performing echocardiography on a non-dialysis day may help by reducing the effect of volume fluctuations. Other imaging techniques may improve accuracy further.
### Table 1-3. Comparison of 2D echocardiographic methods to calculate LV volume

<table>
<thead>
<tr>
<th>Description of method</th>
<th>Area-Length</th>
<th>Truncated Ellipsoid</th>
<th>Biplane Simpson’s Rule</th>
<th>Multiple Diameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In parasternal short-axis view, endocardial border is traced in end-diastrolic CSA; then in apical 4-chamber view, distance from center of mitral valve annulus to LV apex in end-diastole, gives VL: EDV= ((5\times\text{CSA}\times\text{VL})/6). Process is repeated by tracing around epicardial border instead of endocardial border to obtain epicardial volume.</td>
<td>Method is similar to area-length method; measurements are as for area-length method, however, volume calculation used: (\text{EDV} = 8\times(\text{CSA})^2/(3\times\pi\times\text{VL})) Process is repeated by tracing around epicardial border instead of endocardial border to obtain epicardial volume.</td>
<td>Principle of summation of disks. Trace around the endocardium of the LV during end diastole in apical 4 chamber and apical 2 chamber views. The echocardiography software will split the ventricle into ‘discs’. The volume is calculated by the summation of the discs. Total volume is total EDV in apical 2 chamber and apical 4 chamber views.</td>
<td>Calculates epicardial and endocardial volumes with an average of multiple diameters of LV in end-diastole.</td>
</tr>
</tbody>
</table>

| Strengths             | More accurate than using linear dimensions in a single plane in calculating volume. | More accurate than using linear dimensions in a single plane in calculating volume. | More accurate that area-length and truncated ellipsoid methods for abnormally shaped ventricles | Useful if apical views and endocardial borders are suboptimal; useful if LV is not uniform. |

| Limitations           | Makes the geometric assumption that the ventricle is uniform; hence any assymmetry or regional wall abnormality will lead to overestimating volume. | Makes the geometric assumption that the ventricle is uniform because it assumes that the ventricle is a truncated ellipsoid shape. | Complete endocardial border needs to be traced, which may not be possible when images are inadequate and volumes may be overestimated; similarly, the cardiac apex may not be visible, leading to foreshortening of ventricle and underestimation of volume; this method may be more time consuming than area-length and truncated ellipsoid methods. | Time-consuming method; may not be reproducible. |

**Abbreviations:** 2D, 2-dimensional; CSA, cross-sectional area; LV, left ventricle; EDV, end diastolic volume; VL, end-diastolic long-axis length.

**Note:** To calculate: \(\text{LV mass} = (\text{epicardial volume/total volume} - \text{EDV})\times\text{myocardial density (1.05). Based on data from Leeson et al. (16).} \)
Cardiac magnetic resonance imaging is considered the gold standard technique for LV geometric measurements because geometric assumptions are not required (36). Currently, it is still expensive and not widely available. It is not suitable for patients who are claustrophobic or those with mobile ferromagnetic implants. Alternatively, 3-dimensional (3D) echocardiography is an advanced technique that also makes no geometric assumptions and benefits from reduced inter-observer variability (37) (Table 1-4). The ventricular image is built up from multiple images in different directions rather than from just 2 planes. Ventricular volume is measured directly rather than calculated with geometric assumptions (Figure 1-4). LV volume and mass measurements using 3D echocardiography are comparable to cardiac magnetic resonance imaging without contrast (38). However, 3D echocardiography is limited by image quality dependent upon the patient’s acoustic window. Single-beat real-time 3D technology now allows image acquisition in a single contraction, but other limitations include suboptimal spatial resolution (39) and the need for a breath hold, which can be problematic for some dialysis patients with dyspnoea. At present, 3D echocardiography is commonly performed in patients with ESKD and therefore is a research opportunity of great interest (40).

Figure 1-4. Three-dimensional transthoracic echocardiographic image of the left ventricle, direct measurements may be taken without geometric assumptions.
Table 1-4. Comparison of 2D transthoracic echocardiography, 3D transthoracic echocardiography and cardiac magnetic resonance imaging

<table>
<thead>
<tr>
<th>Functions</th>
<th>2D Transthoracic Echocardiography</th>
<th>3D Transthoracic Echocardiography</th>
<th>Cardiac MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple method to provide detailed assessment of gross cardiac structure and function; gross quantification of LV structure and geometry; measurement of cardiac systolic and diastolic function</td>
<td>Accurate measurement of cardiac structure and function with direct measurement of volumes; can provide detailed assessment of valvular heart disease</td>
<td>Gold standard for determining LV volume, mass and geometry in dialysis patients; can detect and quantify inter-myocardial fibrosis (gadolinium enhancement)</td>
</tr>
<tr>
<td>Some indications in practice</td>
<td>Investigation in cause of dyspnea and chest pain; cardiac risk stratification; assessment of effect of therapeutic intervention; assessment for suitability for renal transplant</td>
<td>Research; planning for cardiac surgery such as valvular surgery; accurate assessment of LV volume when left ventricle is asymmetrical, such as after myocardial infarction</td>
<td>Research; poor echocardiographic candidates with inadequate images, i.e., due to obesity; accurate determination of ventricular structure and function is important; assessing myocardial perfusion, myocardial scar, cardiac viability</td>
</tr>
<tr>
<td>Advantages</td>
<td>Non-invasive; inexpensive to perform; widely available; easy to perform; safe</td>
<td>Non-invasive; no geometric assumption, reduced error magnification; reduced inter-observer variability compared with 2D echocardiography; increased reproducibility compared with 2D echocardiography</td>
<td>Non-invasive; makes no geometric assumptions, directly measures cardiac mass</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>M-mode echocardiography may overestimate LV mass because of geometric assumptions, difficulties in estimation of LVMI when indexing to body surface area in dialysis patients; subjective interpretation; inter-observer variability</td>
<td>Requires patients to hold breath in order to obtain images, which may be problematic for dyspnoeic patients; images will be inadequate if there are artefacts; limited acquisition angle, difficult if there is a large dilated left ventricle; cannot be performed in arrhythmias</td>
<td>Costly; not widely available; claustrophobia; contraindicated in patients with internal metal work or pacemakers; gadolinium contrast may give risk to nephrogenic systemic fibrosis in ESKD patients</td>
</tr>
</tbody>
</table>

Abbreviations: 2D, 2-dimensional; 3D, 3-dimensional; MRI, magnetic resonance imaging; LV, left ventricular; LVMI, left ventricular mass index; ESKD, end stage kidney disease
1.4 Left Ventricular Systolic Function

LV systolic dysfunction has a prevalence between 15% (18) and 28% (41) in dialysis patients. The cause is often multifactorial including ischaemic heart disease, volume overload and anaemia (42). A longitudinal analysis of echocardiograms was performed in the Chronic Renal Insufficiency Cohort (CRIC) (43). Patients who progressed to ESKD (defined as starting dialysis therapy) with echocardiograms which were performed in the pre-dialysis phase (estimated glomerular filtration rate < 20mL/min/1.73m^2) and also after commencing ESKD were included in the analysis. In the 190 patients with a full set of data, mean LVMI remained the same, whereas ejection fraction (EF) decreased (53% to 50%, \( P = 0.002 \)) between the 2 measurements (43). It is uncertain whether EF reduction would have been greater had renal replacement therapy not occurred. Although the absolute reduction in EF was small, this potentially is clinically significant. In one study of peritoneal dialysis patients, a 1% reduction in EF led to a significant increased risk of SCD (6%; \( P = 0.004 \)) (44).

1.4.1. Prognostic Implications

Reduction in LV systolic function is predictive of death, cardiovascular events, and chronic cardiac failure in patients with ESKD (5). A prospective study of 1,254 haemodialysis patients involved echocardiography performed within 1 month of initiation of dialysis therapy; 13% had EF ≤50% at baseline. After risk-factor adjustment and a 7-year follow-up, reduced EF at baseline was an independent predictor of cardiovascular death (45). This risk may partly be related to the dialysis procedure itself. In a study of 105 haemodialysis patients who had undergone 2D echocardiography before, during and after dialysis, haemodialysis-induced regional LV systolic dysfunction occurred in 27%, with half of these patients affected within the first hour of dialysis. After a median follow-up of 16.4 months, mortality was higher in those who demonstrated haemodialysis-induced LV dysfunction (46). Patients who develop these intra-dialytic wall motion abnormalities have a LVEF at 1 year that is lower than baseline, supporting the hypothesis that repeat haemodialysis-induced myocardial stunning may lead to myocardial fibrosis and permanent systolic dysfunction (47).
Dialysis patients with EFs <30% have a 5-year SCD risk of 60% (48). Interestingly, dialysis patients with LVEF ≥50% also had a 5-year SCD risk of 28% (48), showing that dialysis patients with preserved EF still have considerable mortality. The adverse prognosis associated with poor systolic function persists after renal transplantation. Echocardiograms performed on the eve of renal transplantation were studied in 141 patients. After median follow-up of 6.5 years, impaired systolic function was independently predictive of mortality (41). Currently there is limited evidence for effective therapies for treatment of systolic dysfunction in haemodialysis patients (49).

1.4.2. Challenges and Limitations

There are 2 common methods of calculating LVEF:

I. The area-length method estimates volume by using the cross sectional area computed by planimetry in an equation with the measured long-axis ventricular length (from LV apex to mitral valve annulus).

II. Modified biplane Simpson’s rule determines the volume by tracing around LV endocardial borders in both apical 2 and 4 chamber views. The left ventricle cavity is divided into discs with the LV volume summated as the stack of elliptical discs (50).

The volumes deduced by either method then are used to calculate EF: (end-diastolic volume – End systolic volume)/ EDV x 100%. Normal EF defined as >50%.

As for LVMI, limitations of these methods involve inaccuracies associated with unclear delineation of endocardial borders and the assumption of geometric uniformity. In patients with concentric LVH, contractility may be overestimated. In these patients midwall fractional shortening may be a more sensitive measurement of systolic function. In a study comparing 6 healthy individuals and 6 patients with LVH (51), measurements of the left ventricle size and endocardial fiber shortening velocities were the same in both groups. However, midwall fractional fiber lengthening rates were reduced in patients with LVH (2.3±0.4s⁻¹ in normal versus 1.6±0.4s⁻¹ in LVH, P < 0.02).
1.5 Sub-clinical Systolic Dysfunction with Normal Ejection Fraction

Dialysis patients with preserved EF still have high mortality rates. This may be because of abnormal contraction patterns. LV systolic function has 3 patterns of contraction: longitudinal, radial and circumferential (Figure 1-5) (52). Two applications that may detect these contraction-relaxation patterns are: tissue Doppler imaging and speckle tracking.

Figure 1-5. Schematic representation of the 3 components of left ventricular contraction.

Note: From the end-diastolic position of maximum volume (A), the ventricle will shorten longitudinally from the base to the apex (B to C), undergo torsion/twisting around its longitudinal axis (D) and radial strain that causes a thickening of the myocardial wall and a narrowing of the perpendicular radius (E to F). The resultant change in the left ventricular cavity volume (A to G) represents the ejection fraction. Reproduced from Green et al. (52), NDT 2012, with permission of Oxford University Press.

Tissue Doppler imaging filters high-velocity signals from blood cells and focuses on low-velocity, high-signal amplitudes of myocardial tissue and valves. Therefore, it can detect abnormal longitudinal or radial contraction and assess diastolic dysfunction and systolic function (53). This application is readily available on most modern echocardiography machines. Tissue Doppler imaging assessment of longitudinal contraction is a sensitive way to assess subclinical left ventricular dysfunction, and has been utilised in dialysis patients with normal EF (54). A main limitation of tissue Doppler imaging is that it can only measure velocity in the plane of the ultrasound beam; thus, it is angle dependent and
cannot discriminate passive (translation or tethering) from active (fibre shortening or lengthening) motion (55).

Speckle tracking is a novel echocardiographic application that is increasingly used in the general population. It uses images from pre-obtained echocardiograms and is an angle-independent technique. Images are formed by tracking the dislocation of acoustic markers throughout the cardiac cycle, thus tracing the movement of specific myocardial fibres. It is able to quantitatively describe all 3 spatial myocardial deformations. The main limitation of speckle tracking is that it depends on obtaining a high-quality image over at least 3 regular consecutive heartbeats and hence it cannot be used when arrhythmia is present. Yan et al. (56) applied speckle tracking to 36 with dialysis, 17 with non-dialysis-dependent CKD, and 18 controls with preserved LVEF >60% and demonstrated that longitudinal, radial, and circumferential strains were all reduced in the kidney disease groups compared with controls. Hence, specific strain pattern abnormalities may be present despite preserved EF in CKD patients.

Another form of subclinical cardiac dysfunction is LV dyssynchrony which is associated with uncoordinated regional cardiac contractions that may result in mechanical insufficiency (57) and increased risk of arrhythmia (58). LV dyssynchrony is associated with LVH, reduced EF, and fluid overload (59). In a study of 145 pre-dialysis and dialysis patients, LV dyssynchrony was present in 54% of all patients, with no significant differences between stages of CKD (59). LV dyssynchrony improved in 50% of patients after a single dialysis session. This was particularly marked in patients with lower LVMIs and higher myocardial systolic velocities. In another study of 144 patients with ESKD, the 2-year mortality was higher in patients with baseline LV dyssynchrony (mean LVEF 48±12%) (60).

1.6 Diastolic Dysfunction

Diastolic dysfunction results from a reduction in compliance and impaired relaxation of the left ventricle, which increases ventricular filling pressure and leading to left atrial enlargement. Patients with diastolic dysfunction may be highly susceptible to fluctuations in ventricular volume. A minor increase in fluid load may cause pulmonary congestion, whilst slight hypovolaemia may cause intra-dialytic hypotension (61). The underlying
pathology of diastolic dysfunction is related to diffuse intermyocardiocytic fibrosis in ESKD (62). Reduction in ventricular compliance is associated with age, hypertension, diabetes mellitus, LVH, coronary artery disease and infiltrative cardiomyopathy (63), factors common in ESKD. The prevalence of diastolic dysfunction is reported to be between 48% - 73% in dialysis patients (63).

1.6.1. Prognostic Implications

Diastolic dysfunction, independently and in combination with other clinical and echocardiographic parameters, has been demonstrated to be a predictor of adverse cardiovascular outcome and mortality (64,65). A cohort of 129 haemodialysis patients (aged 52±16 years) showed diastolic dysfunction to be present in 73%. After a follow-up of 17±7 months, advanced diastolic dysfunction was associated with higher mortality (P=0.012) and was predictive of cardiovascular events (adjusted Cox regression multivariate analysis hazard ratio [HR], 2.2; confidence interval [CI], 1.1-4.3; P=0.021) (66). In a heterogeneous group of patients with CKD (mean estimated glomerular filtration rate, 18.8±7.0mL/min), 62% of whom were receiving dialysis, diastolic tissue velocity (HR, 0.8; P = 0.05) was an independent predictor of outcome after a follow-up of more than 2.4 years (67). To date, no study has shown that improvement in diastolic dysfunction translates into improved survival.

1.6.2. Challenges and Limitations

Mitral transvalvular flow commonly is used to estimate diastolic dysfunction. In studies of echocardiography in CKD, this is most often expressed using the E/A ratio. The E wave is the rate of mitral transvalvular flow during early diastolic left ventricular filling. The A wave is the flow during atrial contraction. Early diastolic dysfunction, representing impaired ventricular relaxation as in LVH, is manifest by a reversed ratio of passive (E) to active (A) diastolic filling components of the left ventricle: E/A < 1. With hypervolaemia and increased filling pressures E will increase, leading to what is known as pseudo-normalization of mitral E/A; the ratio is normal, but due to pathological reasons. E/A ratio is therefore volume dependent, and the prevalence of diastolic dysfunction may be underestimated if pseudo-normalization is not taken into account. Other parameters of diastolic dysfunction should be assessed (Table 1-5).
Tissue Doppler imaging provides advantages as the mitral annulus tissue Doppler (E’) is affected less by preload than conventional mitral transvalvular flow (68–71). Moreover, the ratio of early diastolic velocity of the mitral flow (E) to tissue Doppler diastolic velocity (E’), known as E/E’, has been validated with invasive measures of diastolic pressures (72). Tissue Doppler imaging has demonstrated a high prevalence of right ventricular dysfunction amongst pre-dialysis patients. Prospective data are lacking for whether these patients are more likely to progress to congestive cardiac failure (73).

Table 1-5. Echocardiographic parameters that may indicate diastolic dysfunction.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Normal Ranges</th>
<th>Diastolic Dysfunction</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E/A ratio</strong></td>
<td>Early mitral flow velocity/atrial mitral flow velocity</td>
<td>0.8-2</td>
<td>&lt;1</td>
<td>Load dependent; pseudo-normalisation where E/A&gt;1 but chronic diastolic dysfunction is present</td>
</tr>
<tr>
<td><strong>E/e’ ratio</strong></td>
<td>Early mitral flow velocity/early mitral annulus velocity</td>
<td>&lt;8</td>
<td>8 to ≥13</td>
<td>e’ is a tissue Doppler measurement</td>
</tr>
<tr>
<td><strong>Isovolumic relaxation time</strong></td>
<td>Time from end of aortic outflow to start of mitral inflow</td>
<td>80</td>
<td>&gt;80</td>
<td></td>
</tr>
<tr>
<td>(ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Deceleration time (ms)</strong></td>
<td>Peak to end of the E wave distance</td>
<td>160-260</td>
<td>&gt;260</td>
<td></td>
</tr>
<tr>
<td><strong>E (cm/s)</strong></td>
<td>Mitral E-wave deceleration time</td>
<td>&gt;10</td>
<td>&lt;8</td>
<td></td>
</tr>
<tr>
<td><strong>Pulmonary venous inflow</strong></td>
<td>Trace of the pulmonary vein flow</td>
<td>Systolic wave is dominant or equal to diastolic wave &gt;30cm/s peak velocity of atrial wave and &gt;20-30ms longer atrial wave compared with A wave</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Left atrial volume index</strong></td>
<td>Left atrial volume indexed to body surface area</td>
<td>&lt;28</td>
<td>≥34</td>
<td>Enlarged left atrium is a surrogate marker for chronically elevated LV diastolic pressure</td>
</tr>
<tr>
<td>(mL/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: A, atrial mitral flow velocity; E, early mitral flow velocity; e’ = early mitral annulus velocity; LV, left ventricular. Based on data from Leeson et al. (16).
With speckle tracking, a cutoff point of mitral early diastolic velocity/diastolic strain rate during the iso-volumetric relaxation period ≥236 has been shown to be indicative of diastolic dysfunction (74). In 77 asymptomatic dialysis patients, speckle tracking identified diastolic dysfunction in 48% of patients with preserved LVEF (mean 52%±7%). LV mass (Odds Ratio [OR], 1.02; 95% CI 1.00-1.04; P= 0.014) and pulse wave velocity (OR, 1.34; 95% CI 1.07 – 1.68; P= 0.01), a marker of arterial stiffness, were independent determinants of diastolic dysfunction (63). The prevalence of diastolic dysfunction in dialysis populations may be even higher as this study only included patients with life expectancy of greater than 1 year.

1.7 Other Echocardiographic Considerations in Patients Undergoing Haemodialysis

1.7.1. Left Atrial Size

A dilated left atrium may be a surrogate marker of diastolic dysfunction because left atrial volume (LAV) is associated with the chronicity of LV dysfunction (75). There are other possible reasons for left atrial enlargement in haemodialysis patients (Table 1-6). In the general population, left atrial dysfunction predicts heart failure hospitalisation in patients with coronary artery disease and normal systolic function (76).

Table 1-6. Causes of an enlarged left atrium in haemodialysis patients

<table>
<thead>
<tr>
<th>Increased preload to left atrium</th>
<th>Increased afterload to left atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Volume overload</td>
<td>• Left ventricular systolic dysfunction</td>
</tr>
<tr>
<td>• Mitral valve regurgitation</td>
<td>• Mitral stenosis</td>
</tr>
<tr>
<td>• Arteriovenous fistula</td>
<td>• Hypertension</td>
</tr>
<tr>
<td>• High-output states: chronic anaemia</td>
<td>• Left ventricular hypertrophy</td>
</tr>
</tbody>
</table>

Left atrial size may be described via a diameter, area or volume measurement. The simplest method measures the antero-posterior diameter of the left atrium in the parasternal long-axis view perpendicular to the left atrial wall. As expected, it is open to wide-ranging inaccuracies because measurements taken at different points may yield different values. A modification of this method is to calculate the left atrial area by tracing around the left atrial border, but this only provides 2D data. LAV may be estimated from the biplane Simpson’s rule. As for LV volumes, patients with varying BSA will have corresponding
different sizes of left atrium, and it has been proposed that LAV should also be indexed to height$^{2.7}$ (77).

Enlarged LAV predicts mortality and cardiovascular events (atrial fibrillation and cerebral thrombosis (78)) in dialysis patients (79). Tripepi et al. (77) carried out a prospective cohort study in 249 dialysis patients. Height$^{2.7}$-indexed LAV was significantly increased in dialysis patients compared with age- and sex-matched healthy controls (mean height$^{2.7}$-indexed LAV was 12mL/m$^{2.7}$ in dialysis patients and 7.5mL/m$^{2.7}$ in healthy controls; P<0.001). This parameter was independently predictive for all-cause mortality even when LVH and LVEF were included in a Cox regression model (HR for each 1ml/m$^{2.7}$ was 1.05; 95% CI, 1.01-1.09; P = 0.03), a finding confirmed by other groups (79–81). Tripepi et al. (82) also demonstrated that an increase in LAV by 1ml/m$^{2.7}$ per year predicted cardiovascular events (P < 0.001) independent of baseline LAV and LV function or mass. LAV is a strong nonspecific indicator of cardiovascular pathology and an ideal simple marker of diastolic dysfunction that can easily be identified by physicians in everyday clinical practice.

1.7.2. Valve Annular Calcifications

Mitral valve annular calcification is common in haemodialysis patients, the prevalence being between 19% to 84% (83). Braun et al. (84) demonstrated cardiac valvular calcification in over 50% of dialysis patients, the severity increasing after 1 year of re-testing. There are a variety of proposed contributing factors such as aging, hyperphosphataemia, elevated calcium-phosphorus product values, and inflammation (85).

Valvular calcification can be seen as a bright echogenicity on echocardiography. Studies have used >1mm of bright echoes on cardiac valves as indicative of its presence (86). Some cardiac calcification may be missed, depending on the angle of the transducer. More accurate methods involve electron-beam tomography and multi-detector computed tomography that report coronary calcium score quantitatively, representing the calcium density on the valve.

In a study of 140 patients with ESKD (61 pre-dialysis, 50 haemodialysis, and 29 peritoneal dialysis) with mean follow-up 2.3 ± 0.7 years, the presence of mitral annular calcification at echocardiography was associated with increased cardiac mortality and cardiovascular
events (P = 0.01) (87). Its importance as a prognostic marker led to another study group using this measurement in a prognostic risk score post-kidney transplantation (88). Valvular calcification may be a good prognostic marker of poor outcome because it is a marker of vascular calcification (89). Calcium-phosphate product value is an independent predictor of heart valve calcification, (90) and it may be that treatment of CKD-MBD may ameliorate valvular calcification.

1.7.3. **Pericardial Disease and Inferior Vena Cava Size**

Echocardiography can detect pericardial effusion, the presence of which in dialysis patients may indicate pericarditis, systolic dysfunction, or hypervolaemia. Patients with ESKD may have uraemic pericarditis (pericarditis in patients who have been on dialysis therapy for <8 weeks) or dialysis-associated pericarditis (pericarditis in patients who have been on dialysis therapy for >8 weeks) (91). Uraemic pericarditis, occurs in 6 - 10% of patients around initiation or during the first few weeks of dialysis therapy (92). Dialysis-associated pericarditis occurs in ~13% of patients (93). Pericardial effusions associated with dialysis have good prognosis and resolve with frequent dialysis (94). Other specific rare causes of pericardial effusion, such as viral pericarditis, malignant pericardial effusion, or chylous effusions (95) may occur in dialysis patients and should not be overlooked.

The inferior vena cava size correlates with central venous pressure, right atrial pressure and circulating blood volume (96–99). This also is visualised easily using echocardiography. If the inferior vena cava does not fully collapse with inspiration then this may indicate hypervolaemia. Use of echocardiography to assess inferior vena cava compressibility and pericardial effusion therefore may help assess fluid status and guide dialysis prescriptions, but wider discussion of echocardiography in assessing the volume status of dialysis patients is beyond the scope of this review.

**1.8 Conclusions**

Echocardiography is an invaluable tool in the management and prognostic prediction of dialysis patients. However, interpretation of echocardiographic changes in this setting should be different from the general population. Haemodialysis patients may have wide fluctuations in fluid and nutritional status, and measurements such as LVMI and LVEF are
fraught with inaccuracies. Special considerations are required, such as performance of echocardiography on a non-dialysis day, at dry weight, and with measurements, such as LVMI, indexed to height. Currently, there is limited data regarding the effect of improvement of echocardiographic parameters and outcomes. However, some serially changing echocardiographic parameters are associated with worse prognosis. There is no consensus for the optimal frequency of performing echocardiograms in dialysis patients. However, there is logic in performing 2D echocardiography in all patients at initiation of dialysis such that structural abnormalities can be considered and changes in parameters pursued by serial echocardiograms when appropriate. Ideally, patients would undergo annual review with imaging, when at dry weight on a non-dialysis day, as part of a larger battery of tests assessing fluid status, hypertension, and hidden coronary artery disease. The benefit of such a protocol is unproven, and it is unlikely to become routine practice on the grounds of cost-effectiveness in the present era. In practice, if echocardiography was only performed to investigate clinical changes such as dyspnoea, oedema and arrhythmia, it would still be performed at least annually for many patients. The symptom burden faced by haemodialysis patients is vast and perhaps too few investigations are performed because clinicians assume that symptoms such as dyspnoea are simply a feature of ESKD, and due to inadequate ultrafiltration or excess volume intake. Nephrologists perhaps should habitually ask themselves whether they would undertake echocardiography for the patient in front of them if he or she was not a haemodialysis patient.

1.9 References


24. Mall G, Huther W, Schneider J, Lundin P, Ritz E. Diffuse intermyocardiocytic


57. Spragg DD, Kass DA. Pathobiology of left ventricular dyssynchrony and...


73. Said K, Hassan M, Baligh E, Zayed B, Sorour K. Ventricular function in patients


Chapter 2

Hypothesis and Objectives

2.1 Hypothesis

It has been discussed in chapter 1 that patients with end-stage kidney disease, undergoing haemodialysis, have a high mortality particularly from cardiovascular disease. Bedside investigations such as transthoracic echocardiography, electrocardiography and pulse wave velocity may detect early cardiovascular changes that are predictive of mortality. However, as explained in chapter 1, there are challenges and inaccuracies of using tools such as standard 2-dimensional (2D) transthoracic echocardiography in patients undergoing haemodialysis. Currently, there is no gold standard technique for risk stratification in this high cardiovascular risk population.

There are limited or no data as to whether some emerging 2D and 3-dimensional (3D) echocardiography techniques provide added or better prognostic information than the current measurements taken during standard 2D transthoracic echocardiography.

The purpose of this thesis is to test the hypothesis that novel transthoracic echocardiographic techniques are predictive of outcomes, in particular, mortality and major cardiac events.

The hypotheses to be tested are that:

1. Left ventricular global longitudinal strain (GLS) determined by speckle tracking echocardiography is a better prognostic indicator for mortality, cardiac death and cardiac events than standard 2D echocardiographic parameters and pulse wave velocity in patients undergoing haemodialysis evaluated as a single population, and that this prognostic benefit will differ in sub-groups of patients selected according to left ventricular ejection fraction and mass.

2. Speckle tracking echocardiography determined tissue motion mitral annular displacement is comparable to GLS as a strain-based prognostic marker of all-cause mortality, cardiac death and cardiac events.
(3) 3D echocardiographic determination of left ventricular mass and volume is superior to 2D echocardiographic measures in predicting all-cause mortality.

(4) 3D echocardiographic determined left ventricular dyssynchrony is predictive of all-cause mortality, cardiac events and heart failure admissions.

2.2 Objectives

To address the hypotheses, this thesis will:

(1) Describe the study design, in haemodialysis patients, incorporating bedside investigations such as 12-lead electrocardiogram, Vicorder™, 2D and 3D transthoracic echocardiogram.

(2) Provide analysis of the study population, comparing patients who were recruited versus non-participants.

(3) Investigate whether GLS is predictive of mortality, cardiac death and cardiac events in a multivariable model including pulse wave velocity, in haemodialysis patients with an unselected left ventricular ejection fraction.

(4) Investigate whether GLS is predictive of outcome in a multivariable model including pulse wave velocity for mortality in different subgroups, namely patients with left ventricular hypertrophy and those with preserved ejection fraction.

(5) Investigate whether tissue motion mitral annular displacement is predictive of mortality, cardiac events and deaths. In addition, how this parameter compares with GLS measurements as a prognostic tool.

(6) Compare the prognostic value of 3D echocardiographic derived left ventricular mass and volume compared with 2D transthoracic echocardiographic measurements in predicting all-cause mortality.

(7) Assess the predictive value of 3D echocardiography derived left ventricular dyssynchrony indices for mortality, cardiac events and heart failure admissions.

(8) Provide a summary of the most promising novel markers associated with mortality in haemodialysis patients, how this may be applied in clinical practice and how the data may be used in future research projects.
Chapter 3

Methods

3.1 Preface

This chapter outlines the overall study protocol for the haemodialysis patient sub-study of the Salford Kidney Study (SKS, formerly the Chronic Renal Insufficiency Standards Implementations Study), the study to which patients were recruited for the investigations in this thesis. The chapter provides a detailed description of the methodology and assessments used to address the hypotheses and aims outlined in chapter 2. The rationale for the study design is also included, when appropriate. The details of the specific imaging analyses (e.g. global longitudinal strain, tissue motion mitral annular displacement, left ventricular mechanical dyssynchrony) are described in the methods section of each relevant results chapter, along with details of the statistical method for the analyses in that chapter. These subsequent results chapters will contain a brief overview of the Salford Kidney Study as per this chapter, in line with the expectations of methodological detail for a submitted manuscript. This will lead to some overlap between this and subsequent chapters as discussed in the “Thesis Format” section above.

3.2 Study Design

This prospective observational study is an extension of the Salford Kidney Study, a longitudinal observational study of outcome in patients with Chronic Kidney Disease (CKD). The Salford Kidney Study, previously named the Chronic Renal Insufficiency Standards Implementations Study (CRISIS), has been ongoing since 2003. Adult patients aged over 18 years, with CKD stages 3-5, receiving treatment at Salford Royal Foundation Trust, UK, nephrology services are approached for inclusion and enrolled if written informed consent is gained. Patients who agree to participate in the study undergo annual clinical assessment including a detailed medical history and a non-invasive arterial stiffness measurement. Bloods are taken annually for routine biochemistry and haematology tests. Additional blood samples are taken including serum, plasma, citrate plasma, and whole blood samples which are frozen for future genomic and biomarker
analysis. These data are used to explore the factors affecting CKD progression and outcomes.

This sub-study that forms the thesis focuses only on CKD stage 5 patients who are established on maintenance haemodialysis therapy. In addition to the investigations described above, patients recruited to the sub-study also undergo two-dimensional (2D) and three dimensional (3D) transthoracic echocardiography and 12-lead electrocardiography on a non-dialysis day. Participants for this sub-study was consented separately, therefore patients who were already in the Salford Kidney Study were re-consented if patients were interested to participate.

3.3 Participants and Eligibility Criteria

All incident and prevalent adult (≥18 years) patients receiving maintenance haemodialysis between 13 March 2012 and 30 March 2014 at Salford Royal Hospital NHS Foundation Trust or any of its four satellite units (Bolton, Wigan, Rochdale and Oldham) were approached to enter the study. The inclusion criteria were patients who were willing and able to give consent to attend for an extra visit for assessment. In order for transport to be arranged, patients needed to be mobile. The exclusion criteria were if they refused consent, were unable to consent, or if they required stretcher transport. The rationale for including a broad range of patient phenotypes was because this study was designed to investigate predictive factors for mortality and cardiovascular events and these outcomes may occur in any dialysis patient, irrespective of age or co-morbidity. All patients were offered paid transport to attend for cardiac investigations in order not to discriminate any patient who could not otherwise attend without paid transport facilities and considering the investigations are performed on a non-dialysis day, at an extra study visit.

As standard, all patients received haemodialysis 3 times weekly for 3-4 hours with an adequate dialysis clearance (urea reduction ratio >65%). In all patients, cardiac tests were conducted on a non-dialysis day, after the short inter-dialytic break. This is because some echocardiographic measurements, as explained in chapter 1, such as left ventricular ejection fraction (LVEF) are affected by patient’s fluid status (1). By performing investigation on a non-dialysis day, this will allow time for fluid equilibration post-dialysis.
3.4 Ethical Considerations

Ethical approval from the local ethics committee was obtained for this study (REC 05/Q1404/188) and all investigations adhered to the principles of the Declaration of Helsinki (2013). Written and verbal consent were obtained from all enrolled patients after at least 24 hours to consider the patient information sheet. This study was funded by a project grant from Kidney Research, UK (RP35/2011).

3.5 Data Collection

Baseline demographics, co-morbidities and medications were obtained from a patient self-reported questionnaire (on the day of assessments), review of electronic patient medical records, and contact with the patient's primary care physician. Dialysis records and blood test results were obtained from electronic medical records. Because dialysis prescriptions and blood results act as potential confounding factors, information such as ultrafiltration volumes, inter-dialytic weight gain and blood pressures were obtained as part of the data collection. The mean averaged 3 months dialysis prescription parameters and blood results prior to the assessment date was used. This was felt to be more representative of the effect of dialysis and bloods on cardiac function because gross structural cardiac changes are likely to happen over time.

3.6 Definitions

The following definitions were used for data collection:

*Diabetes mellitus* was defined as a history of diabetes mellitus, previously diagnosed by a physician, with fasting plasma glucose $>$ 7mmol/L or glycosylated HBA1c of $>$ 48mmol/mol (6.5%) or diabetic symptoms and random plasma glucose of $\geq$ 11mmol/l or use of anti-diabetic medication. Type 1 diabetes mellitus was defined as diagnosed diabetes mellitus that was of abrupt onset, in the young (<30 years), that required insulin from diagnosis and/or autoantibodies detected. Type 2 diabetes mellitus was defined as diagnosed diabetes mellitus in the older patients (>30 years), that required insulin or anti-diabetic medications and with no auto-antibodies detected.

*Smoker* was defined as current smoking or previous smoker for more than 6 months.
Myocardial infarction was defined as cardiac sounding chest pain or ischaemic symptoms with consistent electrocardiographic changes such as ST elevation in 2 or more contiguous leads. This is associated with a significant diagnostic increase in Troponin (I or T).

Angina was defined as cardiac sounding chest pain on exertion and prescribed anti-anginal medication, diagnosed by a physician, and documented in medical records. Prevalent coronary artery disease was defined as documented history of myocardial infarction, angina, coronary artery bypass grafting, or coronary percutaneous intervention.

History of congestive cardiac failure was defined as diagnosed and documented history of heart failure by a physician with previous symptoms of exertional dyspnoea, paroxysmal nocturnal dyspnoea or orthopnoea and/or echocardiographic evidence of left ventricular ejection fraction <50%, or diastolic dysfunction in the presence of the above symptoms.

History of peripheral vascular disease was defined as history of peripheral claudication or peripheral lower limb revascularization.

History of cerebrovascular disease/transient ischaemic attack was defined as acute onset neurological symptoms with cerebral bleed or thrombosis demonstrated by computer tomography. For transient ischaemic attack, the neurological symptoms are completely resolved in 24 hours.

3.7 Follow-up

All patients were followed-up prospectively from day of assessments until death, kidney transplantation, moved out of the country or study end date (16th November 2015). Patients were questioned regarding significant medical and cardiovascular events that have occurred at an annual cardiovascular assessment visit. Data were verified and further information obtained from review of electronic medical health records and contact with primary care physicians. Where possible, mortality data was confirmed on death certificates issued from data recorded on hospital records or details from post mortem findings. Cause of deaths occurring in the community or outside of Salford Royal hospital were obtained from primary care physicians. All events, deaths and cardiovascular events were validated by 2 independent assessors.

3.8 Follow-up Definitions

The main primary outcome was all-cause mortality and the secondary outcomes were cardiac deaths, major cardiac events and heart failure admissions.
(1) **Deaths:**

*All-cause mortality:* death from any cause.

*Cardiac death* was defined as death due to myocardial infarction, heart failure, arrhythmia or sudden cardiac death.

2. **Major cardiac events (MCEs)** were defined as any of the following:

*Arrhythmia:* Arrhythmic event or unexplained syncope requiring hospital admission or medical intervention.

*Myocardial infarction:* Electrocardiographic changes consistent with myocardial infarction plus a significant rise in Troponin T or I (≥ 2x upper limit of normal), with or without cardiac sounding chest pain.

*Acute coronary syndrome:* A rise in Troponin T or I from the patients’ baseline value unrelated to non-cardiac precipitating illness such as sepsis or pulmonary embolus, plus either of cardiac chest pain or acute ischaemic electrocardiogram changes.

*New angina:* Absence of angina diagnosis at recruitment, but during study period develops exertional chest pain with evidence such as positive exercise stress test or myocardial perfusion scan.

*Heart failure admission:* Heart failure requiring admission to hospital. The patient must present with symptoms of heart failure: orthopnoea, paroxysmal dyspnoea and/or shortness of breath; signs consistent with heart failure i.e. raised jugular venous pressure, crepitations bilaterally at base of the chest and/or peripheral oedema and requiring an urgent extra session of dialysis or extra fluid removal on ultrafiltration with improvement of heart failure symptoms (2).

*Coronary artery revascularization or coronary artery bypass procedure:* Not electively planned prior to recruitment to study.

### 3.9 Initial Sample Size Estimate

There were 320 prevalent patients with 120 new patients starting haemodialysis per year at Salford Royal Hospital and its satellite units. Initial recruitment window was 24 months from March 2012 to March 2014. This gave 560 patients who can be approached for inclusion. In a previous feasibility exercise undertaken in January 2011, 105 patients were approached, 60% indicated willingness to participate, with 45% agreeing to echocardiography. On this basis, it was estimated that 252 patients will agree to undergo echocardiography. Previous pilot data carried out at Salford Royal Hospital show an annual mortality of 16.3% in our haemodialysis population.
On initial submission of a Kidney Research UK grant application, the study team were particularly interested in detecting sudden cardiac death as an end point. As United States Renal Data System (USRDS) data indicate that 26% of dialysis patient deaths are due to sudden cardiac death (3) this suggests an annual hazard for sudden cardiac death of 4.4% in our population. A power calculation was then performed based on echocardiographic measurements such as left ventricular mass index which may be expected to differ between sudden cardiac death and non-sudden cardiac death patients, to determine a between group difference as a measure of sudden cardiac death risk stratification. Our pilot data suggested that approximately 45% of patients will have an abnormal left ventricular mass index measurement at baseline, and that the hazard for death in the abnormal group is approximately 3.3 times that of the normal group. Under this scenario, the power to detect a hazard ratio of 2, 3 and 4 between the abnormal and normal groups is 32%, 70% and 88%, respectively. Given the conservative nature of this calculation (it assumes that the optimal combination of multivariate repeated measurements would give no better a prediction of the hazard for sudden cardiac death than a single baseline measurement of left ventricular mass index and that the rate of elevated left ventricular mass index is no greater in sudden cardiac death than in all-cause mortality despite it being highly likely that it is, the study was expected to have adequate power to detect hazard ratios less extreme than those observed in our pilot data (which involved only retrospective data and clinically determined non-systematically performed echocardiographs and electrocardiograms).

Although the initial aim was to recruit 252 patients, 219 patients were eventually enrolled. However, for the end-point of all-cause mortality, 219 patients provides sufficient power to detect between group differences as described with a power >90%.

3.10 Assessments

At each assessment session, all patients had their weight recorded without shoes and stripped to the waist wearing a gown only. In addition, the patients had their height measured using a standard meter tape. The height and weight measurements were required to calculated body mass index and for correction of measurements such as left ventricular mass.
3.11 Echocardiographic Assessment

Echocardiography was performed on a non-dialysis day when the patient was clinically stable and reached fluid equilibration post-dialysis. Either a trained experienced technician (Janet Sewell, JS) or a consultant cardiologist (Dr. Nik Abidin, NA) performed two-dimensional transthoracic echocardiography. All patients are examined in the left lateral decubitus position using echocardiographic equipment (Philips inc., Andover, Massachusetts, EUA) with 2 to 4 MHz transducers with M-mode, 2D and tissue Doppler (pulsed, continuous, colour and tissue) functions. Measurements and calculations are performed according to the guidelines of the European Society of Echocardiography (4,5). At least 3 consecutive heartbeats are acquired with each view. The echocardiographic measurements taken included:

- Left ventricular systolic internal diameter measured using M-mode.
- Left ventricular end diastolic diameter; dilatation was defined as >54mm for women and >56mm for men
- Left ventricular posterior wall thickness in diastole
- Left ventricular mass; M-mode measurements provided data to derive left ventricular mass from the Devereux equation (LV mass = 0.8*(1.04 [{LV internal diameter + septal wall thickness + posterior wall thickness\}^3 - LV internal diameter^3]) + 0.6g), then indexed to height to the power of 2.7. Left ventricular mass was indexed to height because this has been reported to be predictive of mortality in patients with kidney disease (6) and is a better measure of true LV mass because body surface area may be affected by fluid status.
- Left ventricular hypertrophy; this was defined as left ventricular mass/height^{2.7} >46.7g/m^{2.7} for women and >49.2g/m^{2.7} for men (7,8)
- Inter-ventricular septum thickness
- Relative wall thickness; < 0.45 mm was considered as normal
- Left ventricular ejection fraction; this was calculated by determining the left ventricular volume using the biplane method of disks (modified Simpson's rule). This was used for estimation of volume as it is more accurate than linear methods which has geometric assumptions. The best focussed, with clear endocardial borders, apical left ventricular 4-chamber view was selected. Using the measurement function, the endocardial border of the left ventricle was traced in diastole. The trackball was then rolled to the end-systolic frame in the same cardiac cycle and the left ventricular systolic endocardial border was traced. In selection of
the left ventricular chamber, particular care was taken to ensure that the left ventricle was not fore-shortened if possible. If the patient was in atrial fibrillation, the volumes were averaged over several cardiac cycles. Using the Philips Xcelera R4.1 image management system, the volume of the left ventricular cavity was calculated, Figure 3-1. This is then repeated for the left ventricle in the 2-chamber view. To calculate the left ventricular ejection fraction, this formula was used:

\[
\text{Left ventricular ejection fraction} = \frac{\text{End-diastolic volume} - \text{End-systolic volume}}{\text{End-diastolic volume}}
\]

An impaired left ventricular ejection fraction was defined as <50%.

- **Left ventricular diastolic function;** mitral trans-valvular flow is recorded in 4 chamber apical view with the cursor positioned at the tips of the mitral valve cusps. From here, measurements of early filling velocity, deceleration time (E wave) and atrial contraction velocity (A wave) and E:A ratio was calculated. Then this was repeated with tissue Doppler velocities. The sampling position is performed consecutively on the junction between left ventricular lateral and septal walls with the mitral annulus. The early (E’) and late (A’) diastolic mitral annular velocities were taken, E’/A’ and E/E’ (average of septal and lateral annulus sides) ratios were calculated. Normal (E/A ratio <1, E’<10cm/s), pseudonormal and restrictive flow (E/A ratio >2 and E’ < 8cm/s).

- **Anteroposterior diameter of left atrium and volume;** left atrial volume was determined in 2D using Simpson’s biplane technique (Figure 3-2) and indexed for height\(^2\).

- ** Inferior vena cava diameter;** inferior vena cava diameter was obtained at end of expiration in a sub-xiphoid location.

All the aforementioned parameters were obtained in real-time except left ventricular mass index, left ventricular and atrial volumes. Offline measurements were measured and calculated by the author (DC).
Figure 3-1. Biplane's Simpson's method of discs in deriving left ventricular volumes.

(a) Adequate apical 4 chamber view selected

(b) Left ventricular endocardial border was traced at the end of diastole.

(c) Cursor rolled to the left ventricle in the end systolic view in the same cardiac cycle

(d) Left ventricular endocardial border was traced. The left ventricular ejection fraction is calculated from the derived volumes, in this case 53%.
Figure 3-2. Using biplane's Simpson's method of discs, the volume of the left atrium was determined.

Note: The volume was taken as an average of the left ventricular volume taken in 2 chamber and 4 chamber view. (a) A 2 chamber view of the left atrium was obtained. (b) The left atrium was then split into discs by first manually tracing the endocardial border, then the QLAB 9.0 (Philips, Andover, Massachusetts, USA) software calculates the left atrial volume by splitting the area into discs.
3.12 Speckle Tracking Analysis

All obtained two-dimensional images were analysed offline in Xclera software, QLAB 9.0 (Philips, Andover, Massachusetts, USA). Echocardiographic images were anonymised before analysis. Using apical 4-chamber and 2-chamber views, the endocardial border was traced by the software with the positions of the mitral annulus and apex determined by the operator (the author). Tracking of the endocardial speckles from the endocardial border were performed by the software in end-diastole and end-systole. Imprecise tracking was manually modified by the interpreter to ensure accurate tracking of the left ventricular wall. A mean of all segmental strains of all 17 segments provided the global longitudinal strain value (Figure 3-3). If patients were in atrial fibrillation, the index beat selection method was used to obtain the global longitudinal strain (9). The representative beat chosen for analysis was where the preceding and the pre-preceding cardiac beat are of almost equal duration. Then the global longitudinal strain analysis was performed as described above.

Global longitudinal strain is expressed as the percentage change in left ventricular longitudinal dimension between end diastole and end systole, the less negative the value the more impaired the strain (10,11). The range for 'normal' global longitudinal strain in the non-renal population has been reported to vary between -15.9% and -22.1% (12). Published articles have utilised a cut off of -15% as pathological for patients with kidney disease (10), therefore this cut off as been used, for comparison, when appropriate.

Although speckle tracking was performed on all obtained 2D echocardiographic images, some left ventricular segments could not be tracked adequately by the software and therefore considered as inadequate images so were not analysed.
Figure 3-3. Speckle tracking echocardiography assessment in the determination of global longitudinal strain.

3.12.1. Tissue Mitral Valve Annular Displacement (TMAD)

Tissue mitral valve annular displacement (TMAD) is a novel method of assessment of left ventricular systolic function by determining the movement of the mitral valve during the cardiac cycle. This can be determined by the same QLAB software as used for speckle tracking analysis (described above). Using the TMAD function of QLAB, the interpreter determined three points in the diastolic frame, in the lateral and medial margin of the mitral annulus and ventricular apex, QLAB then tracks the mitral valve annular displacement in millimetres (medial, lateral and midpoint of the mitral valve annulus motion). An average of the midpoint displacement in 4-chamber and 2-chamber views was then calculated (Figure 3-4).
Figure 3-4. TMAD measurements.

Note: TMAD measurements were taken in (a) 4 chamber view and (b) 2 chamber view. Measurements of TMAD required placement of 3 points (the lateral borders of the mitral annulus and the apex). The excursion of the mitral valve is an estimate of left ventricular systolic function.

3.12.2. Three-dimensional Transthoracic Echocardiography

For acquisition of three-dimensional transthoracic image dataset, a Phillips iE33 (Philips, Andover, Massachusetts, USA) with a 1-3 MHz X3-1 matrix-array transducer was used. This was performed after obtaining two-dimensional echocardiographic images. The transducer acquired 4 real-time pyramidal wedge-shaped volumes (93-21°) during a single 5-7 seconds of breath hold in the apical view. This dataset was used to build up a full single larger pyramidal left ventricular volume (up to 90x90°). The acquisition of the left ventricular sub-volumes are triggered by the occurrence of every R wave on the electrocardiogram of every cardiac cycle. There was a total acquisition time of 4 heart beats. As it is triggered by electrocardiogram, it required a relatively stable R-R interval in order to avoid significant translation artefacts. An attempt to obtain three-dimensional echocardiography images on all patients however, there were some where this was not possible i.e. because of body habitus. If there were an inadequate number of segments (2 or more segments) visualised then these images could not be analysed. In accordance with the recommendation of the American Society of Echocardiography (5), the temporal resolution of the image set was to ≥ 20 frames/s when sector width was optimised. The full volume left ventricular images were stored digitally on the echocardiography machine and there is a link to the work station.
The images were analysed offline using QLAB. The image was optimised with magnification and gain settings. Analysis was based on the two-dimensional approach, three apical views were identified (4-, 3-, 2-chamber) with apex seen (non-foreshortened). The left ventricular endocardial border was manually traced during end-diastole (first frame of sequence) and end-systole (frame with the smallest left ventricular cavity and just ahead of the mitral valve closure). During the tracing of the endocardium, the papillary muscles and endocardial trabeculae were included in the left ventricular cavity. Five points were applied to trace the endocardial border- mitral annulus at the anterior, inferior, lateral and septal annuli and the left ventricular apex in the 2 chamber and 4 chamber view. The QLAB software automatically re-constructs the full left ventricular volume frame by frame throughout the cardiac cycle. This is from a detection procedure by a preset mathematic model by Philips QLAB. Manual alterations can be applied to adjust the left ventricular border, if necessary i.e. if the tracing is suboptimal. Once the three-dimensional image is created, the left ventricle can be visualised from any angle and it is divided into 17 coloured segments. Global and segmental volumes are generated. The following is automatically calculated: left ventricular end-diastolic volume, left ventricular end-systolic volume, left ventricular ejection fraction and volume-time curves for entire left ventricle and the left ventricle was divided into 17 segments (six basal, six middle and five apical segments), (Figure 3-5).

Left ventricular systolic mechanical dyssynchrony indices were automatically generated for 16 segments (six basal, six middle and four apical segments, excludes apical cap, segment 17). These indices included: standard deviation of time interval to the minimum systolic volume (Tmsv-SD) for 16 segments, 12 segments and 6 basal segments separately. The results were also presented as Tmsv 16-SD%, Tmsv 12-SD% and Tmsv 6-SD% which is the percentage after adjustment for heart rate (Figure 3-5).
Figure 3-5. An example of tracing of endocardial border of left ventricle using three-dimensional transthoracic echocardiography.

(a) There is automatic calculation of the left ventricular volumes and ejection fraction (illustrated on the right panel) once the operator defines five points manually - mitral annulus at the anterior, inferior, lateral and septal annuli and the left ventricular apex in the 2 chamber and 4 chamber view.

(b) A report generated with time interval to the minimum systolic volume ($T_{msv}$) of each segment adjusted to the cardiac cycle, expressed as a percentage. Bottom panel is a graphic representation of the time interval to minimum systolic volume.
In addition, there were automated measurements for excursion parameters (time to maximum, minimum, average and standard deviation of volume of each segment). This was represented as a parametric image displayed as a colour-coded bull eye map of regional contraction times (Tmsv) in each segment. The parts that are in green displays the timing reference which is defined by the global Tmsv (variable between patients). Segments in blue refer to early segments and red/yellow segments are late segments (Figure 3-6).

**Figure 3-6. Parametric imaging of the same patient as in the previous figures.**

![Parametric Imaging Diagram](image)

*Note: From the parametric diagram it can be seen that it is not homogenously green, there are some late segments in some of the basal segments.*

There is currently no universally agreed pathophysiological cut off for left ventricular mechanical dyssynchrony. However, in an extensive review of 12 published studies investigating left ventricular dyssynchrony (13), the majority of study groups agree that an average Tmsv-SD of 3% is found amongst healthy individuals. Therefore a cut-off of Tmsv-SD > 3% has been considered abnormal, where appropriate.
3.13 Intra-Observer and Inter-Observer Variability

The author, DC, performed all two-dimensional and three-dimensional echocardiographic off-line analyses. In 20 random patients, 3 months after initial measurements, DC repeated the analyses and used Bland Altman plots to determine the intra-observer variability for each offline analysis. Dr John Hughes, JH, another trained researcher, performed biplane's Simpson's method of discs for left ventricular volume, left atrial volume, global longitudinal strain, TMAD and three dimensional measurements of left ventricular volume and left ventricular mechanical dyssynchrony in a random selection of 20 patients. Using Bland Altman analysis, inter-observer variability was determined between DC and JH analysis of offline measurements.

3.14 Assessment of Pulse Wave Velocity

Pulse wave velocity, was performed using a Vicorder™ system (Skidmore Medical Limited, Bristol, United Kingdom) on the same day as echocardiography. The Vicorder™ system is a small, portable and operator independent device. It is a well validated method of determining aortic stiffness (14). In accordance with the manufacturer's specifications, the assessment was performed after 5 minutes of rest, not immediately after meal. The patient was positioned semi-prone (at about 35°) to prevent the waveform from the venous system interfering with the readings. A neck (positioned around patient's carotid area loosely) and femoral cuff (over the patient's upper right thigh) were applied to the patient over their clothing. The aortic path length was measured as the distance between the patient's supra-sternal notch and midpoint of thigh cuff, measured with a metre tape along the patient's side of the body. Both cuffs were automatically inflated to 65mmHg and the corresponding oscillometric signal from each cuff was digitally analysed to accurately record, in real time, the pulse time delay and the consequent aortic pulse wave velocity. The measurement was carried out twice and the results are averaged for each reading. This procedure took about 5 minutes to carry out (Figure 3-7).
Figure 3-7. Performing Vicorder\textsuperscript{TM} measurements to ascertain pulse wave velocity.

Note: Pulse wave analysis was performed with the patient semi-prone. A cuff was applied to the neck and thigh (a+c). The aortic path length was measured as the distance between the patient’s supra-sternal notch and midpoint of thigh cuff (b) This Vicorder\textsuperscript{TM} device uses the oscillometric signal from each inflated cuff to calculate the pulse wave velocity.

3.15 Dialysis Prescription and Blood Sampling

Blood samples were taken pre-dialysis with blood drawn immediately after insertion of access needles or permcath connection to dialysis lines. Mean serum values were taken over 3 months prior to cardiovascular assessments as part of departmental protocol monthly sample. Extra blood for biomarkers and genomics are taken once, annually, with routine bloods. Biochemical and haematological parameters analysed are detailed in Table 3-1.
Table 3-1. Biochemical and haematological tests performed

<table>
<thead>
<tr>
<th>Biochemical and haematological tests performed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean serum values from pre-dialysis samples over 3 months prior to cardiovascular assessments:</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>Potassium</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>Sodium</td>
<td>Chloride</td>
</tr>
<tr>
<td>Urea</td>
<td>Albumin</td>
</tr>
<tr>
<td>Corrected calcium</td>
<td>Phosphate</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Glucose</td>
</tr>
<tr>
<td>Serum and EDTA bloods for potential future biomarker / metabolomic or genomic studies $^5$</td>
<td></td>
</tr>
</tbody>
</table>

Note: 1 x 7.5ml EDTA tube sample and 1 x 7.5ml Gel serum tube sample were sent to the central haematology and biochemistry laboratories at Salford Royal Hospital for processing within 4 hours of collection. Routine haematology and biochemistry parameters were measured using a multi-channel autoanalyser.

$^5$ 1 x 7.5ml EDTA tube sample were frozen at -80°C within 1 hour of collection. 1 x 7.5ml Gel serum tube sample, 1 x 7.5ml Li-Heparin sample, 1x 2.9ml citrate sample; these underwent centrifugation at 14000g. Serum from the gel serum tube and plasma from the Li-Heparin and coagulation tubes were aliquoted into 2ml cryovials and frozen at -80°C within 1 hour of collection.

All blood tests are run on the Cobas 8000 analyser (Roche). Immunoassays were performed by the laboratories at Salford Royal Hospital and are detailed in Table 3-2.

Table 3-2. Blood assays performed.

<table>
<thead>
<tr>
<th>Blood Test</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein</td>
<td>Latex enhanced immunoturbidimetry.</td>
</tr>
<tr>
<td>Albumin</td>
<td>Colorimetric/dye binding (bromocresol green)</td>
</tr>
<tr>
<td>Calcium</td>
<td>Colorimetric using NM-BAPTA complexation</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Colorimetric using molybdate complexation</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>Electrochemiluminescent immunoassay using antibodies towards intact parathyroid hormone</td>
</tr>
</tbody>
</table>
3.16 Electrocardiographic Analysis

A standard 12 lead electrocardiogram was taken immediately after the echocardiography assessments. A hard copy was stored in the site files and electronic versions (PDF and HTML files) were downloaded to a secure, password protected and encrypted external hard drive. These results were not directly applied to this thesis project and will be utilised for future studies.

3.17 Statistical Analysis

In the following results chapters, depending on the analysis there will be a detailed description of the statistical analysis used for that particular analysis. However, in general, baseline characteristics are expressed with categorical data as n (%) and continuous data as mean ± standard deviation, SD, or by median with 25th and 75th percentiles, depending on the distribution of data. Normality was assessed using the Shapiro-Wilk test. Comparisons between two groups were made using Mann-Whitney U test for continuous variables and Pearson chi-squared test or Fisher's exact test for categorical variables. Analysis of variance were used for differences between three or more groups. For continuous variables, linear regression and binary variables, multiple logistic regression, were used to evaluate association with echocardiogram and pulse wave velocity parameters with outcome measures. Factors with P<0.05 on univariate analysis and any factors decided a priori to affect primary outcome according to existing literature or clinical acumen were included in multivariate models. Kaplan-Meier survival plots and Cox proportional hazards modelling were used to evaluate baseline associations with mortality, cardiac deaths and cardiac events. Censoring occurred at time of death, renal transplantation, moving out of the country or date of last follow-up (16th November 2015). The level of statistical significance was set at P<0.05. The SPSS software version 22.0 (SPSS, inc., Chicago, Illinois, USA) for Windows was used for all statistical analysis.

3.18 Strengths and Limitations of the Study

This was a prospective, observational study designed to identify whether novel echocardiographic parameters were associated with all-cause mortality, cardiac death and cardiac events in maintenance haemodialysis patients. The factors investigated include many new techniques that had never been explored in this patient population such as
TMAD and three dimensional echocardiography determined left ventricular mechanical dyssynchrony and other parameters where there is currently limited data such as global longitudinal strain.

The strengths of this study include a long follow-up time with inclusion of currently the largest group of patients with these cardiovascular assessments performed. This provides strength for the validity of the results. Furthermore, all cardiac investigations were performed on a non-dialysis day. Previously, many published studies have performed echocardiography on a dialysis day. This may lead to inaccuracies in some measurements, for example, it has been shown that left ventricular mass index and end diastolic diameter may be reduced by 26.2-36.1g/m$^2$ and 4-8.4mm respectively before compared with after dialysis (15,16). Moreover, some analyses such as global longitudinal strain may be load dependent. Therefore performing cardiac investigations on a non-dialysis day provides a more accurate reading.

This study has some weaknesses. First, it is an observational study design and therefore no direct causal links can be made between the measured parameters and the end points. Second, due to logistic reasons (because we could not provide transport), patients who required ambulance transport were not eligible to be consented for this study. This will bias the results (see Chapter 4). Finally, the final recruitment figure was not adequate to provide sufficient power to explore sudden cardiac death as an end point as originally intended by the study team. However, the study is well powered to explore all-cause mortality and cardiac events.

This study was designed to answer the hypotheses set out in chapter 2. It is hoped that the data generated will provide insight into the most useful novel echocardiographic assessment techniques for patients undergoing haemodialysis. This may enable a targeted approach to treatment such as earlier cardiology referral and reduction in deaths amongst this high risk population.
3.19 References


Chapter 4

Non-Recruitment To and Selection Bias in Studies using Echocardiography in Haemodialysis Patients

Diana YY Chiu, Darren Green, Nik Abidin, John Hughes, Smeeta Sinha, Philip A Kalra

4.1 Preface

This chapter compares the baseline characteristics and survival differences between patients who were recruited into the Salford Kidney Study and those who did not participate (those who declined consent or who met the exclusion criterion). The aim of this chapter was to determine if the study population was reflective of the wider haemodialysis population. This is important because it would affect the interpretation of results in subsequent chapters in respect of how generalizable the findings would be to the 'general' haemodialysis population. The evidence in other clinical research fields such as cancer research is that that patients who participate in trials proclaim a better survival. However, the same investigations in renal studies have never been explored. Furthermore, many published literature have involved interventional trials and clearly the relevance in prospective observational studies would be different.

4.2 Abstract

Background: It is unknown whether patients recruited to renal cardiac imaging studies are fully representative of the investigated population and whether there are differences in characteristics and survival between participants and non-participants (excluded or declined consent). Any differences may bias results.

Methods: 435 patients were screened for participation in an observational, prospective echocardiographic study of maintenance haemodialysis patients. Baseline demographics, laboratory results and social deprivation scores was obtained from electronic patient record. All patients were followed-up until death, renal transplantation or 16th November 2015. Analysis of variance was utilised to assess the baseline differences between groups
of patients who were recruited, excluded or declined consent. Cox regression analysis adjusted for factors significant on univariate analysis (P < 0.05) was utilised to explore the relationship to survival in the recruited, declined consent and excluded groups.

**Results:** 44 patients were excluded (reasons were 16 due to language barrier, 10 mental incapacity, 9 due to severe co-morbid illness and 9 because of immobility), 172 patients declined consent (84% because of a reluctance to attend for an extra visit) and 219 patients were recruited. There were more males (67% vs 64% vs 52%) and smokers (66% vs 41% vs 43%) in recruited patients compared with those who refused consent or were excluded, respectively. Excluded patients had a lower mean haemoglobin (10.2g/dL vs 10.7g/dL), phosphate (1.34mmol/L vs 1.53mmol/L), albumin (36 g/L vs 38g/L) and higher C-reactive protein (3.1mg/dL vs 1.7mg/dL) compared with recruited patients. No difference was identified between groups for Charleston co-morbidity index (P = 0.115) or social deprivation scores. After a median follow-up of 29.7 (25th-75th centile, 21.1-34.3) months, there were 141 deaths. In a multivariable Cox regression model adjusting for body mass index, age, Charleston co-morbidity index, haemoglobin, albumin, smoking status and diabetes mellitus, patients who declined consent had an adjusted hazard ratio, HR of 1.70, 95% CI 1.10-2.62, P = 0.016 and excluded patients had an adjusted HR of 1.36, 95% CI 0.77-2.41, P = 0.287 for all-cause mortality compared with recruited patients. Patients declining consent lived further away from the base hospital. In a sub-group analysis of the excluded group, survival was significantly better in patients excluded because of language barriers (P = 0.02) rather than due to co-morbidity, immobility or mental incapacity.

**Conclusions:** Patients recruited to the study had longer survival compared to non-participants. Research studies should document phenotypes of non-participants to aid interpretation and generalizability of results.

### 4.3 Introduction

Echocardiography is the most readily available cardiac imaging modality used to detect the structural and functional abnormalities of uraemic cardiomyopathy, a major predictor of cardiovascular mortality in haemodialysis patients, even though its use in patients undergoing haemodialysis does present challenges that are also seen in research studies(1). Standard experimental practice is that echocardiography is performed on a non-dialysis day because of the potential for pre- or post-dialysis imaging to be significantly affected by volume status and dialysis associated ischaemia. The typical requirement of 3 hospital or
satellite clinic haemodialysis visits per week means that some patients are reluctant to participate in studies that require an extra visit for echocardiography and inevitably some do not attend agreed study imaging appointments. As well as the burden of additional hospital visits, logistical reasons, such as requiring ambulance transportation, prevents others from joining studies; exclusion criteria also prevent certain patients from being recruited. Together, these barriers to recruitment and participation in studies of echocardiography in haemodialysis patients have the potential to introduce selection bias when reporting the prevalence of cardiac abnormalities and associated outcomes.

This study aimed to determine whether participants in an observational investigation of two-dimensional (2D) echocardiography were representative of the entire haemodialysis population in terms of phenotype and outcome, by comparing them with non-participating patients (those who declined consent or who were excluded).

The study involved the following:

1. description of the rates of non-participation (declined consent and excluded patients) for a study involving 2D-echocardiography in haemodialysis patients and the different reasons for non-participation.

2. comparison of baseline patient characteristics and survival outcomes between patients who participate versus non-participants.

4.4 Methods

4.4.1. Patient Population

This was a prospective, observational study named the Salford Kidney Study. It involved annual, standardised, non-contrast 2D transthoracic echocardiography, Vicorder™ measurement of aortic stiffness and 12-lead electrocardiogram of consenting maintenance haemodialysis patients, at an extra study visit on a non-dialysis day, to investigate factors associated with mortality and cardiovascular events. Between March 2012 and 2014, all patients receiving maintenance haemodialysis at Salford Royal NHS Foundation Trust or any of its four satellite units were approached to enter the study. The inclusion criteria were wide and simple: maintenance haemodialysis patients aged ≥18 years, able to attend for the study on an inter-dialytic day and with capacity to consent. The exclusion criteria were incapacity to consent and patients physically unable to attend because of illness or
poor mobility that precluded non-ambulance transport. Standard transportation by taxi was provided for all consenting patients to attend for assessment, unless they preferred to use their own transport. Written and verbal informed consent were obtained from all enrolled patients. If patients declined consent, they were verbally asked if they were willing to give a reason for declining. Patients were free to refuse to comment. The study adhered to the Declaration of Helsinki; local ethics committee approval allowed for recording of minimal anonymised demographic data and the reasons for non-inclusion in all screened potential participants to allow characterization of non-participating patients (REC 05/Q1404/188).

4.4.2. Data Collection

Baseline demographics, co-morbidities, and 3 month averaged dialysis and laboratory records were obtained from review of electronic patient medical records of all patients screened. From the co-morbidity data, a Charleston co-morbidity index was determined – an index which is strongly predictive of mortality in dialysis patients (2). As standard, all patients received 3-4 hours haemodialysis, three times a week. As routine, all patients had monthly bloods for haematological and biochemical parameters taken from the haemodialysis circuit immediately before the midweek haemodialysis session. The mean values were calculated for each parameter in all patients during the 3 months prior to the date of approach. To give an estimation of the impact of social deprivation, illness burden and education levels at recruitment, deprivation scores from the United Kingdom 2010 census mapped to patient's residential post codes were used (3). For each score, the higher the number, the more deprived the residential area. The overall index had been weighted according to the average scores as predetermined by the Department of Communities and Local Government (3). In addition, the direct distance from the patient's residence to the base hospital was calculated to explore the impact of distance on participation.

All patients screened were followed-up for the same length of time regardless of whether they chose to participate in the study (until death, renal transplantation or end of follow-up, 16 November 2015). The start date for follow-up was the date patients consented to the study, refused consent or for excluded patients, the 13 March 2012 (this was the first date of screening). All-cause mortality during follow-up (March 2012 to November 2015) was obtained from review of hospital electronic medical records. All patients (participants and non-participants) underwent the aforementioned data collection and patients recruited to
the study underwent an annual echocardiography assessment at extra visits at Salford Royal Hospital.

4.4.3. Statistical Analysis

Clinical and laboratory characteristics were compared between groups of patients who were recruited, declined consent or excluded from the study using the analysis of variance. Test of normality for the distribution of variables used the Shapiro-Wilk test. Any continuous non-normally distributed data is shown as median and 25th-75th centile. Normally distributed data is presented as mean (standard deviation, SD). Categorical data is presented as numbers (percentages).

Survival analyses for all-cause mortality used Cox-regression proportional hazard models and cumulative survival plots. All factors significant on univariate analysis (P < 0.05) and factors decided a priori to be prognostic of death (history of diabetes mellitus and former or current smoker) were included in the multivariable Cox regression models. All statistical analysis was performed using SPSS v.22.0 (SPSS, Inc, Chicago, Illinois) and a two-sided P value <0.05 was considered significant.

4.5 Results

4.5.1. Patient Characteristics

During the recruitment time period, there were 435 patients who received maintenance haemodialysis at Salford Royal Hospital Foundation Trust and its satellite clinics that were considered for the study. There were 44 patients excluded from the study (16 (36%) patients could not understand English, 10 (23%) patients lacked mental capacity to consent, 9 (20%) were deemed too unwell and 9 (20%) were immobile). One hundred and seventy two (39.5%) patients declined consent to the study. The reasons given for declining consent included not wanting to attend for an extra hospital visit (145, 84.3%), not interested in participating in research (7, 4.1%), patient feeling too unwell (5, 2.9%), patient due to change dialysis modality (2, 1.2%) or patient too busy (8, 4.7%); only 5 (2.9%) patients declined to give a reason. There were 219 (50.3%) patients who agreed to participate in the study and who subsequently underwent echocardiographic assessment at
an extra study visit. Figure 4-1 shows the recruitment criteria and the reasons for non-participation.

Figure 4-1. Flow diagram illustrating the numbers of patients who were screened, excluded, declined consent and recruited; with associated reasons.

Baseline phenotypic differences between the groups is illustrated in Table 4-1. Patients who were recruited represented a typical United Kingdom haemodialysis cohort; the
majority were Caucasian (81%) and male (67%) with a median age of 64.1 (53.0-72.7) years. Compared with those patients who declined consent or whom were excluded, study participants were more likely to be male (recruited 67.1% vs refused consent 64% vs excluded 52.3%, P = 0.05) and smokers (65.8% vs 41.3% vs 43%, P < 0.001). There were no significant differences between groups in marital status (P > 0.05) or in Charleston co-morbidity index (P = 0.115).

Table 4-1. Baseline clinical demographics, co-morbidities and laboratory results of patients who were recruited, refused consent and excluded from the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients Recruited</th>
<th>Declined Consent</th>
<th>Excluded from Study</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.1 (53.0-72.7)</td>
<td>67.5 (55.3-77.0)</td>
<td>67.0 (55.3-77.8)</td>
<td>0.152</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>147 (67)</td>
<td>110 (64)</td>
<td>23 (52)</td>
<td>0.050</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>177 (81)</td>
<td>127 (74)</td>
<td>34 (77)</td>
<td>0.258</td>
</tr>
<tr>
<td>Ethnicity (% Asian)</td>
<td>35 (16)</td>
<td>38 (22)</td>
<td>9 (21)</td>
<td>0.427</td>
</tr>
<tr>
<td>Ethnicity (% Afro-Caribbean)</td>
<td>7 (3)</td>
<td>7 (4)</td>
<td>1 (2)</td>
<td>0.565</td>
</tr>
<tr>
<td>Married (%)</td>
<td>126 (58)</td>
<td>94 (55)</td>
<td>22 (50)</td>
<td>0.669</td>
</tr>
<tr>
<td>Divorced (%)</td>
<td>24 (11)</td>
<td>16 (9)</td>
<td>5 (11)</td>
<td>0.845</td>
</tr>
<tr>
<td>Widowed (%)</td>
<td>33 (15)</td>
<td>27 (16)</td>
<td>9 (21)</td>
<td>0.501</td>
</tr>
<tr>
<td>Single (%)</td>
<td>36 (16)</td>
<td>35 (20)</td>
<td>8 (18)</td>
<td>0.951</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>144 (66)</td>
<td>71 (41)</td>
<td>19 (43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol consumption (%)</td>
<td>75 (34)</td>
<td>130 (76)</td>
<td>39 (89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9 (6.1)</td>
<td>25.8 (6.0)</td>
<td>27.9 (8.4)</td>
<td>0.005</td>
</tr>
<tr>
<td>Haemodialysis vintage (months)</td>
<td>29.2 (41.0)</td>
<td>38.8 (38.7)</td>
<td>36.5 (43.3)</td>
<td>0.070</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>87 (40)</td>
<td>51 (30)</td>
<td>22 (50)</td>
<td>0.019</td>
</tr>
<tr>
<td>Myocardial infarction (%)</td>
<td>25 (11)</td>
<td>25 (15)</td>
<td>7 (16)</td>
<td>0.552</td>
</tr>
<tr>
<td>Congestive cardiac failure (%)</td>
<td>36 (16)</td>
<td>24 (14)</td>
<td>7 (16)</td>
<td>0.793</td>
</tr>
<tr>
<td>Peripheral vascular disease (%)</td>
<td>17 (8)</td>
<td>16 (3)</td>
<td>4 (9)</td>
<td>0.855</td>
</tr>
<tr>
<td>Cerebrovascular disease/ TIA (%)</td>
<td>23 (11)</td>
<td>13 (8)</td>
<td>10 (23)</td>
<td>0.014</td>
</tr>
<tr>
<td>Cardiovascular disease (%)</td>
<td>77 (35)</td>
<td>53 (31)</td>
<td>19 (43)</td>
<td>0.281</td>
</tr>
<tr>
<td>Dementia (%)</td>
<td>0 (0)</td>
<td>2 (1.2)</td>
<td>1 (2.3)</td>
<td>0.159</td>
</tr>
<tr>
<td>COPD (%)</td>
<td>24 (11)</td>
<td>20 (12)</td>
<td>6 (14)</td>
<td>0.877</td>
</tr>
<tr>
<td>History of malignancy (%)</td>
<td>23 (11)</td>
<td>9 (5)</td>
<td>7 (16)</td>
<td>0.046</td>
</tr>
<tr>
<td>Charleston co-morbidity index</td>
<td>6 (4-8)</td>
<td>6 (4-7)</td>
<td>7 (5-8)</td>
<td>0.115</td>
</tr>
</tbody>
</table>

Abbreviations: COPD, chronic obstructive pulmonary disease; TIA, transient ischaemic attack; BMI, body mass index.
Note: Cardiovascular disease includes myocardial infarction, congestive cardiac failure, peripheral vascular disease and cerebrovascular disease.
There was no difference between the groups for deprivation scores considering multiple deprivation, education and income related to postcode (Table 4-2). However, patients who lived further away from the hospital were more likely to decline consent (mean distances of residence from hospital were 18.7 km, 19.6 km and 13.5 km; P = 0.001, for recruited, declined consent and excluded patients, respectively).

Table 4-2. Residential postcode related deprivation scores and distance from Salford Royal Hospital in patients recruited, declined consent and excluded patients.

<table>
<thead>
<tr>
<th></th>
<th>Patients Recruited N=219</th>
<th>Declined Consent N=172</th>
<th>Excluded from the Study N=44</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple deprivation score</td>
<td>32.79 (17.85)</td>
<td>32.83 (19.13)</td>
<td>38.40 (15.89)</td>
<td>0.155</td>
</tr>
<tr>
<td>Income score</td>
<td>0.22 (0.12)</td>
<td>0.22 (0.12)</td>
<td>0.26 (0.11)</td>
<td>0.075</td>
</tr>
<tr>
<td>Employment score</td>
<td>0.18 (0.09)</td>
<td>0.19 (0.09)</td>
<td>0.21 (0.08)</td>
<td>0.172</td>
</tr>
<tr>
<td>Educational skills score</td>
<td>31.11 (20.34)</td>
<td>31.52 (20.92)</td>
<td>37.51 (18.18)</td>
<td>0.157</td>
</tr>
<tr>
<td>Health deprivation and disability score</td>
<td>0.91 (0.70)</td>
<td>0.88 (0.73)</td>
<td>1.13 (0.61)</td>
<td>0.101</td>
</tr>
<tr>
<td>Distance from Salford Royal Hospital (Km)</td>
<td>18.7 (10.55)</td>
<td>19.6 (8.78)</td>
<td>13.5 (8.07)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: Data presented as mean (standard deviation). From the 2010 United Kingdom national census, the English Indices of Deprivation uses different indicators that are split across different domains. The higher the deprivation score, the more deprived the area.

When 3 month averaged blood results were compared at time of consent, patients who were excluded from the study had lower mean haemoglobin (10.2 g/dL), phosphate (1.34mmol/L) and albumin (36 g/L) and higher C-reactive protein (3.2mg/dL) than the other two groups (Table 4-3). Patients who declined consent to the study had a higher pre-dialysis systolic blood pressure and although statistical significance was not detected, the mean ultra-filtration volumes was higher in the excluded and declined consent groups (mean ultra-filtration volume was 1.7 L vs 2.3 L vs 2.2 L, P = 0.520 in the recruited, declined consent and excluded group, respectively).
Table 4-3. Mean blood and dialysis prescription results 3 months prior to consent.

<table>
<thead>
<tr>
<th></th>
<th>Patients Recruited N=219</th>
<th>Declined Consent N=172</th>
<th>Excluded from Study N=44</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.7 (1.3)</td>
<td>10.4 (1.3)</td>
<td>10.2 (1.2)</td>
<td>0.015</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.38 (0.14)</td>
<td>2.40 (0.13)</td>
<td>2.36 (0.13)</td>
<td>0.290</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.53 (0.45)</td>
<td>1.50 (0.47)</td>
<td>1.34 (0.28)</td>
<td>0.039</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>38 (4)</td>
<td>37 (5)</td>
<td>36 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>1.7 (2.2)</td>
<td>2.4 (2.5)</td>
<td>3.1 (3.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ultra-filtration Volume (L)</td>
<td>1.70 (6.88)</td>
<td>2.27 (1.03)</td>
<td>2.23 (0.99)</td>
<td>0.520</td>
</tr>
<tr>
<td>Pre-dialysis systolic blood pressure (mmHg)</td>
<td>142 (26)</td>
<td>145 (22)</td>
<td>142 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-dialysis diastolic blood pressure (mmHg)</td>
<td>76 (17)</td>
<td>81 (25)</td>
<td>75 (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Death</td>
<td>48 (22)</td>
<td>70 (41)</td>
<td>23 (52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Deaths/100 patient years</td>
<td>9.3</td>
<td>19.5</td>
<td>19.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Follow-up time (months)</td>
<td>28.3 (8.9)</td>
<td>25.1 (11.5)</td>
<td>31.5 (13.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: Continuous data presented as mean (standard deviation).

Abbreviation: CRP, C-reactive protein.

In a sub-group analysis of just patients who were excluded (N=44), there was no difference between the baseline characteristics of patients for different exclusion reasons except that patients who were immobile had a lower haemoglobin compared with other groups (mean haemoglobin was 9.2 vs 10.9 vs 10.1 vs 10.6 g/dL, P=0.011 for excluded patients due to immobility, deemed too unwell, language barrier and mental incapacity) (Table 4-4).
Table 4-4. Baseline characteristics of patients who were excluded and reasons for exclusion.

<table>
<thead>
<tr>
<th></th>
<th>Immobility (N=9)</th>
<th>Patients Deemed Unfit (N=9)</th>
<th>Language Barrier (N=16)</th>
<th>Mental Incapacity (N=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.0 (55.3-77.8)</td>
<td>74.0 (49.5-79.5)</td>
<td>63.0 (56.0-73.5)</td>
<td>57.5 (53.5-78.0)</td>
<td>0.295</td>
</tr>
<tr>
<td>Male (%)</td>
<td>5 (56)</td>
<td>6 (67)</td>
<td>8 (50)</td>
<td>4 (40)</td>
<td>0.700</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>5 (56)</td>
<td>3 (33)</td>
<td>9 (56)</td>
<td>2 (20)</td>
<td>0.239</td>
</tr>
<tr>
<td>Myocardial Infarction(%)</td>
<td>1 (11)</td>
<td>2 (22)</td>
<td>1 (6)</td>
<td>3 (30)</td>
<td>0.388</td>
</tr>
<tr>
<td>Congestive Cardiac Failure (%)</td>
<td>2 (22)</td>
<td>2 (22)</td>
<td>1 (6)</td>
<td>2 (20)</td>
<td>0.620</td>
</tr>
<tr>
<td>Diabetes Mellitus (%)</td>
<td>1 (11)</td>
<td>1 (11)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0.738</td>
</tr>
<tr>
<td>Charleston Co-morbidity Score</td>
<td>8 (7-8)</td>
<td>7 (5-9)</td>
<td>5 (4-7)</td>
<td>7 (4-8)</td>
<td>0.217</td>
</tr>
<tr>
<td>Cardiovascular Disease (%)</td>
<td>6 (67)</td>
<td>5 (56)</td>
<td>3 (19)</td>
<td>5 (50)</td>
<td>0.083</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>67 (12)</td>
<td>75 (56)</td>
<td>77 (11)</td>
<td>80 (11)</td>
<td>0.061</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>138 (29)</td>
<td>156 (15)</td>
<td>144 (15)</td>
<td>143 (25)</td>
<td>0.377</td>
</tr>
<tr>
<td>Multiple Deprivation Score</td>
<td>29.0 (13.4)</td>
<td>40.2 (17.05)</td>
<td>37.6 (13.7)</td>
<td>46.5 (17.5)</td>
<td>0.111</td>
</tr>
<tr>
<td>Distance from Hospital (km)</td>
<td>10.2 (6.7)</td>
<td>14.5 (8.7)</td>
<td>14.2 (8.3)</td>
<td>14.6 (8.7)</td>
<td>0.591</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>33 (7)</td>
<td>35 (3)</td>
<td>38 (5)</td>
<td>35 (7)</td>
<td>0.277</td>
</tr>
<tr>
<td>Corrected Calcium (mmol/L)</td>
<td>2.29 (0.10)</td>
<td>2.33 (0.18)</td>
<td>2.39 (0.12)</td>
<td>2.41 (0.12)</td>
<td>0.217</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>4.2 (3.3)</td>
<td>4.4 (3.1)</td>
<td>2.5 (3.3)</td>
<td>2.0 (2.6)</td>
<td>0.343</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>9.2 (1.1)</td>
<td>10.9 (1.3)</td>
<td>10.1 (1.0)</td>
<td>10.6 (1.2)</td>
<td>0.011</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.26 (0.17)</td>
<td>1.44 (0.31)</td>
<td>1.28 (0.23)</td>
<td>1.42 (0.38)</td>
<td>0.334</td>
</tr>
<tr>
<td>Ultrafiltration volume (L)</td>
<td>2.41 (1.20)</td>
<td>2.52 (0.94)</td>
<td>2.14 (0.95)</td>
<td>1.97 (1.00)</td>
<td>0.654</td>
</tr>
<tr>
<td>Deaths (%)</td>
<td>8 (89)</td>
<td>7 (78)</td>
<td>5 (31)</td>
<td>5 (50)</td>
<td>0.020</td>
</tr>
<tr>
<td>Deaths/100 patient years</td>
<td>48.2</td>
<td>30.8</td>
<td>9.6</td>
<td>23.8</td>
<td>0.020</td>
</tr>
<tr>
<td>Mean follow-up time (months)</td>
<td>22.1 (13.8)</td>
<td>30.3 (9.7)</td>
<td>39.2 (9.5)</td>
<td>25.2 (15.3)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Note: Continuous data is presented as mean (standard deviation) for normally distributed variables and median (25th-75th centile) for non-normally distributed variables; binary variables presented as n (%). Cardiovascular disease includes myocardial infarction, congestive cardiac failure, peripheral vascular disease and cerebrovascular disease.
4.5.2. Follow-up

After a median follow-up of 29.7 (21.1-34.3) months there were 9.3 deaths/100 patient-years in the recruited group, 19.5 deaths/100 patient-years in patients who refused consent and 19.9 deaths/100 patient-years in the excluded group (P<0.001). There were 69 (15.9%) patients who received a renal transplant during this period, 44 (20%) of the recruited patients, 22 (13%) of the declined consent group and 3 (7%) of the excluded patients.

On univariate Cox regression analysis patients who declined to enter the study (Hazard ratio, HR 2.03, 95% confidence interval, 95% CI, 1.42-2.90, P < 0.001) and patients who were excluded from the study (HR 1.97, 95% CI 1.21-3.20, P = 0.007) had an increased risk for all-cause mortality compared to study participants. In a multivariable Cox regression model adjusting for body mass index, age, Charleston co-morbidity index, haemoglobin, albumin, smoker and history of diabetes mellitus (univariate analysis shown in (Table 4-5), patients who were in the declined consent group had the worst survival compared to excluded and recruited patients. Compared with the recruited patients, those declining consent had an adjusted HR of 1.70, 95% CI 1.10-2.62, P = 0.016 and excluded patients had an adjusted HR of 1.36, 95% CI 0.77-2.41, P = 0.287 for all-cause mortality. The final Cox regression model is shown in Table 4-6.

Table 4-5. Univariate analysis for some tested variables for all-cause mortality in the whole group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.90</td>
<td>0.64-1.26</td>
<td>0.545</td>
</tr>
<tr>
<td>Smoker</td>
<td>1.26</td>
<td>0.91-1.75</td>
<td>0.164</td>
</tr>
<tr>
<td>Body mass index</td>
<td><strong>0.95</strong></td>
<td><strong>0.93-0.98</strong></td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Age</td>
<td><strong>1.06</strong></td>
<td><strong>1.04-1.07</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.92</td>
<td>0.63-1.35</td>
<td>0.679</td>
</tr>
<tr>
<td>Charleston co-morbidity index</td>
<td><strong>1.21</strong></td>
<td><strong>1.14-1.30</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance from hospital from home</td>
<td>0.99</td>
<td>0.97-1.00</td>
<td>0.298</td>
</tr>
<tr>
<td>Index of multiple deprivation score</td>
<td>0.99</td>
<td>0.98-1.00</td>
<td>0.105</td>
</tr>
<tr>
<td>Health deprivation and disability score</td>
<td>0.87</td>
<td>0.69-1.09</td>
<td>0.218</td>
</tr>
<tr>
<td>Albumin</td>
<td><strong>0.92</strong></td>
<td><strong>0.89-0.95</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Corrected calcium</td>
<td>1.95</td>
<td>0.56-6.79</td>
<td>0.292</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td><strong>0.98</strong></td>
<td><strong>0.97-1.00</strong></td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.79</td>
<td>0.53-1.17</td>
<td>0.239</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.00</td>
<td>0.99-1.00</td>
<td>0.938</td>
</tr>
<tr>
<td>Ultra-filtration volume</td>
<td>1.01</td>
<td>0.97-1.05</td>
<td>0.661</td>
</tr>
</tbody>
</table>

*Note: Parameters in bold were statistically significant and were included in the multivariable Cox regression model.*
Table 4-6. Final Cox regression model for all-cause mortality.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.04</td>
<td>1.02-1.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Charleston Co-Morbidity Index</td>
<td>1.10</td>
<td>1.00-1.21</td>
<td>0.037</td>
</tr>
<tr>
<td>Serum Albumin</td>
<td>0.94</td>
<td>0.91-0.98</td>
<td>0.001</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>0.97</td>
<td>0.94-1.00</td>
<td>0.051</td>
</tr>
<tr>
<td>Declined Group*</td>
<td>1.70</td>
<td>1.10-2.62</td>
<td>0.016</td>
</tr>
<tr>
<td>Excluded Group*</td>
<td>1.36</td>
<td>0.77-2.41</td>
<td>0.287</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>0.99</td>
<td>0.97-1.00</td>
<td>0.130</td>
</tr>
<tr>
<td>Smoker</td>
<td>1.42</td>
<td>0.98-2.05</td>
<td>0.062</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.11</td>
<td>0.70-1.77</td>
<td>0.650</td>
</tr>
</tbody>
</table>

Note: Groups (recruited, declined and excluded patients) were adjusted for all factors that were significant on univariate analysis for all-cause mortality (body mass index, age, Charleston co-morbidity index, haemoglobin, albumin) and factors decided a priori to be important (smoker and history of diabetes mellitus).

*Hazard ratio is in comparison with recruited group (reference group) with all-cause mortality.

Figure 4-2 illustrates the adjusted cumulative survival plot for the three groups. The risk of death varied according to the different reasons for exclusion from the study (Table 4-4).

For example, the death rate per 100 patient years was 48.2 in patients who were immobile, 30.8 in those too unwell for study inclusion, 23.8 for patients who had mental incapacity compared with 9.6 deaths excluded because of a language barrier (P = 0.020).

Figure 4-2. Adjusted cumulative survival plot for the groups (recruited, declined consent and excluded patients).

Note: Patients who were recruited had better survival compared with patients who were excluded or declined consent into the study.
4.6 Discussion

These data obtained from a typical observational 2D-echocardiographic study of haemodialysis patients have important implications when interpreting such cardiac imaging studies in this population. Firstly, insights are provided into possible reasons for study non-participation. One major barrier for patients (84.3% of those who declined consent) was the need to attend for the echocardiogram as an extra visit on a non-dialysis day. A factor that may have contributed to their decision was that these patients lived further away from the base hospital, where the echocardiograms were performed. Inconvenience to attend for an extra visit may have several explanations, including a need to arrange for childcare and need to attend work. Our study did not have barriers such as lack of transportation (which was provided free, if required, to non-stretcher patients) or any health risk from participation. However, a patient's perceived risk of participation or the benefits of enrolling were not explored; these may well differ from actual circumstances. In some randomised controlled trials a perceived risk of participating in research has appeared to be a barrier to recruitment, especially in women (4,5). It is important for investigators to ensure that patients fully understand the implications of participation and that patients have an accurate perception of the study. Further qualitative research is needed to explore the psychological reasons for non-participation.

Secondly, we identified some phenotypical differences between those who were recruited and those who declined consent or who were excluded, and this introduces a bias which must be taken into account during interpretation of results, especially if these are to be generalised to the entire population. In the recruited group there was an under-representation of women (P = 0.05) and ethnic minority patients were slightly less represented (percentage of Caucasians were 81%, 74% and 77% in recruited, declined consent and excluded groups). This finding is consistent with other reports of difficulties in...
recruiting women and ethnic minority groups to clinical trials (6–8). Overall, women account for only 38% of participants in reported cardiovascular trials (7), which may be related to both the added costs of child care and perceived risk of research studies. For ethnic minorities, reasons for non-participation may include language barriers and cultural non-acceptance of medical research. In our study, the major reason for exclusion from the study was the language barrier (16/44) that precluded valid consent. Strategies to consider in future studies to reduce bias against these groups include use of interpreters, providing patient information leaflets in different languages and extra funding for child care expenses.

One magnetic resonance imaging study found that participants were more highly educated compared with non-participants (9). When we compared deprivation scores, including educational skills score, we did not identify any difference between participants and non-participants. Although deprivation scores have been utilised in some similar studies (10), it may be that postcode assignment is not sensitive enough to represent the actual patient characteristic. Individualised social background information may be necessary in future investigations.

Of high significance was the fact that survival was not only worse in patients who were excluded, but also in those who declined consent to the study, compared to recruited patients even after adjusting for factors such as co-morbidity, laboratory and clinical data. The mortality difference was non-significant in the excluded group, compared to recruited patients but this may have been influenced by group size (44 patients). It would have been hypothesised that patients who were too unwell or immobile would have higher Charleston co-morbidity score and would be 'sicker' compared with patients excluded because of language barriers. Table 4-4 does show that co-morbidity characteristics were different,
although non-significant because of sample size, and mortality was indeed significantly lower in patients excluded because of language barriers. This finding is of interest and needs to be validated in a larger cohort.

This is the first study of a haemodialysis population to show a reduced survival amongst patients who either could not or who declined to participate. Some studies in cancer patients and a few in cardiovascular research have shown that trial participation is associated with improved survival compared with non-participants (10–12). In an analysis of 21 studies in cancer patients with poor prognosis, survival was reduced in non-participants compared with participants (HR=0.74, 95% CI=0.84, P<0.001) (13). The authors concluded that this may be related to the exclusion of patients with higher co-morbidity who were not approached for the studies due to study criteria. In contrast we demonstrated that there was no difference in Charleston co-morbidity index between groups and that patients who declined consent still had worse survival. Other factors that might improve survival in trial participants include receiving a beneficial drug, trial clinicians providing better care or trial patients receiving more intensive supervision leading to better outcomes (11). However, our study was observational and non-interventional and yet survival was still poorer for patients declining consent. We hypothesise that reasons more specific to a renal population help explain this finding. For example, depression is the most common psychiatric illness in end stage kidney disease patients (14,15), and is a factor that may influence patients' choice to participate in a study: depression has been associated with poorer survival (15,16). Patients who declined consent had a higher pre-dialysis blood pressure and so we can speculate about the negative influence of poorer adherence with treatments such as anti-hypertensive medications and fluid and nutritional restrictions. A non-adherent patient may be less likely to agree to participate in research, as shown in a review of screening logs from 15 cardiovascular
randomised trials in which a history of non-adherence was a factor for non-participation (17). Non-adherent patients may have a poorer survival. Further in-depth studies exploring the psychosocial factors that influence study participation are needed.

Our study has limitations. Patients were only verbally asked their reasons for non-consent and in-depth psychosocial reasons were not explored. In addition, no individualised social background information was collected. These omissions preclude further detailed analysis of non-participation. There were far fewer patients in the excluded group (N=44), and although survival of this group was not significantly worse than the recruited group, this was most likely because of differences in outcome within this sub-group, as patients with language barriers had survival comparable with recruited patients, whereas those excluded due to immobility or deemed too unwell fared worse.

In summary there were characteristic differences between participants and non-participants in this imaging study of haemodialysis patients. The recruited population may not be truly representative of the whole population. In line with the STROBE guidelines (18), when reporting studies researchers should also collect and provide demographic data on those who were excluded or who declined participation to allow accurate interpretation of results. Where inferences are being made, such as in clinical trials, consideration should be given to using statistical techniques to account for these effects.

4.7 References


Chapter 5

Novel Approach to Cardiovascular Outcome Prediction in Haemodialysis Patients


Karger IPR policy: http://www.karger.com/info/Disclaimer

5.1 Preface

The first novel two-dimensional transthoracic echocardiographic parameter to be investigated is global longitudinal strain determined by speckle tracking echocardiography. This technique has been developed within the last decade and is becoming readily available in clinical practice. Speckle tracking tracks the ultrasonographic acoustic reflections occurring naturally in the myocardium and can determine the strain (myocardial deformation). This analysis may be applied to pre-obtained two-dimensional echocardiographic images. There is emerging evidence in patients with chronic kidney disease that abnormal global longitudinal strain is an early and sensitive marker of all-cause mortality. This has also been demonstrated in haemodialysis patients with preserved left ventricular ejection fraction. However, it unknown whether this is the case in haemodialysis patients with a variable and unselected left ventricular ejection fraction. It is also well established that pulse wave velocity, a measure of aortic stiffness, is important in predicting all-cause mortality in chronic kidney disease. However, no study has compared echocardiographic parameters such as global longitudinal strain with pulse wave velocity in their predictive value for all-cause mortality using a single multivariable model. This chapter addresses these aims. This manuscript has been accepted for publication in the American Journal of Nephrology, January 2016.
5.2 Abstract

**Background:** Cardiovascular mortality is high in haemodialysis patients. Arterial stiffness and global longitudinal strain (GLS) are important non-atheromatous cardiovascular risk predictors. No study has encompassed both parameters in a combined model for prediction of outcomes in haemodialysis patients. Important, because left ventricular (LV) dysfunction can result from fibrotic remodelling secondary to increased arterial stiffness.

**Methods:** 219 haemodialysis patients had pulse wave velocity (PWV) and echocardiography (including GLS) assessments. Patients were followed-up until death, transplantation or 16th November 2015. Pearson's correlation coefficient was used to determine factors associated with PWV and GLS. A multivariable Cox regression model investigated factors associated with all-cause, cardiac death and events.

**Results:** 198 haemodialysis patients had full datasets (median age 64.2 years, 69% males) with mean LV ejection fraction (LVEF) of 61.7±10.1% and GLS -13.5±3.3%; 51% had LV hypertrophy. 48 deaths (15 cardiac) and 44 major cardiac events occurred during a median follow-up of 27.6 (25th-75th centile, 17.3-32.7) months. In separate survival models, PWV and GLS were independently associated with all-cause mortality; however, in a combined model, LVM/HT^{2.7} (adjusted HR 1.02, 95% CI 1.00-1.04) and PWV (adjusted HR 1.23, 95% CI 1.03-1.47) were significant. PWV was not associated with cardiac death nor events. However, GLS was associated with cardiac death (adjusted HR 1.24, 95% CI 1.00-1.54) and cardiac events (adjusted HR 1.13, 95% CI 1.03-1.25).

**Conclusions:** PWV and LVM/HT^{2.7} were superior to GLS in prediction of all-cause mortality. However, GLS was associated with cardiac death and events even when accounting for LVEF and LVM/HT^{2.7}.

5.3 Introduction

It is well recognised that patients undergoing haemodialysis suffer a higher mortality rate compared to the age and sex matched general population (1). Death from cardiac causes accounts for almost half of all cases. This is driven by a complex interaction between atherosclerotic and non-atherosclerotic cardiovascular risk factors. Many cardiac parameters have been reported to be associated with mortality in end-stage kidney disease. These include left ventricular hypertrophy and impaired left ventricular ejection fraction (2). However, left ventricular systolic function is more often than not well preserved in
patients with end stage kidney disease. Technological advances in echocardiographic analysis have identified sub-clinical abnormalities in systolic function, such as reduced global longitudinal strain (GLS) (3–5), which appear to be a more sensitive marker of cardiovascular risk than left ventricular ejection fraction.

Alongside echocardiography, other non-invasive bedside measures of cardiovascular risk are available. An example is pulse wave velocity (PWV), a measure of arterial vascular stiffness. PWV is also a well recognised predictor of all-cause mortality in patients undergoing haemodialysis (6). Cross-sectional studies have shown a relationship between PWV and echocardiographic parameters. For example, between PWV and left ventricular mass index and diastolic heart failure (7,8). However, there is a paucity of literature exploring the relationship between GLS and PWV (9), in contrast to the way that the relationship between PWV and left ventricular hypertrophy has been established in haemodialysis patients. Furthermore, there are no published reports investigating which echocardiographic parameters (including GLS) and/or PWV are most closely associated with mortality if included in a single multivariable survival analysis. There are also limited data on association of GLS with cardiac death and events. Therefore the aims of our study were to:

1. establish the cross-sectional association of GLS and PWV, and the association of these parameters with other clinical, laboratory and echocardiographic parameters in haemodialysis patients;
2. determine which of GLS, other echocardiographic parameters, and PWV are independently predictive of all-cause mortality, cardiac death, and major cardiac events if included in a single multivariable survival model.

5.4 Methods

5.4.1. Study Population

All incident and prevalent adult patients (≥18 years) receiving maintenance haemodialysis at Salford Royal Hospital NHS Foundation Trust, UK and its four satellite units between March 2012 and 2014 were approached to enter the study, and were enrolled if written informed consent was gained. All patients received three times weekly dialysis for 3-4 hours. Exclusion criteria included an inability to provide informed consent or if the patient
required ambulance transportation for hospital visits. The study adhered to the Declaration of Helsinki and ethical approval was obtained (UK, REC 05/Q1404/188).

Baseline demographics, co-morbidities, medications, and dialysis records were obtained from patient self-reported questionnaire and review of electronic patient medical records. Two-dimensional transthoracic echocardiography and PWV measurements were then performed at a single visit on a non-dialysis day during a short inter-dialytic break, with patients clinically stable. Laboratory data were determined from routine monthly blood collections from the haemodialysis circuit immediately before the mid-week session. The mean values for biochemistry and haematological results for the 3 months prior to the echocardiography visit were calculated and used. Likewise, dialysis prescription data (ultra-filtrate, pre-dialysis blood pressure etc.) were also taken from the mean of all available data from routine outpatient dialysis over the same 3 month period.

Follow-up was from the date of echocardiography to death, renal transplantation, relocating dialysis to a different country, or 16th November 2015. Events data were obtained from patient self-reporting, hospital medical records and patients’ primary care physicians. The outcome variables included all-cause mortality, cardiac death (defined as death due to myocardial infarction, heart failure, arrhythmia or sudden cardiac death) and major cardiac events (including myocardial infarction, new angina, hospitalisation due to heart failure or arrhythmia, coronary revascularisation/coronary bypass surgery or cardiac death). Cause of death and events were independently verified by two assessors (DC and KV).

5.4.2. Two-Dimensional Echocardiography

Transthoracic echocardiography was performed only by an experienced technician (JS) or a consultant cardiologist (NA). All patients were examined in the left lateral decubitus position using echocardiographic equipment with 3.5-MHz transducers with M-mode and 2D capabilities (Philips Medical Systems, Philips UK Ltd, United Kingdom). Greyscale images were obtained at a frame rate of 50-90 per second. Measurements were performed in accordance with the guidelines of the European Society of Echocardiography (10–12). At least three consecutive heartbeats were acquired with each view. Digital loops with the three standard apical views (i.e. apical 4-chamber, apical 2-chamber, and apical long-axis) were saved on the Philips Xcelera R4.1 image management system for offline analysis. Biplanes Simpsons method of discs was applied to apical 4- and 2- chamber views to
calculate volumes for determining LVEF and left atrial volumes indexed to height\(^{2.7}\) (LAVi). Impaired left ventricular ejection fraction was defined as <50%. M mode left ventricular mass was calculated using the Devereaux formula: left ventricular mass = \(0.8 \times (1.04 \times \left(\frac{\text{left ventricular internal diameter} + \text{septal wall thickness} + \text{posterior wall thickness}}{3}\right)^3 - \text{left ventricular internal diameter}^3) + 0.6\) g. Left ventricular mass was indexed to height\(^{2.7}\) (LVMI/HT\(^{2.7}\)). Left ventricular hypertrophy was defined as LVMI/HT\(^{2.7}\) >46.7 g/m\(^{2.7}\) for females and >49.2 g/m\(^{2.7}\) for men (13,14).

Two-dimensional images were analysed offline in Philips QLAB version 9 by a cardiologist (NA) and researcher (DC). All echocardiographic images were anonymised before analysis. Using apical 4-chamber and 2-chamber views, the endocardial border was traced by the software with the positions of the mitral annulus and apex determined by the operator. Tracking of the endocardial speckles from the endocardial border were performed by the software in end-diastole and end-systole. Imprecise tracking was manually modified by the interpreter to ensure accurate tracking of the left ventricular wall. A mean of all segmental strains of all 17 segments provided the GLS value (Chapter 3, Figure 3-3). The index beat selection method was utilised to determine GLS in patients with atrial fibrillation as described by Su et al.(15). GLS is expressed as the percentage change in left ventricular longitudinal dimension between end diastole and end systole, the less negative (hence more positive) the value the more impaired the strain (4,16). The range for 'normal' GLS in the non-renal population has been reported to vary between -15.9\% and -22.1\%(17). Published articles have utilised a cut off of -15\% as pathological for patients with kidney disease (4), therefore this cut off has been used, for comparison, where appropriate. Intra-rater and inter-rater variability were ascertained for measurements of Biplane Simpson's left ventricular ejection fraction and GLS for 20 randomly selected patients using Bland-Altman analysis of agreement. GLS measurement had significantly less intra- and inter-observer variability compared to left ventricular ejection fraction measurement. The inter-observer variability for left ventricular ejection fraction had a bias of -1.41±3.04, 95\% limits of agreement (LOA) was -7.37 to 4.54. Intra-observer variability for left ventricular ejection fraction had bias of -0.50±4.31 (95\% LOA was -8.95 to 7.96). Inter-observer variability for GLS had a bias 0.54±4.73 (95\% LOA -8.73 to 9.81) and intra-observer variability was -0.36±1.00, 95\% LOA -2.33 to 1.60.
5.4.3. **Pulse Wave Analysis**

At the same session as the echocardiographic studies, aortic stiffness was measured by PWV using the Vicorder™ device (Skidmore Medical Ltd, Bristol, UK). Patients were examined in a semi-recumbent position at approximately 35°, and a neck and femoral cuff were applied to the patient over their clothing. The aortic path length was measured as the distance between the patient's supra-sternal notch and midpoint of thigh cuff. Both cuffs were automatically inflated to 65mmHg and the corresponding oscillometric signal from each cuff digitally analysed to accurately record, in real time, the pulse time delay and the consequent aortic PWV. The measurement was carried out twice and a mean result was calculated.

5.4.4. **Statistical Analysis**

Where continuous data were non-normally distributed according to the Shapiro-Wilk test they are presented as median (25th-75th centile). Normally distributed data are presented as mean (SD). Categorical data are presented as numbers (percentage of total).

Pearson's correlation coefficient was performed to determine baseline factors associated with GLS and PWV. Factors that were associated with GLS and PWV on univariate analysis at P<0.05 were included in a multiple linear regression analysis.

Survival analysis for all-cause mortality, cardiac death, and major cardiac events were performed using multivariable Cox proportional hazard models. Factors associated with outcome at P < 0.05 on univariate analysis, and variables determined *a priori* to be potentially important if not found to be statistically significant on univariate analysis (age, diabetes, history of coronary artery disease, LVM/HT²/², left ventricular ejection fraction) were then included in the model. All statistical analysis was performed using the statistical package SPSS software (version 22, Chicago, Illinois, USA).
5.5 Results

5.5.1. Patient Characteristics

From 219 patients enrolled into the study, 198 patients had adequate echocardiographic and speckle tracking images for analysis. A full breakdown of recruitment and reasons for non-recruitment is shown in Figure 5-1. The population demographic and dialysis prescriptions are presented in Table 5-1, including separation of those who died versus those who did not, with a between group comparison.

Figure 5-1. Summary of recruitment, with inclusion and exclusion criteria.
The study population included a majority of Caucasian males (69%) with a median age of 64.2 (52.8 - 72.9) years. Patients had been treated with dialysis for a median time of 16.7 (5.8 - 44.3) months before recruitment. 78 patients (40%) had diabetes mellitus (3% Type 1 and 37% Type 2 diabetes) and 55 (28%) had prevalent coronary artery disease (defined as history of previous myocardial infarction, angina and/or coronary revascularization).

There were 14 patients with atrial fibrillation (2 paroxysmal) and all other patients were in sinus rhythm. The number of patients on anti-thrombotic therapy was noticeably greater than the number of patients with atrial fibrillation. Although this may reflect alternative diagnoses such as venous thromboembolism, anti-thrombotic therapy is frequently prescribed in haemodialysis patients as primary or secondary prevention of thrombosis of the dialysis arteriovenous fistula.

Patients with valvular heart disease were not excluded from the study in order to be as inclusive of all haemodialysis patients as possible. There were 25 patients with valvular heart disease (some with mixed lesions), all mild to moderate in severity and none severe. Eleven patients had aortic stenosis, 1 mitral stenosis, 6 tricuspid regurgitation, 7 mitral regurgitation, 6 aortic regurgitation and 2 patients with artificial heart valves.

The majority of patients had preserved systolic function (LVEF ≥ 50%, 171 patients, 86.4%). The mean LVEF was 61.7±10.1%. Half of the cohort (51%) had LVH. The median LVMI/HT² was 50.6 (40.9-65.3) g/m² (for men, 52.0 [40.4-66.9] g/m² and women, 49.5 [41.4-60.2] g/m²). 70.2% of the cohort had reduced GLS (≥ -15%). The mean GLS was -13.5±3.3. The mean PWV was 8.8 ± 2.1 m/s. These echocardiographic cross-sectional data and PWV are shown in Table 5-2.
### Table 5-1. Baseline demographics and clinical characteristics of patients with division according to survival status.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=198)</th>
<th>Death (N=48)</th>
<th>Survival (N=150)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.2 (52.8-72.9)</td>
<td>71.7 (65.4-77.2)</td>
<td>60.3 (50.2-70.0)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>136 (69)</td>
<td>35 (73)</td>
<td>101 (67)</td>
<td>0.592</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>129 (65)</td>
<td>39 (81)</td>
<td>90 (60)</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Dialysis Vintage (months)</td>
<td>16.7 (5.8-44.3)</td>
<td>19.1 (6.4-39.2)</td>
<td>15.8 (5.8-44.8)</td>
<td>0.758</td>
</tr>
<tr>
<td>Mean UF volume achieved (L)</td>
<td>2.19 (0.74)</td>
<td>2.18 (0.79)</td>
<td>2.19 (0.73)</td>
<td>0.947</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 (12)</td>
<td>73 (10)</td>
<td>78 (12)</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143 (20)</td>
<td>145 (21)</td>
<td>143 (19)</td>
<td>0.477</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>78 (39)</td>
<td>24 (50)</td>
<td>54 (36)</td>
<td>0.092</td>
</tr>
<tr>
<td>History of congestive cardiac failure (%)</td>
<td>56 (28)</td>
<td>21 (44)</td>
<td>35 (23)</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>55 (28)</td>
<td>17 (35)</td>
<td>38 (25)</td>
<td>0.197</td>
</tr>
<tr>
<td>Anti-platelets (%)</td>
<td>121 (61)</td>
<td>28 (58)</td>
<td>93 (62)</td>
<td>0.386</td>
</tr>
<tr>
<td>Anti-thrombotic agents (%)</td>
<td>16 (8)</td>
<td>7 (15)</td>
<td>9 (6)</td>
<td>0.061</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitor (%)</td>
<td>45 (23)</td>
<td>11 (23)</td>
<td>34 (23)</td>
<td>0.557</td>
</tr>
<tr>
<td>Angiotensin II receptor blocker (%)</td>
<td>33 (17)</td>
<td>10 (21)</td>
<td>23 (15)</td>
<td>0.248</td>
</tr>
<tr>
<td>B-Blocker (%)</td>
<td>91 (46)</td>
<td>22 (46)</td>
<td>69 (46)</td>
<td>1.000</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>124 (63)</td>
<td>34 (71)</td>
<td>90 (60)</td>
<td>0.158</td>
</tr>
<tr>
<td>Nicorandil (%)</td>
<td>7 (4)</td>
<td>5 (10)</td>
<td>2 (1)</td>
<td><strong>0.010</strong></td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.7 (1.2)</td>
<td>10.5 (1.1)</td>
<td>10.8 (1.3)</td>
<td>0.205</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.37 (0.14)</td>
<td>2.40 (0.13)</td>
<td>2.37 (0.14)</td>
<td>0.264</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.55 (0.45)</td>
<td>1.52 (0.47)</td>
<td>1.56 (0.45)</td>
<td>0.704</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39 (36-41)</td>
<td>38 (36-40)</td>
<td>40 (37-41)</td>
<td><strong>0.010</strong></td>
</tr>
<tr>
<td>C-reactive Protein (mg/dL)</td>
<td>9.4 (2.9-21.0)</td>
<td>10.8 (2.1-25.0)</td>
<td>8.3 (3.1-21.0)</td>
<td>0.586</td>
</tr>
<tr>
<td>Follow-up duration (months)</td>
<td>24.8 (10.5)</td>
<td>19.9 (10.1)</td>
<td>26.3 (10.1)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
</tbody>
</table>

Data are presented as mean (SD), n (%) or median (25th percentile, 75th percentile). Abbreviations: UF, ultrafiltration volume. Antiplatelet agents include aspirin and/or clopidogrel. Anti-thrombotic agents include warfarin or sinthrone.
Table 5-2. Echocardiographic and pulse wave velocity results for whole cohort, and for patients who died versus survivors.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=198)</th>
<th>Death (N=48)</th>
<th>Survival (N=150)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>61.7 (10.1)</td>
<td>59.1 (11.9)</td>
<td>62.5 (9.4)</td>
<td>0.085</td>
</tr>
<tr>
<td>Preserved LVEF (%)</td>
<td>171 (86)</td>
<td>33 (69)</td>
<td>138 (92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI/HT(^{2.7}) (g/m(^{2.7}))</td>
<td>50.6 (40.9-65.3)</td>
<td>53.5 (43.6-70.2)</td>
<td>49.9 (39.2-64.0)</td>
<td>0.079</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>101 (51.0)</td>
<td>28 (58.3)</td>
<td>73 (48.7)</td>
<td>0.322</td>
</tr>
<tr>
<td>MV E/A</td>
<td>0.82 (0.68-1.10)</td>
<td>0.81 (0.68-1.10)</td>
<td>0.82 (0.68-1.10)</td>
<td>0.958</td>
</tr>
<tr>
<td>E/A TDI</td>
<td>0.86 (1.54)</td>
<td>1.3 (3.3)</td>
<td>0.8 (0.4)</td>
<td>0.346</td>
</tr>
<tr>
<td>E/e' TDI</td>
<td>10.96 (6.52)</td>
<td>13.1 (5.4)</td>
<td>12.1 (5.6)</td>
<td>0.335</td>
</tr>
<tr>
<td>LAVi (ml/m(^{2.7}))</td>
<td>14.3 (11.5-17.9)</td>
<td>14.9 (12.2-18.8)</td>
<td>14.1 (11.3-17.4)</td>
<td>0.127</td>
</tr>
<tr>
<td>GLS (%)</td>
<td>-13.5 (3.3)</td>
<td>-12.8 (2.7)</td>
<td>-13.7 (3.4)</td>
<td><strong>0.041</strong></td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>8.81 (2.08)</td>
<td>10.0 (2.2)</td>
<td>8.5 (1.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD), n (%) or median (25th percentile, 75th percentile). Abbreviations: LVEF, left ventricular ejection fraction, LVMI/HT\(^{2.7}\), left ventricular mass indexed to height\(^{2.7}\), LVH, left ventricular hypertrophy, MV E/A, early to late transmitral velocity ratio, E/e', early transmitral velocity to tissue Doppler mitral annular early diastolic velocity ratio, TDI E/A, tissue Doppler transmitral velocity early to late ratio, LAVi, left atrial volume indexed to height\(^{2.7}\), GLS, global longitudinal strain, PWV, pulse wave velocity.

5.5.2. Cross-Sectional Associates of GLS and PWV

On univariate analysis the factors that showed significant association with a more positive (hence more abnormal) GLS included higher systolic blood pressure (r=0.322, P < 0.001), lower plasma albumin (r= -0.268, P < 0.001), lower left ventricular ejection fraction (r= -0.383, P < 0.001) and higher LVMI/HT\(^{2.7}\) (r=0.377, P < 0.001). PWV was not associated with GLS (r=0.070, P = 0.336). In a multivariable linear regression model, the above factors remained significantly associated with GLS with the model having an \(r^2\) of 0.36, F stat of 22.40, P < 0.001 (Table 5-3).
Table 5-3. Multivariable linear regression model for factors significantly associated with global longitudinal strain (GLS) and pulse wave velocity (PWV).

<table>
<thead>
<tr>
<th></th>
<th>GLS</th>
<th>PWV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model: F stat 22.40, P &lt; 0.001, r squared 0.36</td>
<td>F stat 14.462, P &lt;0.001, r square 0.310</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Beta</td>
</tr>
<tr>
<td>Constant</td>
<td>-8.181</td>
<td>0.007</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.033</td>
<td>0.204</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>-0.171</td>
<td>-0.204</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>-0.101</td>
<td>-0.317</td>
</tr>
<tr>
<td>LVMI/HT².⁷ (g/m².⁷)</td>
<td>0.050</td>
<td>0.293</td>
</tr>
</tbody>
</table>
| Sodium (mmol/L) | -0.147 | -0.179 | 0.007 | -0.253 to -0.040 | Mean PWV for the whole cohort was 8.8 ±2.1 m/s. On univariate analysis for a higher PWV the significant factors included older age (r=0.405, P <0.001), higher systolic blood pressure (r=0.206, p=0.005), lower creatinine (r= -0.164, P = 0.034), higher serum corrected calcium (r=0.181, P = 0.019) and lower plasma sodium (r=-0.168, P = 0.021). Notably, no echocardiographic parameters were associated with PWV, examples being LVEF (r=0.03, P = 0.660) and LVMI/HT².⁷ (r=0.051, P = 0.440). In the multivariable linear regression model, the factors that remained significantly associated with PWV were age, systolic blood pressure and sodium (Table 5-3).
5.5.3. Survival Analysis

After a median follow-up of 27.6 (25\textsuperscript{th} - 75\textsuperscript{th} centile, 17.3 - 32.7) months there were 48 deaths. There were 15 (31.3\%) deaths from cardiac causes of which 8 were from myocardial infarction, 6 sudden cardiac death and 1 from heart failure secondary to coronary artery disease. Other deaths included 10 from infection, 1 from stroke, 6 from cancer, 7 from withdrawal of haemodialysis and 9 deaths from other causes. 2 patients had moved out of the country therefore follow-up ended at that point. 34 patients received a renal transplant during follow-up. One patient changed modality from haemodialysis to peritoneal dialysis and 9 patients changed to home haemodialysis. There were 44 patients who had one or more major cardiac events.

On univariate Cox regression analysis both a more positive, hence more abnormal GLS (unadjusted hazard ratio, HR 1.12, 95\% confidence interval, CI 1.02-1.22, P = 0.014) and higher PWV (unadjusted HR 1.18, 95\% CI 1.07-1.31, P = 0.001) were independently associated with all-cause mortality. Figure 5-2 shows unadjusted Kaplan-Meier plots for GLS and PWV demonstrating worse survival for patients with above median GLS and PWV. A more positive, hence more abnormal GLS was also associated with cardiac death (unadjusted HR 1.18, 95\% CI 1.01-1.37, P = 0.034), but PWV was not (unadjusted HR 1.11, 95\% CI 0.89-1.39, P = 0.380). This was similarly the case for major cardiac events, a more positive GLS was associated (unadjusted HR 1.13, 95\% CI 1.04-1.24, P = 0.006) whilst PWV (unadjusted HR 1.02, 95\% CI 0.88-1.17, P = 0.802) was not (Table 5-4).
Figure 5-2. Unadjusted Kaplan-Meier survival plots for global longitudinal strain (GLS) and pulse wave velocity (PWV) in prediction of all-cause mortality.

Follow-up Duration (months) | 0 | 6 | 12 | 24 | 31
---|---|---|---|---|---
GLS ≥ median | 77 | 72 | 67 | 54 | 28
GLS < median | 119 | 112 | 101 | 62 | 30

Note: (a) Cumulative survival plot of global longitudinal strain above and below median (-13.7%). Log-rank (mantel-cox), chi square was 6.73, p=0.009. (b) Cumulative survival plot of pulse wave velocity (PWV) above and below the median value (8.37m/s). Log-rank (mantel-cox), chi-square was 6.59, p=0.010. The table represents the number of patients remaining at each time point.
Table 5-4. Outcomes of patients separated into a cut-off of abnormal global longitudinal strain (GLS) ≥ -15%.

<table>
<thead>
<tr>
<th></th>
<th>Abnormal GLS (GLS ≥ -15%)</th>
<th>Normal GLS (GLS &lt; -15%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N= 139</td>
<td>N=59</td>
<td></td>
</tr>
<tr>
<td>Primary Outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>41 (30)</td>
<td>7 (12)</td>
<td>0.010</td>
</tr>
<tr>
<td>Cardiac death</td>
<td>14 (10)</td>
<td>1 (2)</td>
<td>0.043</td>
</tr>
<tr>
<td>Secondary Outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revascularisation/cardiac bypass surgery</td>
<td>4 (3)</td>
<td>0 (0)</td>
<td>0.320</td>
</tr>
<tr>
<td>Admissions due to heart failure</td>
<td>21 (15)</td>
<td>5 (9)</td>
<td>0.255</td>
</tr>
<tr>
<td>Admissions due to angina</td>
<td>4 (3)</td>
<td>1 (2)</td>
<td>1.000</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>16 (12)</td>
<td>1 (2)</td>
<td>0.195</td>
</tr>
<tr>
<td>Major cardiac events</td>
<td>37 (27)</td>
<td>7 (12)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Data expressed as n (%) representing each patient, therefore some patients may have had more than one myocardial infarction but is only counted once in this table. P value from a Chi squared or Fisher's exact test analysis. Major cardiac events is defined as myocardial infarction, new angina, hospitalisation due to heart failure or arrhythmia, coronary revascularisation/coronary bypass surgery or cardiac death. Patients with an abnormal GLS had a higher rate of all-cause mortality and cardiac death. Major cardiac events were also more common.

The factors which were significant on univariate analysis (P < 0.05) and factors which were decided a priori to be important were included in a combined model (Table 5-5). In a multivariable Cox regression model for all-cause mortality, the significant factors were age (adjusted HR 1.08, 95% CI 1.03-1.13, P = 0.002), LVMI/HT².7 (adjusted HR 1.02, 95% CI 1.00-1.04, P = 0.038) and PWV (adjusted HR 1.23, 95% CI 1.03-1.47, P = 0.020). In a multivariable Cox regression model for cardiac death, the significant factors were a more positive hence abnormal GLS (adjusted HR 1.24, 95% CI 1.00-1.54, P = 0.050) and diabetes (adjusted HR 4.44, 95% CI 1.18-16.8, P = 0.028). For major cardiac events, age (adjusted HR 1.04, 95% CI 1.00-1.07, P = 0.034), angiotensin receptor blocker usage (adjusted HR 2.25, 95% CI 1.12-4.51, P = 0.023) and a more positive GLS were the only significant factors (adjusted HR 1.13, 95% CI 1.03-1.25, P = 0.012). A table listing factors in the final Cox regression models is shown in Table 5-6. As PWV was not significant on univariate analysis for cardiac death or major cardiac events, it was not included in the final multivariable model for these.
Table 5-5. Univariate analysis in relation to outcome

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All-Cause Mortality</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.07</td>
<td>1.04-1.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.31</td>
<td>1.12-4.77</td>
<td>0.024</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>1.62</td>
<td>0.92-2.86</td>
<td>0.094*</td>
</tr>
<tr>
<td>History of Coronary Artery Disease</td>
<td>1.39</td>
<td>0.77-2.52</td>
<td>0.277*</td>
</tr>
<tr>
<td>History of congestive cardiac failure</td>
<td>1.84</td>
<td>1.04-3.26</td>
<td>0.036</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.97</td>
<td>0.95-0.99</td>
<td>0.041</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.93</td>
<td>0.86-0.99</td>
<td>0.031</td>
</tr>
<tr>
<td>Left ventricular mass indexed to height²/²</td>
<td>1.02</td>
<td>1.00-1.03</td>
<td>0.031</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>0.96</td>
<td>0.94-0.99</td>
<td>0.014</td>
</tr>
<tr>
<td>Pulse wave velocity</td>
<td>1.18</td>
<td>1.07-1.31</td>
<td>0.001</td>
</tr>
<tr>
<td>Global Longitudinal Strain</td>
<td>1.11</td>
<td>1.02-1.22</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Cardiac Death</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>3.04</td>
<td>1.04-8.89</td>
<td>0.043</td>
</tr>
<tr>
<td>History of Coronary Artery Disease</td>
<td>1.19</td>
<td>0.41-3.49</td>
<td>0.750*</td>
</tr>
<tr>
<td>Left ventricular mass indexed to height²/²</td>
<td>0.99</td>
<td>0.97-1.02</td>
<td>0.622*</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>1.01</td>
<td>0.96-1.07</td>
<td>0.626*</td>
</tr>
<tr>
<td>Global longitudinal strain</td>
<td>1.18</td>
<td>1.01-1.37</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Major Cardiac Events</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>1.01-1.07</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>2.05</td>
<td>1.13-3.73</td>
<td>0.019</td>
</tr>
<tr>
<td>History of Coronary Artery Disease</td>
<td>2.87</td>
<td>1.59-5.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium channel blocker use</td>
<td>2.10</td>
<td>1.13-3.40</td>
<td>0.019</td>
</tr>
<tr>
<td>Angiotensin receptor blocker use</td>
<td>2.40</td>
<td>1.27-4.56</td>
<td>0.007</td>
</tr>
<tr>
<td>Global longitudinal strain</td>
<td>1.13</td>
<td>1.04-1.24</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Note: Univariate Cox regression analysis was performed on all parameters in relation to the outcomes all-cause mortality, cardiac death and major cardiac events. The following parameters were significant on univariate analysis and therefore included in the multivariable Cox regression model illustrated in Table 5-6. In addition, factors that were not significant, but were considered a priori to be important for the outcome were also included *. 

102
Table 5-6. Final multivariable Cox regression model illustrating the significance of each included variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All-cause mortality (N=48)</th>
<th>Cardiac death (N=15)</th>
<th>Major cardiac event (N=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio (Confidence Interval)</td>
<td>P</td>
<td>Hazard Ratio (Confidence Interval)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.08 (1.03-1.13)</td>
<td>0.002</td>
<td>-</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.62 (0.67-4.00)</td>
<td>0.296</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>1.85 (0.91-3.74)</td>
<td>0.088</td>
<td>4.44 (1.18-16.78)</td>
</tr>
<tr>
<td>History of Coronary Artery Disease</td>
<td>0.65 (0.29-1.46)</td>
<td>0.297</td>
<td>0.82 (0.22-2.98)</td>
</tr>
<tr>
<td>CCF</td>
<td>0.97 (0.45-2.11)</td>
<td>0.943</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>1.01 (0.98-1.05)</td>
<td>0.485</td>
<td>-</td>
</tr>
<tr>
<td>CCB</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ARB</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.93 (0.86-1.01)</td>
<td>0.092</td>
<td>-</td>
</tr>
<tr>
<td>LVM/HT2.7 (g/m2.7)</td>
<td>1.02 (1.00-1.04)</td>
<td>0.038</td>
<td>0.98 (0.95-1.01)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.97 (0.94-1.01)</td>
<td>0.114</td>
<td>1.04 (0.98-1.11)</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>1.23 (1.03-1.47)</td>
<td>0.020</td>
<td>-</td>
</tr>
<tr>
<td>GLS (%)</td>
<td>1.00 (0.86-1.17)</td>
<td>0.984</td>
<td>1.24 (1.00-1.54)</td>
</tr>
</tbody>
</table>

Abbreviations: CCF, history of heart failure, CCB, calcium channel blocker usage, ARB, angiotensin receptor blocker usage, LVEF, left ventricular ejection fraction, LVM/HT2.7, left ventricular mass indexed to height2.7, GLS, global longitudinal strain, PWV, pulse wave velocity.

Note: The hazard ratios are expressed as per unit increase therefore for GLS, a hazard ratio of 1.24 is per 1% increase in GLS, therefore the more positive the GLS and the more abnormal the longitudinal strain.
The analysis was re-performed with PWV included and this demonstrated similar results (Table 5-7) except that GLS became non-significant for cardiac death, albeit with a marginal change in P value (P = 0.070). This likely reflects the small number of cardiac deaths (n = 15), and by adding PWV the model may be over-adjusted.

Table 5-7. Multivariable model including pulse wave velocity and global longitudinal strain in the same model as Table 5-6.

<table>
<thead>
<tr>
<th></th>
<th>All-cause mortality (N=48)</th>
<th>Cardiac death (N=15)</th>
<th>Major cardiac event (N=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio (Confidence Interval)</td>
<td>P</td>
<td>Hazard Ratio (Confidence Interval)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.08 (1.03-1.13)</td>
<td>0.002</td>
<td>-</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.62 (0.67-4.00)</td>
<td>0.296</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>1.85 (0.91-3.74)</td>
<td>0.088</td>
<td>3.12 (0.78-12.43)</td>
</tr>
<tr>
<td>History of Coronary Artery Disease</td>
<td>0.65 (0.29-1.46)</td>
<td>0.297</td>
<td>1.38 (0.35-5.46)</td>
</tr>
<tr>
<td>CCF</td>
<td>0.97 (0.45-2.11)</td>
<td>0.943</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>1.01 (0.98-1.05)</td>
<td>0.485</td>
<td>-</td>
</tr>
<tr>
<td>CCB</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ARB</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.93 (0.86-1.01)</td>
<td>0.092</td>
<td>0.99 (0.95-1.02)</td>
</tr>
<tr>
<td>LVMI/HT$^{2.7}$ (g/m$^{2.7}$)</td>
<td>1.02 (1.00-1.04)</td>
<td>0.038</td>
<td>0.99 (0.95-1.02)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.97 (0.94-1.01)</td>
<td>0.114</td>
<td>1.06 (0.98-1.14)</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>1.23 (1.03-1.47)</td>
<td>0.020</td>
<td>1.15 (0.87-1.53)</td>
</tr>
<tr>
<td>GLS (%)</td>
<td>1.00 (0.86-1.17)</td>
<td>0.984</td>
<td>1.26 (0.98-1.62)</td>
</tr>
</tbody>
</table>

Note: Although pulse wave velocity was not significant on univariate analysis, the following multivariable Cox regression models have included pulse wave velocity and global longitudinal strain in the same models as illustrated in Table 5-6.

Abbreviations: CCF, history of heart failure, CCB, calcium channel blocker usage, ARB, angiotensin receptor blocker usage, LVEF, left ventricular ejection fraction, LVMI/HT$^{2.7}$, left ventricular mass indexed to height$^{2.7}$, GLS, global longitudinal strain, PWV, pulse wave velocity.
5.6 Discussion

This is the largest study to investigate the relationship between GLS and PWV in haemodialysis patients and to compare the individual value of both parameters in a combined survival model. Both arterial stiffness and left ventricular systolic dysfunction may play a part in the mechanistic pathway of increased risk of death in end stage kidney disease. However, it is unknown what factors are most powerful as a cardiovascular risk prediction tool.

GLS is a measure of myocardial deformation which can be easily ascertained using speckle tracking software on pre-determined 2D transthoracic echocardiographic images. This study is currently the largest study of GLS in an ambulant haemodialysis population. All echocardiographic procedures in this study were performed on a non-dialysis day, which has not been consistently been the case in previous analyses. This is important because some echocardiographic measurements of systolic function can be affected by load manipulation during dialysis and myocardial oedema (18). Nonetheless, the factors that we found to be associated with GLS were consistent with previous studies. Although the associations identified were significant, the correlations were weak. These included higher systolic blood pressure, lower albumin, lower left ventricular ejection fraction and higher LVMI/HT\(^2.7\), associations that were consistent with findings from Wang et al.(13) who investigated 98 patients undergoing haemodialysis and found that impaired longitudinal strain correlated with higher left ventricular mass, relative wall thickness, pre-dialysis systolic blood pressure, calcium-phosphate product and lower mid wall fractional shortening. We also found that a low albumin was associated with GLS. This may reflect hypervolaemia and thereby higher blood pressure affecting longitudinal strain, although the association of albumin with GLS was independent of systolic blood pressure. Another possible reason may be that a low albumin may be representative of a higher inflammatory state that could have impact on cardiac function/structure. These hypotheses remain to be speculative, however.

Increased after-load due to hypertension is a common precursor to cardiac hypertrophy, and left ventricular hypertrophy is associated with myocardial fibrosis which may affect myocardial deformation. This is the likely explanation for the association of left ventricular hypertrophy with reduction in GLS. However, PWV was not associated with GLS, suggesting that increased afterload due to arterial stiffness may not play as significant a
part in left ventricular hypertrophy and reduced GLS in patients undergoing haemodialysis as hypertension and hypervolaemia. This is in contrast to a study by Krishnasamy et al. (9), who reported an association of GLS with aortic PWV in patients with chronic kidney disease stages 3 and 4. The metabolic milieu, inflammation and vascular calcification of the dialysis patient may explain why the associations of GLS and PWV in pre-dialysis and dialysis patients may be very different. Indeed, less patients had left ventricular hypertrophy and abnormal GLS in the aforementioned study compared with our cohort.

Consistent with previous findings, we note that higher age and systolic blood pressure were associated with a higher PWV (19). With increasing age there is central arterial stiffening, remodelling and calcification thus accounting for the increased arterial stiffness. It has been reported that there is accelerated vascular aging in patients with end stage kidney disease (20). As a consequence of arterial stiffening there would be a higher systolic blood pressure. We found that a low sodium was also associated with an increased PWV, again possibly being reflective of hypervolaemia leading to hypertension which may contribute to vascular stiffness. In one study of 49 haemodialysis patients, left ventricular mass index was positively correlated with PWV (r=0.44, P = 0.001)(7). Conversely our study did not find any association between PWV and LVMI/HT$^{2.7}$ (r=0.051, P = 0.440).

PWV has long been recognised to be a predictor of all-cause mortality (6). It has been reported in a cohort of 241 haemodialysis patients that an increase of 1 ms$^{-1}$ of PWV is associated with an adjusted HR of 1.39 (95% CI 1.19-1.62) for all-cause mortality (21). The recognition of GLS as an important prognostic risk factor has only recently emerged (4,5,16,22). In 88 stable haemodialysis patients with a mean follow up of 25±9.9 months Liu et al. (4) reported that a less negative GLS (performed during dialysis therapy) was associated with a 3.57 times higher all cause mortality (95% CI 1.41-9.04; P = 0.01). However, to our knowledge, no study has combined PWV and GLS within the same multivariable model to determine which factor is most strongly associated with all-cause mortality. We report that after adjustment, PWV and LVMI/HT$^{2.7}$ were most predictive of all-cause mortality. However, GLS was associated with cardiac death and events, more so than standard echocardiographic parameters such as left ventricular ejection fraction and LVMI/HT$^{2.7}$. A possible explanation for this is that cardiovascular death is the major cause of death in haemodialysis patients. We hypothesise that PWV and LVMI/HT$^{2.7}$ might be related to other causes of death such as stroke and aneurysm ruptures rather than solely cardiac deaths. These two markers may be more representative of the general state of the
vasculature of the haemodialysis patient, and they may be stronger predictors of mortality in general, whilst GLS may be more sensitive to cardiac mortality and events. Liu et al.(4) also reported that patients with an abnormal GLS had a higher number of cardiac deaths (27%) and events (33%). Their study had relatively low number of events, whilst we were able to demonstrate this relationship using survival analysis.

We also found that angiotensin II blocker usage was positively associated with major cardiac events. This specific finding has not previously been reported. Some other studies have shown a protective effect of renin-angiotensin blockade (23,24) whilst others have failed to show any reduction in cardiovascular events (25). Further, large randomised controlled trials are needed in haemodialysis patients to address this important question (26) and therefore whether our finding is valid or actually reflects hidden confounders.

This study has some limitations. Firstly, for logistic reasons, patients who required ambulance or stretcher transport to hospital were excluded (N=44). This has likely excluded a high cardiovascular co-morbidity group. Second, this was an observational data set which cannot therefore account for residual confounding factors, and causality can only be implied. Thirdly, the number of patients enrolled into the study was small and our findings will need to be confirmed in a larger cohort. Although multicenter recruitment may achieve this and reproducibility for GLS in our series was good, GLS is challenging to achieve and may be less reproducible across centers. Therefore a larger cohort may need to be investigated in a single center. Fourthly, we did not distinguish between non-ischaemic versus ischaemic arrhythmia because these are likely to be of multi-factorial origin in haemodialysis patients. However, none of the 5 arrhythmic end points episodes occurred during a myocardial infarction or acute coronary syndrome event. Finally, the use of statin was high in our cohort, but unfortunately we did not have lipid profile data to provide further information.

Our study is the first to show that PWV and LVM/H\(T^{2.7}\) is strongly associated with all-cause mortality when newer echocardiographic techniques such as GLS are taken into account. Perhaps, measurements of PWV, which can be performed simply and quickly at the bedside, should be used more routinely alongside echocardiographic analyses. GLS has associations with cardiac death and events over and above standard echocardiographic measures such as left ventricular ejection fraction and LVM/H\(T^{2.7}\). Therefore this may be a useful cardiac risk stratification tool in haemodialysis patients. An abnormal GLS may
highlight haemodialysis patients who require more intensive cardiac risk factor management, dialysis prescription review and perhaps earlier referral for appropriate cardiac intervention.

5.7 References


Chapter 6

Cardiovascular Risk Assessment in Haemodialysis Patients with Preserved Left Ventricular Ejection Fraction and Left Ventricular Hypertrophy


6.1 Preface

Chapter 5 detailed a survival analysis including echocardiography and pulse wave velocity in a group of haemodialysis patients with unselected left ventricular ejection fraction and mass. However, it was hypothesised that the prognostic value of factors such as global longitudinal strain will differ in different sub-groups of patients with different echocardiographic phenotype. This chapter investigated whether global longitudinal strain is predictive of all-cause mortality when accounting for pulse wave velocity and echocardiographic parameters in the sub-groups of patients with left ventricular hypertrophy and those with preserved left ventricular ejection fraction. These sub-groups have been selected because they are common in haemodialysis patients: over 75% of haemodialysis patients have left ventricular hypertrophy at dialysis inception and 80% of patients in reported cohorts of haemodialysis patients have preserved ejection fraction. Showing a difference in significance of global longitudinal strain in different sub-groups would be a step towards a more stratified approach to interpretation of imaging findings in different dialysis patients.

6.2 Abstract

Background: Reduced global longitudinal strain (GLS) and increased pulse wave velocity (PWV) are associated with mortality in haemodialysis (HD) patients. Due to different pathophysiological mechanisms and characteristics of patients with left ventricular hypertrophy (LVH) and preserved left ventricular ejection fraction (LVEF), it is
hypothesised that the patterns and prognostic significance of GLS and PWV would be
different in these groups.

**Methods:** Baseline clinical, laboratory, 2D echocardiographic (speckle tracking
determined GLS) and PWV were analysed cross-sectionally in 219 HD patients. Chi-
squared and t-tests were used to compare differences between groups with or without LVH
and preserved versus impaired LVEF (LVEF ≥ or < 50%, respectively). Cox proportional
hazard models investigated factors associated with all-cause mortality in patients with
LVH and preserved LVEF.

**Results:** 198 patients had full datasets and 51% patients had LVH (of which 56% had
concentric hypertrophy and 44% had eccentric hypertrophy). Patients with LVH had a
more abnormal GLS (-12.7 vs -14.4%, P<0.001) compared to patients without. PWV was
not different between groups (8.70 vs 8.93m/s, P=0.442). In patients with LVH only (N=
100), after a median follow-up of 27.1 (25th - 75th centile, 16.8-31.2) months, there were
21 deaths. In a multivariable model adjusting for age, diabetes mellitus (DM), coronary
artery disease (CAD), PWV and GLS, only age (HR 1.07, 95% CI 1.01-1.13, P=0.019)
was predictive of mortality.

171 patients had preserved LVEF; compared to patients with a reduced LVEF, they had a
better GLS (-13.8% vs -10.9, P=0.001). There was no difference in PWV (9.1 vs 8.8 m/s,
P=0.589). After a median follow-up of 28.2 (25th-75th centile, 17.8 - 33.4) months, there
were 33 deaths in the preserved LVEF group. In a combined model adjusting for age,
smoking, CAD and DM; a higher PWV (HR 1.23, 95% CI 1.04-1.45, P=0.015) and a more
abnormal GLS (HR 1.16, 95% CI 1.01-1.33, P=0.031) were predictive of mortality.

**Conclusion:** Abnormalities of GLS are greater in patients with LVH than those without
and in patients with reduced as opposed to preserved LVEF. Neither GLS nor PWV were
predictive of mortality in patients with LVH, but both were sensitive markers of poor
prognosis in patients with preserved LVEF.

### 6.3 Introduction

Mortality from cardiovascular causes is the major mode of death in haemodialysis (HD)
patients (1), and several cardiovascular risk factors are contributory. Increased aortic
stiffness measured by pulse wave velocity (PWV) has been reported to be associated with
all-cause and cardiovascular mortality in end stage kidney disease (ESKD)(2,3). More
recently, global longitudinal strain (GLS) has been demonstrated to be a sensitive, early
marker of increased risk for mortality in patients with chronic kidney disease (CKD) (4,5). We have recently shown that when both PWV and GLS are combined in a multivariable model in a HD population with a range of left ventricular ejection fraction (LVEF), PWV remained as a significant predictor of all-cause mortality whilst GLS did not (6). However, in the renal literature the predictive value of GLS has been emphasised in patients with preserved LVEF (4,5), which supports the value of GLS as a sensitive marker of LV dysfunction, detectable before reduction in LVEF is evident. However, no study has investigated whether combining PWV and GLS in the same multivariable model in patients with preserved LVEF would give the same result as in a HD population with more heterogeneous LV systolic function. The pathophysiology and cardiac risk may be very different in a population with preserved LVEF, and since the majority of HD patients have preserved LVEF (7), it is important to explore this relationship in order to determine which markers are most sensitive for prognosis.

Another sub-group in which the relative importance of PWV and GLS has not been explored is in patients with left ventricular hypertrophy (LVH) (8). Over 75% of dialysis patients have LVH at dialysis inception (9). Different pathological mechanisms lead to concentric and eccentric hypertrophy (10). LVH has been associated with an increased arterial stiffness (11) and there is a plausible mechanistic link between increased after-load, indicated by arterial stiffness, and hypertrophy.

We hypothesised that GLS and PWV will be different and have independent prognostic values in sub-groups of HD patients with either LVH or preserved LVEF because of the different pathophysiological processes involved. Our aims therefore were:
1. to describe the differences in baseline characteristics, laboratory findings, GLS and PWV values in HD patients with versus without LVH, concentric versus eccentric LVH and preserved versus impaired LVEF.
2. In groups of (i) LVH and (ii) preserved LVEF, to investigate the prognostic value of GLS and PWV in a multivariable model for all-cause mortality.

6.4 Methods

6.4.1 Study Population

The Salford Kidney Study is a prospective observational study undertaken at Salford Royal Hospital NHS Foundation Trust, UK, and its four HD satellite units, investigating
the echocardiographic associations of mortality in HD. Prevalent and incident maintenance HD patients were approached for recruitment between March 2012 and March 2014. All patients received standard 3 times weekly, 3-4 hours HD with adequate dialysis clearance (defined as urea reduction ratio (URR) > 65%). Inclusion criteria included a mobile adult (≥18 years) receiving maintenance HD and the patients had to have capacity to consent (written and verbal). Exclusion criteria included lack of ability to consent, and patients that required ambulance transport for echocardiographic study visits, due to logistical reasons. The study adhered to the Declaration of Helsinki and ethical approval was obtained.

Clinical and demographic data were obtained from detailed review of electronic medical records and patient self-reported questionnaire obtained on the day of assessment. All patients were followed-up until death, transplantation, moved out of the country or 16th November 2015. Event data were obtained from patient self reporting, detailed review of hospital electronic health records, and contact with primary care physicians. The primary outcome was all-cause mortality. Cause of death data were independently verified by two independent assessors (DC and VK).

Blood samples were collected on a monthly basis from the HD circuit, immediately before the midweek dialysis session. Mean values for laboratory results from the 3 months prior to echocardiography were used in the analysis. Parameters from the dialysis prescription (e.g. ultrafiltration volume) were also averaged over the 3 months prior to assessment date.

6.4.2. Two-dimensional Transthoracic Echocardiographic Measurements

Conventional transthoracic 2D echocardiography was performed on a non-dialysis day and after the short inter-dialytic break, by either of an experienced technician (JS) or a consultant cardiologist (NA). All patients were examined in the left lateral decubitus position using the Philips Medical Systems echocardiograph (Philips UK Ltd, United Kingdom) with 3.5-MHz transducers using M-mode and 2D. All echocardiographic measurements were performed in accordance with the guidelines of the European Society of Echocardiography (12). Each echocardiographic view consisted of at least three consecutive heartbeats and all three standard apical views (apical 4-chamber, 2-chamber, and 3-chamber) were obtained. All 2D images were saved on the Philips Xcelera R4.1 image management system for offline analysis. In order to determine the volumes for LVEF estimation and left atrial volumes indexed to height².7 (LAVi), Biplanes Simpsons
method of discs was applied to pre-obtained apical 4- and 2- chamber views. Preserved LVEF was defined as ≥50%.

M mode LV mass was calculated using the devereaux formula: LV mass = 0.8*(1.04*[(LV internal diameter+septal wall thickness+posterior wall thickness)^3- LV internal diameter^3]+0.6g. LV mass was then indexed to height^2.7. LVH was defined as LVMI/HT^2.7 >46.7 g/m^2.7 for women and >49.2g/m^2.7 for men (8,13). Relative wall thickness (RWT) was calculated as: (posterior wall thickness x 2)/LV internal diameter at end diastole. It was considered increased if RWT>0.42. Concentric hypertrophy was defined as LVH with a RWT>0.42 and eccentric hypertrophy as LVH with RWT≤0.42 (14).

6.4.3. Global Longitudinal Strain Analysis

The 2D echocardiographic images saved in the image management system were analysed offline using the Philips QLAB version 9 by a trained researcher (DC). Using apical 4-chamber and 2-chamber views, the endocardial border was determined using a semi-automated system, with the positions of the mitral annulus and apex determined manually. This was performed on the left ventricle in the end-diastolic and end-systolic frames. Imprecise tracking was manually modified to ensure accurate capture of the LV wall. The GLS was derived from data from an average of 17 segments of the LV (Chapter 3, Figure 3-3). By convention, the less negative the strain, the more impaired the myocardial deformation.

6.4.4. Pulse Wave Analysis

On the echocardiographic assessment day, aortic stiffness was assessed using a Vicorder™ device (Skidmore Medical Ltd, Bristol, UK). The patient was positioned at 35°, and a neck and femoral cuff were applied over clothing. The aortic path length was measured as the distance between the patient's supra-sternal notch and midpoint of the thigh cuff. The neck and femoral cuffs were inflated simultaneously and the oscillometric signal detected by each cuff is analysed by the Vicorder™ device to give the pulse time delay and thus aortic PWV. The Vicorder™ measurements were carried out twice and the results averaged.
6.4.5. Statistical Analyses

Continuous variables were assessed for normality by the Kolmogorov-Smirnov test and presented as mean (standard deviation, SD). Categorical variables are presented as frequencies and percentages. Comparison of baseline characteristics between patients with and without LVH, with concentric versus eccentric LVH, and preserved versus impaired LVEF were carried out using, where appropriate, unpaired t-test or Mann-Whitney U test for continuous variables, and Fisher's exact test or Chi-squared test for categorical variables. Univariate and multivariable cox regression analysis was performed to determine factors that were associated with all-cause mortality in the sub-groups of patients with LVH and then repeated in those with preserved LVEF. The multivariable Cox regression model was adjusted for factors that were significant on univariate analysis (P<0.05) and also for factors known to be important for prediction of mortality (history of coronary artery disease and diabetes mellitus). PWV and GLS were included to assess the significance of each after adjustment. All statistical analyses were performed in SPSS version 22.0 (SPSS, Inc, Chicago, IL). A two-sided P value of <0.05 was considered statistically significant. Intra- and inter-observer variability were ascertained for measurements of GLS in 20 randomly selected patients using Bland-Altman analysis of agreement by 2 independent observers (DC and JH). Inter-observer variability for GLS had a bias of 0.54±4.73 (95% limits of agreement, LOA, -8.73 to 9.81) and intra-observer variability was -0.36±1.00, 95% LOA -2.33 to 1.60.

6.5 Results

6.5.1. Clinical and Echocardiographic Characteristics

Out of 219 HD patients enrolled there were 198 patients who had adequate echocardiographic images for analysis (Figure 6-1). 68.7% were Caucasian males with a median age of 64.2 (52.8 - 72.9) years. There were 78 (39%) patients who had diabetes mellitus and 55 (28%) had prevalent coronary artery disease. The mean LVEF was 61.7±10.1%, and median LVMI/HT² was 50.6 (40.9-65.3) g/m² (for men, 52.0 [40.4-66.9] g/m² and women, 49.5 [41.4-60.2] g/m²); the mean GLS was -13.5±3.3% and PWV was 8.8 ± 2.1 m/s.
Figure 6.1. Inclusion, exclusion criteria and final number of patients available for analysis.

Using the accepted LVM/Ht².7 cut offs indicated that there were 100 (51%) patients with LVH, 56 with concentric hypertrophy and 44 had eccentric hypertrophy. The clinical, laboratory and echocardiographic characteristics of patients with and without LVH are shown in Table 6-1. Patients who had LVH had a higher body mass index (27.7 vs 25.9 kg/m², P=0.051), higher HD ultrafiltration volume (2.31 vs 2.06 L, P=0.018), higher systolic blood pressure (148 vs 139 mmHg, P=0.001), higher diastolic blood pressure (78 vs 75 mmHg, P=0.027) and greater calcium channel blocker use (55 vs 34%, P=0.001) compared with patients without LVH. Patients with LVH had increased markers of diastolic dysfunction such as tissue doppler determined E/e' (12.0 vs 9.9, P=0.018) and enlarged left atrial volume (15.2 vs 13.8 ml/m².7, P=0.029). There was no difference between LVEF (P=0.944) between patients with and without hypertrophy, although patients with LVH had a less negative (more abnormal) GLS (-12.7 vs -14.4%, P<0.001). PWV was not different between groups (8.70 vs 8.93 m/s, P=0.442).
Table 6-1. Clinical, laboratory, echocardiographic and Vicorder™ characteristics of the study cohort between patients with and without left ventricular hypertrophy.

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Total population (n=198)</th>
<th>Left ventricular hypertrophy (n=100)</th>
<th>No left ventricular hypertrophy (n=98)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.2 (52.8-72.9)</td>
<td>64.9 (56.3-71.5)</td>
<td>63.5 (50.8-74.5)</td>
<td>0.536</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>136 (69)</td>
<td>66 (66)</td>
<td>70 (71)</td>
<td>0.446</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>161 (81)</td>
<td>81 (81)</td>
<td>80 (82)</td>
<td>1.000</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>129 (65)</td>
<td>68 (68)</td>
<td>61 (62)</td>
<td>0.456</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (23.7-30.4)</td>
<td>27.7 (24.8-31.6)</td>
<td>25.9 (22.6-29.6)</td>
<td>0.051</td>
</tr>
<tr>
<td>HD vintage (months)</td>
<td>16.7 (5.8 - 44.3)</td>
<td>13.1 (5.2 - 41.4)</td>
<td>19.4 (7.7 - 46.9)</td>
<td>0.071</td>
</tr>
<tr>
<td>Mean UF volume achieved (L)</td>
<td>2.19 (0.74)</td>
<td>2.31(0.76)</td>
<td>2.06 (0.70)</td>
<td>0.018</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143 (20)</td>
<td>148 (19)</td>
<td>139 (20)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 (12)</td>
<td>78 (12)</td>
<td>75 (11)</td>
<td>0.027</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>78 (39)</td>
<td>43 (43)</td>
<td>35 (36)</td>
<td>0.312</td>
</tr>
<tr>
<td>Prevalent coronary artery disease (%)</td>
<td>55 (28)</td>
<td>31 (31)</td>
<td>24 (24)</td>
<td>0.343</td>
</tr>
<tr>
<td>ACEI/ARB (%)</td>
<td>82 (41)</td>
<td>44 (44)</td>
<td>38 (39)</td>
<td>0.382</td>
</tr>
<tr>
<td>B-Blocker (%)</td>
<td>91 (46)</td>
<td>52 (52)</td>
<td>39 (40)</td>
<td>0.081</td>
</tr>
<tr>
<td>CCB (%)</td>
<td>87 (44)</td>
<td>55(55)</td>
<td>32 (34)</td>
<td>0.001</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>124 (63)</td>
<td>62 (62)</td>
<td>62 (63)</td>
<td>0.881</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.7 (1.2)</td>
<td>10.7 (1.2)</td>
<td>10.7 (1.3)</td>
<td>0.787</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.37 (0.14)</td>
<td>2.38 (0.15)</td>
<td>2.38 (0.14)</td>
<td>0.969</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.55 (0.45)</td>
<td>1.58 (0.44)</td>
<td>1.51 (0.46)</td>
<td>0.226</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39 (36-41)</td>
<td>40 (37-41)</td>
<td>38 (36-41)</td>
<td>0.876</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>9.4 (2.9-21.0)</td>
<td>8.6 (3.0-21.0)</td>
<td>9.2 (2.8-22.9)</td>
<td>0.352</td>
</tr>
<tr>
<td>MV E/A ratio</td>
<td>0.82 (0.68-1.10)</td>
<td>0.82 (0.66-1.00)</td>
<td>0.86 (0.71-1.10)</td>
<td>0.252</td>
</tr>
<tr>
<td>TDI E/e' ratio</td>
<td>11.0 (6.5)</td>
<td>12.0 (7.2)</td>
<td>9.9 (5.6)</td>
<td>0.018</td>
</tr>
<tr>
<td>TDI E/A ratio</td>
<td>0.86 (1.54)</td>
<td>0.71 (0.42)</td>
<td>1.02 (2.14)</td>
<td>0.163</td>
</tr>
<tr>
<td>LAVi (ml/m²)</td>
<td>14.3 (11.5 - 17.9)</td>
<td>15.2 (12.2-19.1)</td>
<td>13.8 (10.9-16.6)</td>
<td>0.029</td>
</tr>
<tr>
<td>LVMI/HT².²7 (g/m²²)</td>
<td>52.5 (18.5)</td>
<td>65.0 (13.3)</td>
<td>39.2 (13.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>61.7 (10.1)</td>
<td>61.6 (8.6)</td>
<td>61.7 (11.5)</td>
<td>0.944</td>
</tr>
<tr>
<td>GLS (%)</td>
<td>-13.5 (3.3)</td>
<td>-12.7 (3.1)</td>
<td>-14.4 (3.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>8.81 (2.08)</td>
<td>8.70 (1.94)</td>
<td>8.93 (2.22)</td>
<td>0.442</td>
</tr>
</tbody>
</table>

Data is presented as categorical data n (%), continuous data as mean (standard deviation), median (25th-75th centile).

Abbreviations: BMI, Body mass index, UF, ultra-filtration, ACE/ARB, angiotensin converting enzyme inhibitor/angiotensin II receptor blocker, CCB, calcium channel blocker, CRP, C-reactive protein, LVEF, left ventricular ejection fraction, LVMI/HT².²7, left ventricular mass indexed to height².²7, MV E/A, early to late transmitral velocity ratio, TDI E/e', early transmitral velocity to tissue Doppler mitral annular early diastolic velocity ratio, TDI E/A, tissue Doppler transmitral velocity early to late ratio, LAVi, left atrial volume indexed to height².²7, GLS, global longitudinal strain, PWV, pulse wave velocity.
When comparison was made between baseline characteristic and echocardiographic differences between concentric and eccentric hypertrophy groups, there was no difference between any parameter. In particular, GLS (-12.6 vs -12.8m/s, P=0.774) and PWV (8.71 vs 8.68 m/s, P=0.929) were very similar (Table 6-2).

Table 6-2. Baseline, laboratory, echocardiographic and Vicorder™ characteristics comparison between patients with concentric versus eccentric left ventricular hypertrophy (LVH).

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Concentric LVH (n=56)</th>
<th>Eccentric LVH (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.8 (54.2-68.3)</td>
<td>68.6 (56.3-74.3)</td>
<td>0.510</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>40 (71)</td>
<td>26 (59)</td>
<td>0.210</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>42 (75)</td>
<td>39 (87)</td>
<td>0.123</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>37 (66)</td>
<td>31 (70)</td>
<td>0.672</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.1 (24.2-31.7)</td>
<td>28.3 (25.4-31.4)</td>
<td>0.792</td>
</tr>
<tr>
<td>Haemodialysis vintage (months)</td>
<td>13.4 (5.0 - 41.4)</td>
<td>13.1 (5.3 - 42.6)</td>
<td>0.938</td>
</tr>
<tr>
<td>Mean UF volume achieved (L)</td>
<td>2.26 (0.69)</td>
<td>2.34 (0.81)</td>
<td>0.603</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>149 (19)</td>
<td>146 (18)</td>
<td>0.544</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 (12)</td>
<td>78 (12)</td>
<td>0.995</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>26 (46)</td>
<td>17 (39)</td>
<td>0.542</td>
</tr>
<tr>
<td>Prevalent coronary artery disease (%)</td>
<td>17 (30)</td>
<td>14 (32)</td>
<td>1.000</td>
</tr>
<tr>
<td>Heart failure (%)</td>
<td>19 (34)</td>
<td>22 (50)</td>
<td>0.151</td>
</tr>
<tr>
<td>ACEI/ARB (%)</td>
<td>26 (46)</td>
<td>18 (41)</td>
<td>0.838</td>
</tr>
<tr>
<td>B-Blocker (%)</td>
<td>30 (54)</td>
<td>22 (50)</td>
<td>1.000</td>
</tr>
<tr>
<td>Calcium channel blocker (%)</td>
<td>34 (61)</td>
<td>21 (48)</td>
<td>0.303</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>37 (66)</td>
<td>25 (57)</td>
<td>0.523</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.7 (1.1)</td>
<td>10.7 (1.2)</td>
<td>0.886</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.38 (0.15)</td>
<td>2.38 (0.15)</td>
<td>0.856</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.61 (0.49)</td>
<td>1.57 (0.38)</td>
<td>0.742</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>38 (40)</td>
<td>38 (40)</td>
<td>0.929</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>15.3 (23.0)</td>
<td>14.4 (13.7)</td>
<td>0.802</td>
</tr>
<tr>
<td>MV E/A ratio</td>
<td>0.89 (0.33)</td>
<td>0.86 (0.28)</td>
<td>0.569</td>
</tr>
<tr>
<td>TDI E/e'</td>
<td>14.0 (6.5)</td>
<td>12.7 (6.03)</td>
<td>0.349</td>
</tr>
<tr>
<td>TDI E/A</td>
<td>0.76 (0.26)</td>
<td>0.73 (0.33)</td>
<td>0.577</td>
</tr>
<tr>
<td>LAVi (ml/m²)</td>
<td>16.5 (6.5)</td>
<td>16.3 (5.9)</td>
<td>0.926</td>
</tr>
<tr>
<td>LVMI/HT²/³ (g/m²)</td>
<td>64.3 (13.5)</td>
<td>66.0 (13.0)</td>
<td>0.522</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>60.9 (7.6)</td>
<td>62.5 (9.9)</td>
<td>0.388</td>
</tr>
<tr>
<td>Global longitudinal strain (%)</td>
<td>-12.6 (3.2)</td>
<td>-12.8 (3.0)</td>
<td>0.774</td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td>8.71 (1.80)</td>
<td>8.68 (2.12)</td>
<td>0.929</td>
</tr>
</tbody>
</table>

Data is presented as categorical data n (%), continuous data as mean (standard deviation) or median (25th-75th centile).

Abbreviations: UF, ultra-filtration, ACE/ARBI, angiotensin converting enzyme inhibitor/angiotensin II receptor blocker, CRP, C-reactive protein, LVEF, left ventricular ejection fraction, LVMI/HT²/³, left ventricular mass indexed to height²/³, MV E/A, early to late transmirtal velocity ratio, TDI E/e', early transmirtal velocity to tissue Doppler mitral annular early diastolic velocity ratio, TDI E/A, tissue Doppler transmirtal velocity early to late ratio, LAVi, left atrial volume indexed to height²/³.
There were 171 (86%) patients with preserved ejection fraction; 66% were male, median age 63.1 (25th-75th centile, 51.2-71.8) years and 81% were caucasian. The median dialysis vintage of the group was 16.1 (5.8 - 45.0) months with a mean systolic blood pressure of 143±19 mmHg and diastolic blood pressure of 77±12 mmHg. There were 62 (36%) patients with diabetes mellitus and 40 (23%) with a history of coronary artery disease.

Table 6-3. Baseline characteristic comparisons between patients with and without preserved LVEF.

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Preserved LVEF (n=171)</th>
<th>LVEF &lt;50% (n=27)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.1 (51.2-71.8)</td>
<td>66.6 (59.2-75.9)</td>
<td>0.032</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>113 (66)</td>
<td>23 (85)</td>
<td>0.039</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>139 (81)</td>
<td>22 (81)</td>
<td>1.000</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>107 (63)</td>
<td>22 (81)</td>
<td>0.051</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.0 (24.2-30.5)</td>
<td>24.0 (22.1-28.7)</td>
<td>0.138</td>
</tr>
<tr>
<td>Haemodialysis vintage (months)</td>
<td>16.1 (5.8 - 45.0)</td>
<td>17.4 (6.4 - 33.8)</td>
<td>0.075</td>
</tr>
<tr>
<td>Mean UF volume achieved (L)</td>
<td>2.21 (0.71)</td>
<td>1.99 (0.77)</td>
<td>0.219</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143 (19)</td>
<td>149 (27)</td>
<td>0.336</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 (12)</td>
<td>71 (13)</td>
<td>0.051</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>62 (36)</td>
<td>16 (59)</td>
<td>0.052</td>
</tr>
<tr>
<td>Prevalent coronary artery disease (%)</td>
<td>40 (23)</td>
<td>15 (56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACEI/ARB (%)</td>
<td>69 (40)</td>
<td>9 (33)</td>
<td>0.815</td>
</tr>
<tr>
<td>B-Blocker (%)</td>
<td>76 (44)</td>
<td>15 (56)</td>
<td>0.344</td>
</tr>
<tr>
<td>Calcium channel blocker (%)</td>
<td>79 (46)</td>
<td>8 (30)</td>
<td>0.345</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>104 (61)</td>
<td>20 (74)</td>
<td>0.334</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.7 (1.2)</td>
<td>10.7 (1.3)</td>
<td>0.964</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.38 (0.14)</td>
<td>2.36 (0.13)</td>
<td>0.662</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.55 (0.46)</td>
<td>1.49 (0.41)</td>
<td>0.538</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39 (40)</td>
<td>37 (40)</td>
<td>0.143</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>15.8 (21.2)</td>
<td>17.2 (23.9)</td>
<td>0.808</td>
</tr>
<tr>
<td>MV E/A ratio</td>
<td>0.91 (0.33)</td>
<td>0.94 (0.41)</td>
<td>0.772</td>
</tr>
<tr>
<td>TDI E/e'</td>
<td>12.1 (5.6)</td>
<td>14.9 (5.0)</td>
<td><strong>0.044</strong></td>
</tr>
<tr>
<td>TDI E/A</td>
<td>0.97 (1.70)</td>
<td>0.74 (0.35)</td>
<td>0.153</td>
</tr>
<tr>
<td>LAVi (ml/m²)</td>
<td>15.2 (5.8)</td>
<td>15.7 (5.8)</td>
<td>0.732</td>
</tr>
<tr>
<td>LVM/HT² (g/m²)</td>
<td>51.8 (18.7)</td>
<td>59.6 (15.8)</td>
<td>0.051</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>63.8 (8.3)</td>
<td>43.8 (5.4)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Global longitudinal strain (%)</td>
<td>-13.8 (3.2)</td>
<td>-10.9 (3.3)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td>8.80 (2.11)</td>
<td>9.10 (2.26)</td>
<td>0.589</td>
</tr>
</tbody>
</table>

Data is presented as categorical data n (%), continuous data as mean (standard deviation), median (25th-75th centile).

Abbreviations: UF, ultra-filtration, ACE/ARB, angiotensin converting enzyme inhibitor/angiotensin II receptor blocker, CRP, C-reactive protein, LVEF, left ventricular ejection fraction, LVM/HT², left ventricular mass indexed to height².⁷, MV E/A, early to late transmitral velocity ratio, TDI E/e', early transmitral velocity to tissue Doppler mitral annular early diastolic velocity ratio, TDI E/A, tissue Doppler transmitral velocity early to late ratio, LAVi, left atrial volume indexed to height².⁷.
In patients with preserved LVEF, the mean LVEF was 63.8±8.3% and GLS was -13.8±3.2%, with mean LVMI/HT $^{2.7}$ 51.8±18.7g/m$^{2.7}$ and PWV 8.80±2.11m/s. A comparison of baseline characteristics of patients with and without preserved LVEF is illustrated in Table 6-3. Patients with a reduced LVEF were older (66.6 vs 63.1 years, $P=0.032$), with greater male preponderance (85% vs 66%, $P=0.039$), significantly more prior coronary artery disease (56% vs 23%, $P<0.001$), diastolic dysfunction with abnormal E/e' (14.9 vs 12.1, $P=0.044$) and a less negative (more abnormal) GLS (-10.9 vs -13.8%, $P=0.001$). There was no difference in PWV (9.10 vs 8.8m/s, $P=0.589$).

6.5.2. Prognostic Value of GLS and PWV in LVH (N=100)

For the sub-group of patients with LVH, the median follow-up duration was 27.1 (25th - 75th centile, 16.8-31.2) months. There were 21 deaths of which 5 were due to cardiac causes. The causes of death included 1 due to myocardial infarction, 4 due to sudden cardiac death, 3 due to ESKD, 6 due to infection, 3 due to cancer and 4 due to other causes. Sixteen patients received a renal transplant.

On univariate analysis, the factors significant for all-cause mortality were age (HR 1.08, 95% CI 1.03-1.14, $P=0.002$) and PWV (HR 1.33, 95% CI 1.08-1.63, $P=0.008$). LVEF and GLS were not significant (HR 0.99, 95% CI 0.94-1.04, $P=0.73$ and HR 0.97, 95% CI 0.84-1.13, $P=0.72$, respectively). A multivariable Cox regression model adjusting for factors significant on univariate analysis (PWV and age) and factors decided a priori to be important (diabetes mellitus, history of coronary artery disease and GLS) was undertaken. The only factor which remained significant for all-cause mortality was age (adjusted HR 1.07, 95% CI 1.01-1.13, $P=0.019$). GLS and PWV were not significant (adjusted HR 0.99, 95% CI 0.83-1.18, $P=0.923$ and HR 1.22, 95% CI 0.98-1.53, $P=0.073$, respectively). The final Cox regression model is shown in Table 6-4.
Table 6-4. Final Cox regression model with all adjusted factors for all-cause mortality in a cohort of haemodialysis patients with left ventricular hypertrophy (LVH) and preserved left ventricular ejection fraction (LVEF) groups respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For LVH group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.07</td>
<td>1.01-1.13</td>
<td><strong>0.019</strong></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.37</td>
<td>0.54-3.48</td>
<td>0.513</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>0.54</td>
<td>0.19-1.57</td>
<td>0.260</td>
</tr>
<tr>
<td>Pulse wave velocity</td>
<td>1.22</td>
<td>0.98-1.53</td>
<td>0.073</td>
</tr>
<tr>
<td>Global longitudinal strain</td>
<td>0.99</td>
<td>0.83-1.18</td>
<td>0.923</td>
</tr>
<tr>
<td><strong>For preserved LVEF group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.08</td>
<td>1.04-1.13</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Smoker</td>
<td>1.65</td>
<td>0.61-4.46</td>
<td>0.328</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.14</td>
<td>0.54-2.40</td>
<td>0.741</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>0.50</td>
<td>0.20-1.22</td>
<td>0.127</td>
</tr>
<tr>
<td>Pulse wave velocity</td>
<td>1.23</td>
<td>1.04-1.45</td>
<td><strong>0.015</strong></td>
</tr>
<tr>
<td>Global longitudinal strain</td>
<td>1.16</td>
<td>1.01-1.33</td>
<td><strong>0.031</strong></td>
</tr>
</tbody>
</table>

Note: The hazard ratios are expressed as per unit increase in variable. Therefore for global longitudinal strain, per 1% increase in global longitudinal strain (the more positive, the more abnormal the strain), there is a 1.16 times higher chance of mortality (in patients with preserved left ventricular ejection fraction). Similarly in the left ventricular hypertrophy group, for a pulse wave velocity with a hazard ratio of 1.22, there is a 1.22 times more likely risk of death per 1 m/s increase in pulse wave velocity. Although this increase is non-significant, it is as one would expect; the greater the pulse wave velocity, more increased arterial stiffness and hence increased risk of mortality.

6.5.3. Prognostic Value of GLS and PWV in Preserved LVEF (N=171)

During a median follow-up of 28.2 (25th-75th centile, 17.8 - 33.4) months there were 33 deaths, of which 10 (30%) were cardiac deaths. The causes of death included 4 due to myocardial infarction, 5 due to sudden cardiac death, 1 due to heart failure, 3 due to ESKD, 9 due to infection, 4 due to cancer and 7 due to other causes. There were 33 patients who received a renal transplant.

On univariate analysis, factors associated with all-cause mortality included age (HR 1.08, 95% CI 1.04-1.12, P<0.001), smoker (HR 2.64, 95% CI 1.09-6.04, P=0.031) and PWV (HR 1.22, 95% CI 1.09-1.37, P=0.001). GLS was not significantly associated (HR 1.10, 95% CI 0.98-1.22, P=0.093). In a multivariable analysis including all factors significant on
univariate analysis (age, smoker, PWV) and factors decided a priori to be important (history of coronary artery disease, diabetes mellitus, GLS), the significant associated factors included age (adjusted HR 1.08, 95% CI 1.04-1.13, P<0.001), PWV (adjusted HR 1.23, 95% CI 1.04-1.45, P=0.015) and GLS (adjusted HR 1.16, 95% CI 1.01-1.33, P=0.031; Table 6-4).

6.6 Discussion

This study has examined the associations and outcomes of two sub-groups of HD patients categorised by their echocardiographic characteristics, patients with LVH irrespective of LVEF and secondly, those with preserved LVEF. GLS and PWV have both independently been shown to be associated with all-cause mortality in CKD patients (4,15,16) and we have recently reported both in a multivariable model (6) in an unselected HD population. We hypothesised that the relative importance of GLS and PWV would be different in patients with either LVH or preserved LVEF, as we assumed that the pathophysiology and baseline characteristics would be different compared to patients without LVH and reduced LVEF, respectively.

We found that patients with LVH had a higher body mass index, systolic and diastolic blood pressures, ultrafiltration volume and were more likely to be taking calcium channel blockers, which are all known associations of LVH in HD patients (10). GLS has been shown to be more abnormal in patients with LVH compared to those without. We chose to study GLS rather than radial or circumferential strain because although LVH can affect all directions of strain (17), longitudinal strain is typically the first to be affected. This has been shown in hypertensives (18) and patients with ischemic myocardium (19). In one study of patients with hypertension, longitudinal deformation abnormalities were present before the hypertension was detected (18). Longitudinal strain was affected before circumferential and radial strain in patients with increased severity of aortic stenosis and LVH (20). It is thought that deformation in the sub-endocardium accounts for longitudinal strain abnormalities; this layer is affected first, and before progressive changes occur through the myocardial wall in cases of increasing pressure overload and ischaemia.

Concentric hypertrophy (increased left ventricular RWT and LV mass) may be a result of pressure overload from hypertension, arterial stiffness due to vascular calcification, or
increased sympathetic activation. The earliest physiological adaptation to pressure overload is 'concentric remodelling' with increase in RWT before increase in LV mass. Conversely, eccentric hypertrophy is due to volume overload from factors such as excess intra-dialytic weight gain, oliguria, renal anaemia, and the effect of high flow arteriovenous fistulae. This involves an increase in LV mass, but little augmentation in RWT.

We found no difference in baseline characteristics when patients with concentric and eccentric hypertrophy were compared in our HD cohort. Moreover, there were no differences in GLS and PWV between these sub-groups which contrasts with a similar sized study in 98 HD patients with preserved LVEF (8). This found that longitudinal strain was lower (more abnormal) in the concentric hypertrophy compared with eccentric hypertrophy group (-15.5 +/- 2.2% vs -17.8 +/- 2.6%). Other study groups have found unexpected associations; for example, in certain hypertensive populations more patients have been found to have eccentric hypertrophy compared with concentric LVH (21,22), which would be contrary to what is expected in patients who presumably have pressure overload as the major pathophysiological mechanism. These were studies performed over ! decades ago. In our study cohort, one potential explanation may be that using 2D transthoracic echocardiography to determine the LV measurements such as RWT may be too insensitive to correctly classify patients into different LV geometries in this population. It relies on patients being at dry weight, having clear endocardial definitions and there will be some operator dependent variability. One group has suggested that eccentric and concentric hypertrophy should be further sub-divided into whether concentricity is increased or left ventricular end diastolic volume is increased (23), as when classified as such there were significant biomarker differences and potential cardiac stress differences between these groups. Therefore it may be that the classification of eccentric and concentric hypertrophy needs to be refined in order to differentiate patients more appropriately.

In our multivariable model for all-cause mortality in patients with LVH, the only significant factor associated was age; PWV and GLS were not independently associated. It may be that in patients with LVH the PWV is similar in the patients and therefore insensitive at discerning higher cardiovascular risk. GLS is likely to have been abnormal in both concentric and eccentric hypertrophy because in LVH, the longitudinal myocardial fibers are adversely affected through inflammation, fibrosis, myocardial capillary
degeneration and myocyte apoptosis (24). Due to the GLS being affected to the same degree in both types of hypertrophy, it may be too insensitive to be prognostic of mortality. To our knowledge, there has been no study investigating the prognostic implications of GLS in different LVH geometry groups.

We proceeded to investigating patients according to whether or not they had preserved LVEF, and found that patients with a reduced LVEF were older, more likely to be male, had a greater prevalence of coronary artery disease and more abnormal GLS. However, there was no difference in PWV between groups (9.10 vs 8.8m/s, P=0.589). These findings are supportive of the hypothesis that repeated myocardial stunning during HD sessions may contribute to subsequent heart failure (25,26), rather than increased aortic stiffness being the predominant mechanism.

In the multivariable model for all-cause mortality for patients with preserved LVEF both PWV (adjusted HR 1.23, 95% CI 1.04-1.45, P=0.015) and GLS (adjusted HR 1.16, 95% CI 1.01-1.33, P=0.031) were found to be independent associated factors. Consistent with current literature, GLS has been consistently reported as an early sensitive marker of all-cause mortality in patients with preserved LVEF. Furthermore in dialysis patients there is an acceleration of arterial ageing with medial calcification, arterial dilatation and increased wall thickness (27,28) and hence it is not surprising that PWV is also a sensitive marker of mortality. However, in our recent study of a HD population including patients with all LVEF values, PWV was a more sensitive marker of all-cause mortality than GLS (adjusted HR 1.23, 95% CI 1.03-1.47) (6). It may be that when patients with poor LVEF are included, the value of GLS as a subtle marker of LV dysfunction is lost.

This study has some limitations. There were some patients who were excluded from the study analysis, including patients who had inadequate images or with poor LV geometry; in these patients GLS could not be assessed. Secondly, for logistic reasons, patients who were immobile could not participate in the study due to transport issues (they would require ambulances) for non-dialysis day tests. This sub-group of patients is likely to have had different co-morbidities.

In conclusion, we report that there are some differences between patients with and without LVH and with and without preserved LVEF. Although GLS is more abnormal in patients with LVH, we could find no difference in this or other characteristics in patients with
eccentric and concentric hypertrophy. Neither GLS nor PWV were shown to have an independent association with all-cause mortality in patients with LVH. In contrast, both are significant predictors of mortality in patients with preserved LVEF. Consistent with our initial hypothesis, the predictive value of GLS and PWV are different in the sub-groups of LVH and preserved LVEF. Due to the majority (86% in our study sample) of prevalent HD patients having a preserved LVEF, we would still recommend that both GLS and PWV could be utilised routinely as rapidly acquired non-invasive and sensitive risk stratification methods in assessing patients undergoing HD.

6.7 References

Chapter 7

Speckle Tracking Determination of Tissue Motion Annular Displacement: Comparison with Strain and Ejection Fraction, and Association with Outcomes in Haemodialysis Patients


7.1 Preface

Chapter 5 and 6 has assessed the prognostic implications of global longitudinal strain, this chapter evaluates the predictive value of another novel cardiac marker for mortality which can be measured by speckle tracking echocardiography- tissue motion annular displacement of the mitral valve (TMAD). TMAD has potential advantages compared to global longitudinal strain in that it can be performed quicker than global longitudinal strain and it does not depend on good endocardial definition. This has never been investigated in an end stage kidney disease population and it is unknown how it compares with global longitudinal strain. If TMAD correlates with global longitudinal strain and is a good prognostic marker, then it may be useful in routine clinical practice. This chapter, therefore, aims to assess the correlation of TMAD with global longitudinal strain and left ventricular ejection fraction and the prognostic value of TMAD for mortality, cardiac death and cardiac events.

7.2 Abstract

Background: Cardiac imaging tools that provide a rapid and easy assessment of left ventricular (LV) systolic function are important in establishing cardiovascular risk in end stage kidney disease (ESKD) patients because cardiovascular mortality is high. Abnormal Global longitudinal strain (GLS) and reduced LV ejection fraction (LVEF) are established poor prognostic risk factors. Tissue motion annular displacement of mitral valve annulus (TMAD), determined by speckle tracking echocardiography, can be performed more rapidly and is also an indicator of LV systolic function, but has been less well explored.
This study aims to compare TMAD with GLS and LVEF and its association with outcomes in ESKD patients.

**Methods and Results:** 198 ESKD patients receiving haemodialysis (median age 64.2 years, 69% men) had analysable 2D transthoracic echocardiography, with speckle tracking determined GLS and TMAD. TMAD had low inter- and intra-observer variability with small biases and narrow limits of agreement [LOA] (bias of -0.01 ± 1.32 (95% LOA was -2.60 to 2.58) and -0.07 ± 1.27 (95% LOA -2.55 to 2.41) respectively). There was a moderate negative correlation between GLS and LVEF (r=-0.383, P<0.001) and a weak positive correlation between TMAD and LVEF (r=0.248, P<0.001). There was strong negative correlation of TMAD with GLS (r=-0.614, P<0.001). In a multivariable Cox regression analysis, TMAD was not associated with mortality (HR 1.04, 95% CI 0.91-1.19, P=0.533), cardiac death (HR 1.03 (95% CI 0.80-1.32, P=0.841) or cardiac events (HR 0.91 (95% CI 0.80-1.02, P=0.111).

**Conclusion:** TMAD did not have independent prognostic value in this ESKD population despite some apparent advantages over conventional methods of systolic functional measurements.

### 7.3 Introduction

Patients with end stage kidney disease (ESKD) have a very high burden of cardiovascular mortality (1). Abnormality of conventional echocardiographic measurements, such as a reduced left ventricular ejection fraction (LVEF), are associated with all-cause mortality(2) in this setting. However, LVEF is generally well preserved in ESKD. For example, one study reported that in the prevalent dialysis population, mean LVEF was between 44.2 and 60.8% (3) and for new starters, LVEF <50%, was observed in just 13.2% of patients (2). Determination of LVEF may be inaccurate in ESKD with conventional two-dimensional (2D) transthoracic echocardiography. This is due to the frequent finding of left ventricular hypertrophy (LVH) [75% of haemodialysis patients] and regional wall motion abnormalities which are not consistent with the assumption that the left ventricle is perfectly ellipsoid when using calculations such as area-length method or Teicholz formula to derive LVEF (4). Biplane Simpson's rule of discs improves upon this estimation by dividing the left ventricle into segments but this can be time-consuming.

There are now emerging technologies that provide rapid sub-clinical (i.e. with preserved LVEF) measurements of systolic function which are also not susceptible to the
inaccuracies found in LVH and regional wall motion abnormalities. Speckle Tracking Echocardiography (STE) is becoming readily available in routine practice. This method quantifies regional myocardial tissue deformation by frame-by-frame tracking of acoustic speckles. From this, LV global longitudinal strain (GLS) can be determined. A less negative, more abnormal, GLS has been shown to be associated with mortality in ESKD patients undergoing haemodialysis independent of LVEF (5,6).

Tissue motion mitral valve annular displacement (TMAD) may also be measured by STE. STE tracks the longitudinal movement of the mitral valve during systole and this measure gives an indication of the LV systolic function. This measure can be determined far more rapidly than GLS - in less than 10 seconds. Furthermore, this method has added advantages over GLS and LVEF measurements because it is load independent and does not require as well-defined endocardial border definitions. This may be particularly useful in ESKD patients where obtaining adequate 2D echocardiographic images may be difficult due to dyspnoea and increased body surface area due to fluid overload. TMAD has been reported to correlate with LVEF determined by magnetic resonance imaging (MRI) in the general population without renal disease (7,8) and in children (9).

No study has explored the use of TMAD in ESKD patients undergoing haemodialysis; it is unknown how TMAD correlates with LVEF and GLS in ESKD or whether it has the same independent prognostic value for all-cause mortality, cardiac death and cardiac events as the two latter measurements. If TMAD was shown to be independently prognostically significant then it may act as a useful tool to replace GLS as a quicker and more reproducible method of LV systolic function assessment. This study therefore aimed to determine, in a prospective single centre observational study of haemodialysis patients:

(1) the relationship between TMAD and LVEF and GLS

(2) the independent association of TMAD with all-cause mortality, cardiac death and major cardiac events
7.4 Methods

7.4.1. Patients and Protocols

This was a prospective observational study involving 2D echocardiography with STE to identify factors associated with outcomes. All incident and prevalent adult (≥18 years) patients receiving maintenance haemodialysis between March 2012 to 2014 at Salford Royal Hospital NHS Foundation Trust or any of its four satellite units were approached to enter the study and enrolled if written informed consent was gained. The only exclusion criteria were if they were unable to consent or if patients were immobile or oxygen dependent as transport was not able to be provided in such cases for a non-dialysis day echocardiography. All patients received thrice weekly in-centre haemodialysis. The study adhered to the Declaration of Helsinki and local ethical approval (UK, REC 05/Q1404/188) had been granted. It was funded by a research grant from Kidney Research UK.

Clinical background data including baseline demographics, co-morbidities and medications were obtained from patient self-reporting, and verified from the hospital electronic medical records and contact with the patients' primary care physician. Dialysis prescriptions and laboratory data was obtained from the electronic medical records. Blood tests were taken from the haemodialysis circuit every month. The mean value of these blood tests for the three months prior to echocardiography was used in the analyses.

7.4.2. Two-Dimensional Echocardiography

Consented patients underwent conventional 2D echocardiography performed by an experienced technician (JS) or a consultant cardiologist (NA) on a non-dialysis day. All patients were examined in the left lateral decubitus position using echocardiographic equipment with M-mode and 2D capabilities with a 3.5 MHz transducer (Philips Medical Systems, Philips UK Ltd, United Kingdom). Greyscale images were optimised at a frame rate of > 60 per second and at least three consecutive heartbeats were acquired with each image. Standard apical views were acquired (i.e. 4 chamber, 2 chamber and 3 chamber). Measurements were performed in accordance to the guidelines of the European Society of Echocardiography (10). All echocardiographic images were saved on the Philips Xcelera R4.1 image management system for offline analysis. Biplanes Simpsons method of discs
was applied to apical 4- and 2- chamber views to determine the LV volumes used to calculated the LVEF by \( \text{LVEF} = \frac{(\text{end-diastolic LV volume - end-systolic volume})}{\text{LV end diastolic volume}} \). An impaired LVEF was defined as <50%. LV mass was indexed to height\(^{2.7}\)(LVMI/HT\(^{2.7}\)). LVH was taken as LVMI/HT\(^{2.7}\) >46.7 g/m\(^{2.7}\) for females and >49.2g/m\(^{2.7}\) for men (11,12).

### 7.4.3. Speckle Tracking Echocardiography- GLS and TMAD

Two-dimensional echocardiographic images were analysed offline in Philips QLAB version 9 software. TMAD function was selected in the software for the apical 4-chamber and 2-chamber views. The interpreter located three points- lateral and medial edge of mitral annulus and the LV apex, in the diastolic frame. QLAB software then tracks the mitral valve annular displacement in millimetres. An average of the midpoint displacement in 4-chamber and 2-chamber views were calculated (Chapter 3, Figure 3-4).

Similarly, using the CMQ function in the QLAB software, in selected 4-chamber, 2-chamber and 3-chamber views, the same three points as for TMAD were determined by the interpreter. The software then automatically tracks the movement of the endocardial speckles from the endocardial border from end-diastole to end-systole. Any part of the myocardium that was imprecisely tracked was manually modified by the interpreter. A strain measurement was given for each individual myocardial segment. An average of all segments then gave the GLS (Chapter 3, Figure 3-3).

All offline analyses were performed by a trained interpreter (DC). In 20 randomly selected patients, another trained interpreter (JH) repeated the measurements for Biplanes' Simpson's method of determination of LVEF and speckle tracking analysis of GLS and TMAD. DC then repeated the same analysis 3 months after initial interpretation for intra-observer variability. Bland-Altman analysis was performed to determine the bias and limits of agreement for inter-observer and intra-observer variability.

### 7.4.4. Follow-up

All recruited patients were followed-up prospectively from time of echocardiographic assessment until death, renal transplantation, re-location out of the country or the 16th November 2015. Cardiac events were collated from patient self-reporting, review of
electronic medical records and contact with the patients' primary care physicians. The primary outcome was all-cause mortality. The secondary outcomes were cardiac death (death due to myocardial infarction, heart failure, arrhythmia or sudden cardiac death) and non fatal major cardiac events (including myocardial infarction, new angina, hospitalisation due to heart failure or arrhythmia, coronary revascularisation/coronary bypass surgery). All events were adjudicated by two independent assessors (DC and VK).

7.4.5. Statistical Analysis

Continuous data are presented as mean (standard deviation, SD) for normally distributed and median (25th-75th centile) for non-normally distributed variables. Categorical data are presented as numbers (percentages). Correlations between echocardiographic measurements of systolic function including LVEF, GLS, and TMAD were ascertained using linear regression with Pearson's correlation coefficient.

Survival analyses were performed for all-cause mortality, cardiac death, and major cardiac events using univariate and multivariable Cox proportional hazards models. Factors that were significant on univariate analysis (p<0.05), and variables that were decided a priori to be important such as diabetes mellitus and history of coronary artery disease, were included in each multivariate model. All statistical analyses were performed in SPSS version 22.0 (SPSS, Inc, Chicago, Illinois, USA). Two-tailed P value of <0.05 was considered statistically significant.

7.5 Results

7.5.1. Study Population

There were 435 patients who were receiving haemodialysis treatment during the recruitment period. Of these, 18 patients were excluded due to being unsuitable for study transport, 26 patients were unable to give consent, and 172 patients refused consent. Subsequently, 219 patients were enrolled into the study and 198 patients had adequate 2D echocardiographic images for analysis. Baseline demographics of the patients are shown in Table 7-1.
Table 7-1. Baseline characteristics, medications, dialysis prescriptions and medications of study cohort (N=198).

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.2 (52.8-72.9)</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>136 (69)</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>161 (81)</td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>16.7 (5.8-44.3)</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>129 (65)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.9 (23.7-30.4)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143 (20)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 (12)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>78 (39)</td>
</tr>
<tr>
<td>History of myocardial infarction (%)</td>
<td>23 (12)</td>
</tr>
<tr>
<td>History of angina (%)</td>
<td>37 (19)</td>
</tr>
<tr>
<td>Cerebrovascular disease (%)</td>
<td>15 (8)</td>
</tr>
<tr>
<td>Peripheral vascular disease (%)</td>
<td>17 (9)</td>
</tr>
<tr>
<td>History of congestive cardiac failure (%)</td>
<td>56 (28)</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>55 (28)</td>
</tr>
<tr>
<td>ACEI/ARB (%)</td>
<td>82 (41)</td>
</tr>
<tr>
<td>B-Blocker (%)</td>
<td>91 (46)</td>
</tr>
<tr>
<td>Calcium channel blocker (%)</td>
<td>87 (44)</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>124 (63)</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.7 (1.2)</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.37 (0.14)</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.55 (0.45)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39 (36-41)</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>9.4 (2.9-21.0)</td>
</tr>
</tbody>
</table>

Data represented as mean (standard deviation) for normally distributed and median (25-75th centile) for non-normally distributed continuous variable. For categorical variables, data presented as n (%).

Abbreviation: ACEI/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker.

In this study population, the median age was 64.2 (52.8 - 72.9) years, and the majority of patients were Caucasian males (69 %). The median length of time that patients had been treated with haemodialysis was 16.7 (5.8 - 44.3) months before recruitment. There were 78 patients (39%) who had diabetes mellitus, 56 (28%) with a history of congestive heart failure and 55 (28%) with prevalent coronary artery disease. Highlighting the high cardiovascular burden of this cohort, almost half of all patients were taking each of an
angiotensin converting enzyme inhibitor or angiotensin receptor blocker, B-Blocker, calcium channel blocker or statin (41%, 46%, 44% and 63% respectively).

To be as inclusive of a real world dialysis population as possible, patients with valvular disease were not excluded from the assessments. Twenty five patients had valve disease (stenosis, regurgitation and artificial heart valves), but no patients had severe lesions. Typical of a UK dialysis cohort, the majority of patients in the study had a preserved LV systolic function (LVEF ≥ 50%; 171 patients, 86%) with a mean LVEF of 61.7±10.1%.

The median LVMI/HT\(^{2.7}\) was 50.6 (40.9-65.3) g/m\(^{2.7}\) (for men, 52.0 [40.4-66.9] g/m\(^{2.7}\) and women, 49.5 [41.4-60.2] g/m\(^{2.7}\)). In the population, 51% had LVH. The mean GLS was -13.5±3.3 %, which is reduced when compared with the general population where a normal GLS has been reported to be between minus 16-22% (13). The mean TMAD was 10.0±2.5mm. In the literature, in normal control groups, mean TMAD has been reported to be 16.9±1.7mm (14), therefore it is reduced in our cohort. These echocardiographic and speckle tracking cross-sectional data are shown in Table 7-2.

**Table 7-2. Echocardiographic and speckle tracking analysis parameters for the study population (N=198).**

<table>
<thead>
<tr>
<th>Echocardiographic parameter</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preserved LVEF (%)</td>
<td>171 (86.4)</td>
</tr>
<tr>
<td>LVMI/HT(^{2.7}) (g/m(^{2.7}))</td>
<td>50.6 (40.9-65.3)</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>101 (51.0)</td>
</tr>
<tr>
<td>GLS (%)</td>
<td>-13.5 (3.3)</td>
</tr>
<tr>
<td>TMAD mid-point, 4-chamber view (mm)</td>
<td>10.1 (2.8)</td>
</tr>
<tr>
<td>TMAD mid-point, 2-chamber view (mm)</td>
<td>9.9 (2.9)</td>
</tr>
<tr>
<td>TMAD mid-point average (mm)</td>
<td>10.0 (2.5)</td>
</tr>
</tbody>
</table>

*Data represented as mean (standard deviation) for normally distributed and median (25-75th centile) for non-normally distributed continuous variable.*

*Abbreviations: LVEF, left ventricular ejection fraction, LVMI/HT\(^{2.7}\), Left ventricular mass index to height\(^{2.7}\), LVH, Left ventricular hypertrophy, GLS, Global longitudinal strain, TMAD, tissue mitral annular displacement.*

### 7.5.2. Reproducibility

TMAD had good inter- and intra-observer variability with small biases and narrow limits of agreement (LOA). For inter-observer variability this had a bias of -0.01 ± 1.32 (95% LOA -2.60 to 2.58) and intra-observer variability of -0.07 ± 1.27 (95% LOA -2.55 to 2.41), Figure 7-1. TMAD had smaller bias and 95% LOA compared with GLS measurements,
although GLS measurements had less intra- and inter-observer variability compared to Biplane Simpson's derived LVEF. For LVEF, the inter-observer variability was -1.41±3.04, 95%, LOA was -7.37 to 4.54 and intra-observer variability for LVEF had a bias of -0.50±4.31, 95%, LOA was -8.95 to 7.96, whilst the inter- and intra-observer variabilities for GLS had a bias of 0.54±4.73 (95% LOA -8.73 to 9.81) and -0.36±1.00, 95% LOA -2.33 to 1.60, respectively.

Figure 7.1. Bland-altman plots for intra- (a) and inter- (b) observer variability for TMAD measurements.

(a)

Inter-observer difference vs. average for TMAD

(b)

Intra-observer Difference vs average for TMAD

Note: For inter-observer variability this had a bias of -0.01 ± 1.32 (95% limits of agreement (LOA) was -2.60 to 2.58) and intra-observer variability of -0.07 ± 1.27 (95% LOA -2.55 to 2.41).
7.5.3. Relationship of TMAD with Two-Dimensional LVEF and GLS

There was a weak positive correlation between TMAD and 2D Biplane Simpson's LVEF ($r=0.248$, $P=0.001$). However, there was a strong negative correlation between TMAD and GLS ($r=-0.614$, $P<0.001$); as TMAD increased in value, GLS became more negative (indicating better longitudinal strain), Figure 7-2.

Figure 7-2. Correlation between tissue motion annular displacement (TMAD) and global longitudinal strain (GLS) and left ventricular ejection fraction (LVEF).

Note: There was a weak positive correlation between TMAD and 2D Biplane Simpson's LVEF ($r=0.248$, $P=0.001$). However, there was a strong negative correlation between TMAD and GLS ($r=-0.614$, $P<0.001$).

7.5.4. Survival Analysis

After a median follow-up of 27.6 (25th - 75th centile, 17.3 - 32.7) months, there were 48 deaths. 15 (31%) deaths were due to cardiac causes (8 as a result of myocardial infarction, 6 from sudden cardiac death and 1 from heart failure secondary to coronary artery disease). There were 44 major cardiac events. During follow-up, 34 patients were censored at renal transplantation, and 2 for relocating out of the country.

On univariate Cox regression analysis, TMAD was not associated with all-cause mortality (unadjusted HR 0.93, 95% CI 0.83-1.05, $P=0.226$) or cardiac death (unadjusted HR 1.00, 95% CI 0.82-.23, $P=0.985$). TMAD was associated with major cardiac events (unadjusted HR 1.13, 95% CI 1.04-1.24, $P=0.006$ and unadjusted HR 0.87, 95% CI 0.77-0.98, $P=0.22$, respectively).
In a multivariable Cox regression model including factors associated with mortality, the only factors that remained significant were age (HR 1.10, 95% CI 1.05-1.15, \( P < 0.001 \)) and reduced LVEF (HR 0.97, 95% CI 0.94-0.99, \( P=0.044 \)). TMAD was not associated with all-cause mortality (HR 1.04, 95% CI 0.91-1.19, \( P=0.533 \)). For cardiac death, in a multivariable Cox regression model, the only significant factor was a history of diabetes mellitus (adjusted HR 4.87, 95% CI 1.28-18.46, \( P=0.020 \)). TMAD was not associated with cardiac death (adjusted HR 1.03, 95% CI 0.80-1.32, \( P=0.841 \)). For major cardiac events, prior significant history of coronary artery disease was significantly associated (adjusted HR 2.27, 95% CI 1.21-4.26, \( P=0.010 \)), whereas TMAD was not associated (adjusted HR 0.91, 95% CI 0.80-1.02, \( P=0.111 \)). The final Cox regression models are shown in Table 7-3.

Table 7-3. The final Cox regression analysis models for all-cause mortality, cardiac death and major cardiac events.

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All-Cause Mortality (N=48)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.10</td>
<td>1.05-1.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.91</td>
<td>0.84-4.35</td>
<td>0.123</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.88</td>
<td>0.96-3.69</td>
<td>0.067</td>
</tr>
<tr>
<td>History of coronary artery disease</td>
<td>0.55</td>
<td>0.26-1.15</td>
<td>0.113</td>
</tr>
<tr>
<td>Congestive cardiac failure</td>
<td>1.71</td>
<td>0.89-3.29</td>
<td>0.111</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>1.01</td>
<td>0.98-1.05</td>
<td>0.446</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.94</td>
<td>0.87-1.01</td>
<td>0.106</td>
</tr>
<tr>
<td>LVMI/HT(^{2.7}) (g/m(^{2.7}))</td>
<td>1.01</td>
<td>0.99-1.03</td>
<td>0.075</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.97</td>
<td>0.94-0.99</td>
<td>0.044</td>
</tr>
<tr>
<td>Average TMAD (mm)</td>
<td>1.04</td>
<td>0.91-1.19</td>
<td>0.533</td>
</tr>
<tr>
<td><strong>Cardiac Death (N=15)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4.87</td>
<td>1.28-18.46</td>
<td>0.020</td>
</tr>
<tr>
<td>History of coronary artery disease</td>
<td>0.95</td>
<td>0.27-3.40</td>
<td>0.940</td>
</tr>
<tr>
<td>LVMI/HT(^{2.7}) (g/m(^{2.7}))</td>
<td>1.01</td>
<td>0.95-1.07</td>
<td>0.718</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>0.99</td>
<td>0.96-1.02</td>
<td>0.586</td>
</tr>
<tr>
<td>Average TMAD (mm)</td>
<td>1.03</td>
<td>0.80-1.32</td>
<td>0.841</td>
</tr>
<tr>
<td><strong>Major cardiac event (N=44)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.61</td>
<td>0.87-3.00</td>
<td>0.130</td>
</tr>
<tr>
<td>History of coronary artery disease</td>
<td>2.27</td>
<td>1.21-4.26</td>
<td>0.010</td>
</tr>
<tr>
<td>Average TMAD (mm)</td>
<td>0.91</td>
<td>0.80-1.02</td>
<td>0.111</td>
</tr>
</tbody>
</table>

**Abbreviations:** LVMI/HT\(^{2.7}\), Left ventricular mass index to height\(^{2.7}\), TMAD, tissue mitral annular displacement, LVEF, left ventricular ejection fraction.

Factors that were significant on univariate analysis \( (P<0.05) \) and variables decided a priori to be important such as diabetes mellitus and coronary artery disease were included.
7.6 Discussion

ESKD patients undergoing haemodialysis pose some specific challenges when undergoing conventional 2D transthoracic echocardiography. As demonstrated in our cohort, the majority of patients have preserved LVEF (86.4%) and LVH is prevalent (51%). This is similar to other dialysis cohorts with LVEF reported between 53-87% (2,3) and 74% with LVH in patients commencing dialysis (15). The high prevalence of LVH means that assumptions held by traditional echocardiographic calculations of LVEF may not necessarily be accurate. A more accurate method of establishing LVEF using biplane's Simpson's method requires adequate visualisation of endocardial borders, which is time consuming and labour intensive. Therefore reduced LVEF, although a well established prognostic marker, may be less than ideal as a risk stratification tool.

STE is becoming widely available in clinical practice. It may be applied to pre-obtained conventional 2D echocardiographic images and tracks reflections from the ultrasound beam known as 'speckles'. From this, measures of myocardial systolic function by GLS or TMAD (tracking of the mitral valve motion through the cardiac cycle) can be derived. There is established data on GLS and poor outcome in ESKD, but TMAD has not been explored. TMAD has added advantages over GLS in that it is less dependent on adequate image quality and it is much quicker to perform (on average taking less than 10 seconds).

In the general population, TMAD has been reported to be good at predicting LVEF (8,14). DeCara et al. studied 65 patients (23 with LVEF <50% of whom 5 had regional wall motion abnormalities) and showed that TMAD was highly correlated with 2D echocardiography biplane LVEF ($r^2 = 0.72$, P <0.001). There was low intra- and inter-observer variability for TMAD derived LVEF (0.6±1.0% and 1.2±0.7% respectively)(7). In a study of 70 healthy children under 10 years of age, it was reported that TMAD midpoint was strongly correlated with the LVEF determined by MRI ($r=0.69$, P <0.001) (9). We report that TMAD is also correlated with biplane LVEF ($r= 0.25$, P =0.001). The difference in degree of correlation compared with the literature may be related to the different population groups and characteristics.

In addition, we think that TMAD gives a better estimation of longitudinal strain compared with overall LVEF. The LVEF was largely preserved in our population whilst the strain was not, therefore TMAD was affected whilst LVEF was not. Supporting this, TMAD had
a strong negative correlation with GLS (r=-0.61, P <0.001). Therefore TMAD may be a promising alternative to GLS for quick and effective estimation of GLS in patients where the images contain suboptimal endo-cardiac definition.

There is limited literature on the prognostic value of TMAD, and this is only available in certain specific populations. In a retrospective study of 167 patients post non-ST elevation myocardial infarction, TMAD was an independent predictor of all-cause mortality (HR 1.36, 95% CI 1.07-1.73, P=0.01) after a follow-up of 48.6±12.1 months (16). More recently, there was a suggestion that patients with sepsis had a higher risk of death if they had reduced TMAD in echocardiography performed in the first week of diagnosis (17). In our study, there was no association of TMAD with mortality (HR 1.04, 95% CI 0.91-1.19), cardiac death (HR 1.03 (95% CI 0.80-1.32) or with cardiac events (HR 0.91, 95% CI 0.80-1.02). In an analysis of the same cohort, GLS was associated with all-cause mortality (unadjusted HR 1.11 per 1% increase in GLS (more abnormal), 95% CI 1.02-1.22, P=0.014) (18). We hypothesise that although TMAD is a quick technique to estimate longitudinal strain, it is too insensitive to identify increased mortality risk in haemodialysis patients. Further studies with a larger cohort and control group are needed to confirm our findings.

To our knowledge this study is the first to compare the correlation of TMAD with GLS and LVEF in ESKD patients. However, this study has limitations. Although it is the largest study of TMAD in ESKD at present, the sample size is still small (198), therefore the results will need to be confirmed in a larger cohort. Due to logistic reasons, because it was not feasible to provide ambulance transport and patients needed to attend on a non-dialysis day, individuals who were immobile or unwell were excluded from the study (N=18). These patients may potentially have the worst outcome, LV strain and ejection fraction. Therefore we may have excluded some extreme values in our analysis. The results need to be interpreted as such as this may not be representative of the whole haemodialysis population.

In conclusion, TMAD has strong negative correlation with GLS, but only a weak positive association with LVEF. This may be because TMAD is a better measure of longitudinal strain, which is frequently affected first before LVEF in pathological states. TMAD is a promising quick, alternative assessment of GLS. However, unlike GLS, it does not confer
prognostic associations with mortality, cardiac death and cardiac events. This may limit its use in ESKD populations.

7.7 References


12. de Simone G, Devereux RB, Daniels SR, Koren MJ, Meyer RA, Laragh JH. Effect of growth on variability of left ventricular mass: Assessment of allometric signals in adults and children and their capacity to predict cardiovascular risk. J Am Coll


Chapter 8

Comparison of Two-dimensional and Three-dimensional Echocardiographic Parameters and Association with Mortality in Haemodialysis Patients


8.1 Preface

Moving on from two-dimensional (2D) transthoracic echocardiographic parameters, this chapter investigates another novel echocardiographic technology: real time three-dimensional echocardiography (RT3DE). RT3DE is becoming readily available in clinical practice. Many 2D echocardiography machines permit the recording of 3D images with the attachment of a separate transducer. In the general population without renal disease, RT3DE measurement of left ventricular mass and volume has been reported to be comparable to cardiac magnetic resonance imaging. Therefore, RT3DE has the potential to be routinely applied in clinical practice to improve the accuracy of echocardiography for the cardiac assessment of patients. However, the prognostic value for mortality of RT3DE determined left ventricular mass and volume in patients with end stage kidney disease is unknown. This chapter compared the left ventricular mass and volume measurements obtained by 2D echocardiography and RT3DE in the same patients to assess the correlation between 2d and RT3DE in haemodialysis patients, and compared the prognostic value of these measurements when obtained by 2D versus RT3DE for all-cause mortality.
8.2 Abstract

**Background:** Real-time three-dimensional echocardiography (RT3DE) is becoming widely available. Yet it is unclear how reproducible RT3DE is and how it compares with standard 2D echocardiographic (2DE) measurements in haemodialysis (HD) patients and whether the measurements of left ventricular (LV) volumes and mass provides the same prognostic implications for mortality.

**Methods:** This was a prospective, observational study performed at a single center. 219 patients were recruited and underwent both 2DE and RT3DE, on a non-dialysis day. Comparison between and within 2DE and RT3DE measurements were made using linear regression and Bland-Altman analysis. Prospective follow-up of patients was undertaken until death, renal transplantation or 16th November 2015. Cox regression analysis was utilised to investigate the relationship with mortality.

**Results:** 198 patients had adequate 2D echocardiograms but only 69 patients (median age 62.1 years, 62% male) had optimal datasets for both 2DE and RT3DE. Diabetic patients (44% vs 30%, P=0.023) and those with a high BMI (27.7 kg/m^2 vs 25.1 kg/m^2, P=0.001) were more likely to have inadequate 3D images.

In patients with full datasets, there was good correlation between 2DE and RT3DE for EDV and LVEF (r: 0.74 and 0.78, respectively) but r was 0.58 for LVMI/HT^{2.7}. Mean LV volumes and LVEF were similar for RT3DE compared to 2DE (97.1mL vs 96.5mL, for EDV and 64.6% vs 61.5%, for LVEF, P<0.001). But 2DE overestimated values for LVMI/HT^{2.7} compared with RT3DE (55.5 g/m^{2.7} vs 28.3 g/m^{2.7}, P<0.001).

RT3DE had smaller bias and limits of agreement compared with 2DE; bias was 0.23 ± 4.58, -1.41± 3.04, -1.17±3.34 for EDV, LVEF and LVMI/HT^{2.7} for 2DE. For RT3DE, it was 0.17 ± 2.19, -1.36 ± 3.48 and 0.69±2.59 respectively. After a median follow-up of 24.2 (25th-75th centile, 17.5-30.7) months, there were 16 deaths. 2DE measurement of a lower LVEF was associated with mortality, adjusted HR 0.94, 95% CI 0.89-0.99, whilst 2DE measure of LVMI/HT^{2.7}, RT3DE measures of LVMI/HT^{2.7} and LVEF were not (HR 1.02, 95% CI 0.99-1.04, HR 1.03, 95% CI 0.98-1.09 and HR 0.95, 95% CI 0.90-1.00, respectively).

**Conclusions:** RT3DE is comparable to 2DE measurements of LV volume and LVEF. But LVMI/HT^{2.7} was overestimated with 2DE. RT3DE had better reproducibility compared with 2DE. However, adequate RT3DE images can be obtained in far fewer patients than 2DE, and this, together with a lack of improved prognostic power, may limit its value in large scale studies in HD patients. However, it may be useful in specific cases.
8.3 Introduction

Cardiovascular death and all-cause mortality is particularly high in patients with end stage kidney disease (ESKD) compared to the general population (1). It is well recognised that a larger left ventricular (LV) mass and reduced ejection fraction (LVEF) determined by two-dimensional echocardiography (2DE) are associated with poor prognosis in patients undergoing haemodialysis (HD). A progressive increase in LV mass over time is the strongest predictor of deaths due to cardiac causes in HD patients (2). Within one month of dialysis initiation, a LVEF ≤30% at baseline had an adjusted hazard ratio of 9.42 (95% confidence interval, 95% CI 3.82 to 23.3) for cardiovascular mortality (3).

Real time three-dimensional echocardiography (RT3DE) is becoming increasingly available in clinical practice. It has been reported to be as accurate as the gold standard cardiac magnetic resonance imaging (CMRI) for determination of LV mass and volume (4,5). RT3DE may have some advantages over CMRI in patients with ESKD because of the risks of use of gadolinium contrast in this group. RT3DE may be able to provide data of similar repeatability without risk from contrast. Data has been limited in the general and cardiac populations, in part also due to the difficulties in obtaining adequate images. Despite the increasing availability of RT3DE, there has been no published study using RT3DE in patients undergoing HD. It is unknown how reproducible the measurements of RT3DE are in HD patients and how the measurements of LV mass, volume and ejection fraction compare with conventional 2DE measurements in this population.

Knowing how frequently adequate RT3DE images can be acquired, and how they compare with 2DE measurements, will aid physicians when planning echocardiography in this patient group. We also need to know whether RT3DE can provide more accurate prognostic information than 2DE in HD patients.

Therefore this study of HD patients aims to determine:
1. the inter-observer agreement of RT3DE and 2DE measurements.
2. the correlation between LV mass, volume and LVEF determined by 2DE and RT3DE.
3. the association of both RT3DE and 2DE measures of LV mass, volume and LVEF with all-cause mortality.
8.4 Methods

8.4.1. Study Participants

Between March 2012 and 2014, all adult (≥ 18 years old) incident and prevalent maintenance HD patients at Salford Royal Hospital or one of its four satellite units were approached to enter a prospective observational study of cardiovascular evaluation. The inclusion criteria were that patients had to have capacity to consent and they had to be sufficiently mobile to facilitate transport by means other than ambulance. The exclusion criteria also included patients being too unwell for extra hospital visits. Informed written and oral consent was obtained from all study participants, and they were given over 24 hours to consider the study protocol. All patients received standard 3-4 times weekly HD. The study was approved by the local ethics committee. All consented patients attended for an extra visit, on a non-dialysis day after the short intra-dialytic break, for assessments outlined below. Routine biochemical and haematological bloods were taken from the dialysis circuit (before dialysis) monthly before the mid-week session. Results were averaged for the 3 month period before echocardiography. Dialysis prescriptions were obtained from electronic patient medical records and values averaged over 3 months prior to the assessment date.

8.4.2. Echocardiographic Measurements

All echocardiographic studies were performed by an experienced echocardiographic technician (JS) or a consultant cardiologist (NA) using a commercial scanner (iE33; Philips Medical System, N.A., Bothell, WA, USA). Patients were examined in the left lateral decubitus position. Measurements were performed in accordance with the guidelines of the European Society of Echocardiography (6). For 2D echocardiograms, a 3.5-MHz transducer with M-mode and 2D capabilities was used (Philips Medical Systems, Philips UK Ltd, United Kingdom). At least three consecutive heartbeats were acquired with each view. Three standard apical views were taken (i.e. apical 4-chamber, apical 2-chamber and long-axis). Images were saved digitally to be analysed offline using Xclera R4.1 image management system. Volumes were determined by the Biplanes Simpsons method of discs on apical 4- and 2-chamber views. LVEF was then calculated by the formula: LVEF= [(LV end-diastolic volume minus LV end-systolic volume)/LV end-diastolic volume]*100.
An impaired LVEF was defined as <50%. M mode LV mass was calculated by the Devereaux formula and then indexed to height^{2.7} (LVMI/HT^{2.7}).

For 2DE, Left ventricular hypertrophy (LVH) was defined as LVMI/HT^{2.7} >46.7g/m^{2.7} for women and >49.2g/m^{2.7} for men (7,8). As left ventricular mass index is often reported indexed to body surface area (LVMI/BSA, calculated by the Du bois formula (9)) these values were included for comparison with left ventricular hypertrophy defined as >116 g/m^2 in men and >104 g/m^2 in women (10). To our knowledge, there is no agreed cut off for LVH for RT3DE with LVMI/HT^{2.7}. In The Multi-Ethnic Study of Atherosclerosis LVH was defined as >45.1g/m^{2.7} for men and >38g/m^{2.7} for women using CMRI with LVMI/HT^{2.7} (11,12). For LVMI/BSA, using CMRI, this has been referenced as >84.1g/m^2 for men and >76.4g/m^2 for women. In acknowledgement of the close agreement between CMRI and RT3DE, these definitions were used in this study for RT3DE measurements for comparison with 2DE (13).

RT3D echocardiograms were obtained using a fully sampled matrix-array transducer (X5-1, Philips Medical Systems) immediately after 2D images were acquired. The aim was to include the entire LV cavity in the pyramidal scan volume if possible. Full-volume acquisition (4 pyramidal wedge-shaped volumes, 93-21°) were obtained during a 5-7 second breath hold in the apical view and over 4 cardiac cycles. Acquisition was triggered by the R wave in every other cardiac cycle. The dataset was then used to build a larger pyramidal LV volume (up to 90x90°). The temporal resolution of the image set was to ≥ 20 frames/s when sector width was optimised. An offline analysis of the data was performed using Xclera software, QLAB 9.0 (Philips, Andover, Massachusetts, USA). Images were optimised in magnification and gain. 3D datasets where 2 or more segments could not be visualised, or those affected by significant translational artefacts, were discarded. Three apical views were identified (4-, 3-, 2-chamber) and the images were optimised in order that the apex was seen. In the end-diastolic and end-systolic frames the LV endocardial borders were manually traced (Chapter 3, Figure 3-5). The QLAB software re-constructed the full LV volume frame by frame from the traced endocardial borders, and any imprecise tracking was adjusted by the operator. Volumes were calculated by QLAB and the following measurements were available: left ventricular end diastolic volume (LVEDV), LV end systolic volume (LVESV), LV ejection fraction (LVEF) and individual segmental volumes. For determining the LV mass, the endocardial and epicardial contours were traced manually at end-diastole with the papillary muscles included in the LV cavity. The
analysis software automatically used the traced contours to determine the volumes to derive the LV mass value. This has been shown to correlate well with LV mass determined by CMRI (14).

8.4.3. Inter-Observer and Intra-Observer Variability

To determine the intra-observer variability in the 2DE and RT3DE evaluations of LV mass, volumes and thus LVEF, all measurements were repeated 3 months later by the same observer (DC) blinded to the previous results obtained. For inter-observer variability the same measurements were repeated by a second trained observer (JH) for 20 randomly selected echocardiograms.

8.4.4. Follow-up

All patients were followed-up from date of echocardiography to death, renal transplantation, re-locating dialysis to a different country, or 16th November 2015. All causes of death and events data were obtained from hospital medical records, patients’ primary care physicians and for non-fatal events, patient self-reporting. Data were validated by two independent assessors (DC and KV). The primary outcome measure was all-cause mortality.

8.4.5. Statistical Analysis

All categorical variables are expressed as n (%) whilst continuous variables were expressed as median (25th-75th centile) for non-normal data and mean (standard deviation) for normally distributed data. Comparisons between 2DE and RT3DE derived categorical variables were performed using the chi-squared test or Fisher's exact test. For continuous variables this was by student's t test. These comparisons were made for patients with and without adequate 3D echocardiographic images. Agreement between each technique was evaluated by use of a paired t test for the mean of parameters and linear regression analysis with Pearson's correlation coefficient. Bland-Altman analysis was utilised to determine the degree of agreement between and within observers for determination of the bias and 95% limits of agreement. Cox regression analysis was utilised to determine the association with all-cause mortality. A multi-variate model utilised factors that were significant on univariate analysis and which were decided a priori to be important such as history of
coronary artery disease and diabetes mellitus. Values of two-sided p <0.05 were considered significant. All statistical analysis was performed using the statistical package SPSS version 22, Chicago, IL.

### 8.5 Results

219 patients were recruited into the study; Figure 8-1 shows the number of patients who fitted within the inclusion and exclusion criteria.

**Figure 8-1. Inclusion and exclusion criteria for the study, in addition to the number of patients with adequate 2DE and RT3DE.**

435 haemodialysis patients were receiving maintenance hemodialysis in Sandford Royal Hospital NHS Trust or one of its four satellite units during recruitment period March 2012-2014

N= 263

N= 219 had 2D transthoracic echocardiograms

N=188 had 2D measurements of LVEF and LVMI/HT².⁷

69 patients had both 2D and 3D echocardiographic measurements of LVEF and LVMI/HT².⁷

Inclusion Criteria:
- Age greater than or equal to 18 years
- Maintenance haemodialysis
- Able and willing to give consent

Exclusion Criteria:
- Unable to give consent or language barrier (n=26)
- Stretcher transport or too ill to participate (n=18)

In 21 patients, 2D measurements of LVEF or LV mass could not be obtained due to inadequate image quality

In 96 patients 3D LVEF could not be obtained. In 129 patients 3D LVMI/HT².⁷ could not be measured.

*Note: 198 patients had 2D measurements of LV mass and ejection fraction. From this group, there were 102 with 3D measurement of LVEF and 69 with LVMI/HT².⁷. Therefore 69 patients had full 2D and 3D measurements.*
There were 198 patients with adequate 2DE for LVEF and LVMI/HT\(^{2.7}\) measurement. The median age was 64.2 (52.8 - 72.9) years, with 69% Caucasian males. There were 78 patients (39%) with a history of diabetes mellitus and 55 (28%) with coronary artery disease. Out of the 198 patients with full data for 2DE, only 102 had adequate 3D measurements of LVEF and 69 patients had adequate LVMI/HT\(^{2.7}\) results. It was more difficult to obtain LVMI/HT\(^{2.7}\) measurements because of the requirement for adequate visualisation of endo and epicardial borders, whilst for LVEF, only adequate endocardial border was required for LV volume assessment. Analyses were therefore undertaken on the 69 patients who had complete datasets for 2DE and RT3DE. The median age for this group was 62.1 years (25th-75th centile, 50.6-71.6 years), 43 (62%) were male and 62 (90%) Caucasian.

Differences in characteristics between the 69 patients who had adequate 3D images versus the 129 patients who did not are shown in Table 8-1. Patients who had complete optimal 3D images were more likely to be female (38 % vs 28 %, \(P=0.039\)), to have lower body mass index (25.1 kg/m\(^2\) vs 27.7 kg/m\(^2\), \(P=0.001\)), higher diastolic blood pressure (80 mmHg vs 76 mmHg, \(P=0.024\)) and were less likely to be diabetic (30 % vs 44 %, \(P=0.023\)).

### 8.5.1. Inter-observer and Intra-observer Variability

The inter-observer variability for acquisition of 2DE and RT3DE measurements is shown in Table 8-2. Overall there was good inter-observer variability for both 2DE and RT3DE measurements. The degree of bias for RT3DE measurements was less than that of 2DE measures and the limits of agreement (LOA) were wider for 2DE measures. For example, for 2DE LVMI/HT\(^{2.7}\) the bias of inter-observer variability was -1.17 ± 3.34 and the 95% LOA was -7.71 to 5.37. For RT3DE measurement of LVMI/HT\(^{2.7}\) the bias was 0.69±2.59 and 95% LOA was -4.38 to 5.77 (Figure 8-2). Intra-observer variability was lower than inter-observer variability for 2DE and RT3DE measurements. For 2DE measurement of LVMI/HT\(^{2.7}\) there was a bias of 0.42±2.71 (95% LOA was -4.89 to 5.72). The intra-observer variability for RT3DE measurement of LVMI/HT\(^{2.7}\) had a bias of -0.12±1.77, with 95% LOA was -3.59 to 3.36.
Table 8-1. Baseline demographics of patients with and without adequate RT3DE.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=198)</th>
<th>Inadequate RT3DE (N=129)</th>
<th>Adequate RT3DE (N=69)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.2 (52.8-72.9)</td>
<td>64.5 (53.1-72.0)</td>
<td>62.1 (50.6-71.6)</td>
<td>0.648</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>136 (69)</td>
<td>93 (72)</td>
<td>43 (62)</td>
<td><strong>0.039</strong></td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>161 (81)</td>
<td>99 (77)</td>
<td>62 (90)</td>
<td>0.058</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>129 (65)</td>
<td>83 (64)</td>
<td>46 (67)</td>
<td>1.000</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (23.7-30.4)</td>
<td>27.7 (24.2-31.2)</td>
<td>25.1 (22.4-28.8)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>16.7 (5.8-44.3)</td>
<td>16.1 (6.0-40.2)</td>
<td>18.0 (5.0-57.6)</td>
<td>0.550</td>
</tr>
<tr>
<td>Mean UF volume achieved (L)</td>
<td>2.19 (0.74)</td>
<td>2.23 (0.70)</td>
<td>2.08 (0.79)</td>
<td>0.197</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 (12)</td>
<td>76 (11)</td>
<td>80 (13)</td>
<td><strong>0.024</strong></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143 (20)</td>
<td>143 (20)</td>
<td>145 (21)</td>
<td>0.500</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>78 (39)</td>
<td>57 (44)</td>
<td>21 (30)</td>
<td><strong>0.023</strong></td>
</tr>
<tr>
<td>History of congestive cardiac failure (%)</td>
<td>56 (28)</td>
<td>40 (31)</td>
<td>16 (23)</td>
<td>0.320</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>55 (28)</td>
<td>25 (19)</td>
<td>30 (44)</td>
<td>1.000</td>
</tr>
<tr>
<td>ACEI/ARB (%)</td>
<td>82 (41)</td>
<td>56 (43)</td>
<td>26 (38)</td>
<td>0.200</td>
</tr>
<tr>
<td>B-Blocker (%)</td>
<td>91 (46)</td>
<td>60 (47)</td>
<td>31 (45)</td>
<td>0.832</td>
</tr>
<tr>
<td>CCB (%)</td>
<td>87 (44)</td>
<td>56 (43)</td>
<td>31 (45)</td>
<td>0.692</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>124 (63)</td>
<td>85 (66)</td>
<td>39 (57)</td>
<td>0.219</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>10.7 (1.2)</td>
<td>10.7 (1.3)</td>
<td>10.7 (1.2)</td>
<td>0.841</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>2.37 (0.14)</td>
<td>2.38 (0.13)</td>
<td>2.39 (0.17)</td>
<td>0.675</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>1.55 (0.45)</td>
<td>1.52 (0.46)</td>
<td>1.56 (0.45)</td>
<td>0.534</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39.0 (36.8-40.7)</td>
<td>39.0 (36.7-41.0)</td>
<td>39.0 (36.5-40.8)</td>
<td>0.722</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>9.4 (2.9-21.0)</td>
<td>16.8 (23.4)</td>
<td>14.7 (18.5)</td>
<td>0.490</td>
</tr>
</tbody>
</table>

Note: Data presented as median (25th-75th centile) for non-normally distributed continuous variables, mean (standard deviation) for normally distributed continuous variables and for categorical variables, n (%).

Abbreviations: BMI, body mass index, ACEI/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker, CCB, calcium channel blocker, CRP, C-reactive protein.
Table 8-2. Inter-observer variability for 20 randomly selected patients by 2 independent interpreters (DC and JH) for 2DE and RT3DE determined parameters. Bias and limits of agreement as determined by Bland-Altman Analysis.

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Bias</th>
<th>Limits of Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D LVEDV (mL)</td>
<td>0.23 ± 4.58</td>
<td>-8.74 to 9.21</td>
</tr>
<tr>
<td>2D LVEF (%)</td>
<td>-1.41 ± 3.04</td>
<td>-7.37 to 4.54</td>
</tr>
<tr>
<td>2D LVMI/HT².⁷ (g/m².⁷)</td>
<td>-1.17 ± 3.34</td>
<td>-7.71 to 5.37</td>
</tr>
<tr>
<td>3D LVEDV (mL)</td>
<td>0.17 ± 2.19</td>
<td>-4.12 to 4.45</td>
</tr>
<tr>
<td>3D LVEF (%)</td>
<td>-1.36 ± 3.48</td>
<td>-8.18 to 5.46</td>
</tr>
<tr>
<td>3D LVMI/HT².⁷ (g/m².⁷)</td>
<td>0.69 ± 2.59</td>
<td>-4.38 to 5.77</td>
</tr>
</tbody>
</table>

Abbreviations: LVEDV, Left ventricular end-diastolic volume, LVEF, left ventricular ejection fraction, LVMI/HT².⁷, left ventricular mass indexed to height².⁷.

8.5.2. Correlation Between 2DE and RT3DE Measurements

There was good correlation between the parameters measured by 2DE and RT3DE, especially so for volumes and LVEF. Correlation between 2DE and RT3DE measurements of LV volumes, LVEF and mass are shown in Table 8-3. However, when the means were compared, there was a significantly higher mean value in 2DE measurements of LV mass compared with RT3DE. This was the case with all measures of LV mass (comparison of 2DE versus RT3DE; LV mass was 225.8g versus 114.6g, LVMI/BSA was 123.7 g/m² versus 62.8 g/m², LVMI/HT².⁷ was 55.5 g/m².⁷ versus 28.3 g/m².⁷, P<0.001). The values for LV volumes and LVEF were similar for 2DE and RT3DE (Table 8-4, Figure 8-3).

Table 8-3. Comparison of 2DE and RT3DE measured parameters (N=69) by linear regression analysis.

<table>
<thead>
<tr>
<th>2DE versus RT3DE</th>
<th>R</th>
<th>R square</th>
<th>P</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDV (mL)</td>
<td>0.739</td>
<td>0.547</td>
<td>&lt;0.001</td>
<td>0.591-0.928</td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>0.861</td>
<td>0.741</td>
<td>&lt;0.001</td>
<td>0.669-0.894</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.775</td>
<td>0.601</td>
<td>&lt;0.001</td>
<td>0.637-0.954</td>
</tr>
<tr>
<td>LVMI/HT².⁷ (g/m².⁷)</td>
<td>0.584</td>
<td>0.341</td>
<td>&lt;0.001</td>
<td>0.678-1.372</td>
</tr>
</tbody>
</table>

Abbreviations: LVEDV, Left ventricular end-diastolic volume, LVESV, Left ventricular end-systolic volume, LVEF, left ventricular ejection fraction, LVMI/HT².⁷, left ventricular mass indexed to height².⁷.
Figure 8-2. Bland-Altman plots for inter-interpreter agreement. The plots show the mean difference (x axis) and the limits of agreement (dotted lines) between interpreters (DC and JH) for left ventricular end diastolic volume, ejection fraction and mass for 2DE and RT3DE in 20 randomly selected patients.
Table 8-4. Mean values for LV volumes, ejection fraction and mass as determined by 2DE and RT3DE. Comparison of means by student paired t test.

<table>
<thead>
<tr>
<th>LV parameter measured</th>
<th>2DE</th>
<th>RT3DE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDV (mL)</td>
<td>96.5 (31.4)</td>
<td>97.1 (96.5)</td>
<td>0.832</td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>35.2 (18.5)</td>
<td>39.3 (20.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>61.5 (9.8)</td>
<td>64.6 (10.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>225.8 (66.9)</td>
<td>114.6 (41.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI/BSA (g/m²)</td>
<td>123.7 (37.2)</td>
<td>62.8 (21.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI/HT².⁷ (g/m²)</td>
<td>55.5 (16.9)</td>
<td>28.3 (9.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: LVEDV, Left ventricular end-diastolic volume, LVESV, Left ventricular end-systolic volume, LVEF, left ventricular ejection fraction, LVMI/HT².⁷, left ventricular mass indexed to height².⁷, LVMI/BSA, left ventricular mass indexed to body surface area.

Figure 8-3. Box and whisker plot comparing measurements of left ventricular volume, ejection fraction and mass between 2DE and RT3DE. The box represents the median and inter-quartile ranges and the bar shows the maximum and minimum values.

Abbreviations: EDV, end diastolic volume, ESV, end systolic volume, LVEF, left ventricular ejection fraction, LVMI/HT².⁷, Left ventricular mass indexed to height to the power 2.7
8.5.3. Association of RT3DE with all-cause mortality

The median follow-up time for these 69 patients was 24.2 (25th-75th centile, 17.5-30.7) months; there were 16 deaths with 3 cardiac deaths (2 sudden cardiac deaths and 1 due to heart failure). There were 16 patients who had major cardiac events which included myocardial infarction, new angina, hospitalization due to heart failure or arrhythmia, coronary revascularisation/coronary bypass surgery or cardiac death. On univariate Cox regression analysis, the factors that were associated with all-cause mortality were age (HR 1.09, 95% CI 1.03-1.15, P<0.001) and a lower LVEF measured by 2DE (HR 0.93, 95% CI 0.88-0.99, P=0.012). Conversely, measurements by RT3DE were not associated with all-cause mortality. LVMI/HT^2.7 by RT3DE had HR 1.02 (95% CI 0.97-1.07, P=0.461) and the HR was 0.96 (95% CI 0.92-1.07, P=0.144) for LVEF. Due to the relatively low number of events, including too many factors in a multivariate model would lead to model instability. Therefore the multivariate model was only adjusted for age, diabetes mellitus and coronary artery disease, because it is well-recognised that the presence of diabetes mellitus (15) and coronary artery disease (16) are important factors associated with mortality in patients undergoing HD. In a multivariate model, lower LVEF as assessed by 2DE was still associated with mortality (HR 0.94, 95% CI 0.89-0.99, P=0.016). 2DE measure of LVMI/HT^2.7 was not associated with death (adjusted HR 1.02, 95% CI 0.99-1.04, P=0.205). RT3DE measures of LVMI/HT^2.7 and LVEF were not associated with mortality despite adjusting for the same factors (HR 1.03, 95% CI 0.98-1.09, P=0.211 and HR 0.95, 95% CI 0.90-1.00, P=0.056, respectively).

8.6 Discussion

RT3DE has been one of the major advances in echocardiographic imaging over the last decade. This technological milestone has allowed the modality to become easily applicable to clinical practice as images can be obtained and interpreted in real-time at the bedside, and usually with the same echocardiographic machine as used for 2D imaging. 3DE has been shown to have similar accuracy and repeatability for cardiac measurements as cardiac MRI (4,5). Patients undergoing HD frequently have abnormal cardiac structure and function (17,18), and hence accurate cardiac imaging is vital for cardiac risk stratification and management. However, few studies have utilised RT3DE in this patient group. Our study is the first to compare this modality with standard 2DE in HD patients and to assess
the usefulness of RT3DE in relation to mortality. We feel that the results have provided important insight into the use of this modality in patients undergoing HD.

The study has highlighted difficulties in obtaining adequate RT3DE images in many patients. When comparing patients who did and did not have adequate RT3DE, it was evident that diabetic patients and those with a high BMI were more likely to have inadequate images. This was due to an inadequate penetration, imaging artefacts and poor acoustic windows for analysis. Furthermore, patients are required to breath-hold for at least 5-7 seconds in order to capture images for 3D re-construction. This may be particularly challenging in obese patients and for HD patients who may already by dyspnoeic for reasons of fluid overload, chronic obstructive airways disease and anaemia. Even when 3D images can be obtained, epicardial visualization may still be sub-optimal, which is especially important for measurement of LV mass. This was exemplified by 102 patients having adequate RT3DE LVEF measurements, but only 69 of these patients having LVMI/HT$^{2.7}$ readings suitable for analysis. All images in this study were obtained by an experienced technician or physician under research conditions, and hence in routine, busy clinical practice, it may be less feasible to obtain adequate RT3DE images for analysis. Other studies of different patient populations have reported similar challenges with 20% of patients having technically difficult images (19) and excellent images were reported in only 16% in one study (20). Secondly, we report that there was good inter- and intra-observer agreement in measurements obtained by RT3DE and 2DE. This was more so for RT3DE measurements which may be explained by the fact that once the viewing planes were determined from the 3D dataset, the inbuilt software automatically applies a deformable shell model, and so, to some degree, the software carries out a semi-automated process. Our values for inter-observer variability were comparable to those of other investigators. In one examining LVEF, the inter-observer bias for 2D echo was 8±10 % (variability 14±17) compared with RT3DE which had a bias of 3±4 (variability of 5±4) (21). In our study we reported bias of -1.36 ± 3.48, (95% LOA -8.18 to 5.46) for LVEF assessed by RT3DE. This good inter-observer agreement supports the use of RT3DE by different assessors in clinical practice where repeated measurements may frequently need to be taken. Furthermore, other studies have demonstrated that RT3DE has good reproducibility over time. In a study with serial RT3DE measures taken one year apart in patients with previous myocardial infarction, this modality was able to detect subtle changes in LV volumes that were not detected on 2DE; change in LV end diastolic volume
detected with RT3DE correlated with CMRI (-4±20, p<0.01), but these were not detected by 2DE (4±19, P=0.09)(22).

A good correlation between 2DE parameters and RT3DE is important if the two echocardiographic modalities are to be utilised interchangeably. Comparing the mean values for 2DE and RT3DE parameters, the volume measurements were similar, with a trend for larger volumes with RT3DE. This is not surprising as one of the limitations of 2DE is that the LV apex may be foreshortened and LV volumes are underestimated whilst RT3DE visualises the LV apex more easily.

However, large differences were seen with LV mass measurements, with 2DE values for LV mass, LVMI/BSA and LVMI/HT\(^{2.7}\) being greater than those assessed by RT3DE; mean differences were 111.2g, 60.9g/m\(^2\) and 27.2 g/m\(^{2.7}\) respectively. No literature is available for RT3DE in dialysis patients, but this finding was consistent with the literature comparing CMRI and 2DE. In one study of 32 haemodialysis patients, CMRI assessed LV mass was much lower compared with 2DE measurements (mean difference in men was 54.7g/m\(^2\) and for women was 43.9g/m\(^2\))(23). In our study, using the different cut-offs for 2DE and RT3DE, there were 37 (54%), 34 (49%), 11 (16%) and 6 (9%) patients classified with left ventricular hypertrophy using 2DE determined LV mass/BSA, LVMI/HT\(^{2.7}\) and RT3DE measure of LVMI/BSA, LVMI/HT\(^{2.7}\) respectively. Therefore 38-40% of patients were reclassified to normal left ventricular mass with RT3DE using CMRI values of LVMI/BSA and LVMI/HT\(^{2.7}\) as cut offs. In the aforementioned study of 32 haemodialysis patients (23), 66% had 2DE defined left ventricular hypertrophy versus 44% using CMRI. An explanation for these findings are that 2DE measurement of LVMI/HT\(^{2.7}\) gives an overestimation of the LV mass, with greater number of patients classified with LVH. Using 2DE, in order to calculate LV mass using the Devereaux formula, LV dimensions are measured using M mode in one plane and the results are cubed therefore magnifying any inaccuracies in initial measurements. Moreover, this formula has been validated in hearts with normal geometry, based on the geometric assumption that the LV is an idealized shape. This is unlikely, especially in patients undergoing HD, as left ventricular hypertrophy is frequently present. Therefore RT3DE is likely to be more accurate as it makes no such assumptions.

The number of patients classified as having LVH was low in our cohort especially when classified by RT3DE. This may be explained by the fact that valid RT3DE measurements
were only available in around 30% of patients and those excluded were more likely to have LVH (eg. obese diabetic males). In an unselected haemodialysis cohort we would expect to see far more patients with LVH.

We found that a lower LVEF as assessed by 2DE, but not by RT3DE, was associated with all-cause mortality in an adjusted Cox regression model. We are unsure of the explanation for this finding, but our sample size was small and hence this result will need to be tested in a larger group of patients. As discussed previously, in view of the difficulties in obtaining adequate RT3DE images, this may require multi-center recruitment of patients.

The main limitations of this study were the small sample size of patients with adequate 3D images for LV mass analysis, which means that the results will need to be confirmed in a larger cohort. Also, patients who were immobile or too unwell to attend on a non-dialysis day for echocardiographic assessment were not included in the study. This may have excluded some patients with poorer LV function. Therefore the imaging performed in this study is unlikely to be generalizable to the entire HD population. We also did not have normal healthy control group to determine a reference range for LVH which will be needed for future studies. In summary, advances in RT3DE provides promising features. It certainly appears to have good reproducibility and correlation with 2DE measurements. This is important for repeated echocardiographic studies in clinical practice. However, we found in this limited sample of HD patients that parameters measured by RT3DE did not have the same prognostic implications as those measured by 2DE. This, together with the demonstrated difficulties of obtaining adequate images in many HD patients, may suggest that its use should be limited for specific cases in hospital practice, rather than widespread use in a research setting. Validation of our findings in a larger population will be important.

8.7 References


18. Chiu D, Green D, Abidin N, Sinha S, Kalra PA. Cardiac imaging in patients with


9.1 Preface

In chapter 8 it was reported that real time 3D echocardiography (RT3DE) measured left ventricular mass and volume were not associated with mortality. RT3DE can measure other parameters such as left ventricular (LV) dyssynchrony. The utilisation of RT3DE for measurement of LV dyssynchrony has not been reported in the literature in haemodialysis patients. Yet there is some indication that LV dyssynchrony may be a useful marker of prognosis in end stage renal disease. LV dyssynchrony is frequently present in patients with coronary artery disease, left ventricular hypertrophy, and abnormal cardiac loading conditions. All three of these factors are common in end stage kidney disease. It has been reported in a small haemodialysis cohort that on a haemodialysis day, prior to dialysis treatment, LV dyssynchrony was present. Therefore, it would be hypothesised that LV dyssynchrony determined by RT3DE would be abnormal in patients undergoing haemodialysis. Further, it is hypothesised that it would be predictive of mortality as demonstrated in some literature published in the general population without renal disease. This chapter aims to characterize LV mechanical dyssynchrony, measured by RT3DE, in patients undergoing haemodialysis, establish factors associated with abnormal LV dyssynchrony in these patients, and determine its association with outcomes (mortality and cardiac events).
9.2 Abstract

**Background:** Left ventricular (LV) mechanical dyssynchrony is associated with coronary artery disease, left ventricular hypertrophy and abnormal loading conditions. This study aimed to characterize LV mechanical dyssynchrony, measured by 3D transthoracic echocardiography, in patients undergoing haemodialysis (HD). Factors associated with an abnormal LV dyssynchrony and its association with outcomes were explored.

**Methods:** Maintenance HD patients had 3D transthoracic echocardiography performed on a non-dialysis day. Unpaired t-tests and Chi-squared tests were utilised to identify differences between baseline characteristics of patients with and without LV mechanical dyssynchrony, defined as a standard deviation (SD) of time to minimum systolic volume corrected to heart rate (Tmsv-16 SD) of >3%. Cox regression analysis was applied to assess the predictive value of LV dyssynchrony to all-cause mortality, cardiac events, and heart failure hospitalisations.

**Results:** Ninety-seven patients had adequate images for analysis (67% male, median age 63 [25th-75th centile, 50-72] years). 94% had preserved LV ejection fraction (LVEF) >50%. Mean Tmsv-16 SD was 3.35 (3.30)%, and 39 patients (40%) had LV mechanical dyssynchrony. There was no difference between patients with and without LV dyssynchrony in any clinical parameter; LVEF (59.6% vs 63.3%, P=0.067); LVMI/HT\(^{2.7}\) (58.7 g/m\(^{2.7}\) vs 54.1 g/m\(^{2.7}\), P=0.229); QRS duration (98.7ms vs 95.8ms, P=0.540). Follow-up time was for a median of 27.6 months (17.9 - 30.8 months), Tmsv-16 SD >3% was not predictive of all-cause mortality (adjusted HR 2.16, 95% CI 0.96-4.89, P=0.063) or cardiac events (adjusted HR 2.06, 95% CI 0.85-4.97, P=0.110). However, it was associated with heart failure hospitalisation (adjusted HR 1.03, 95% CI 1.00-1.06, P=0.046).

**Conclusions:** 40% of HD patients, the majority with preserved LVEF, had LV mechanical dyssynchrony on a non-dialysis day. In this study, LV dyssynchrony was associated with a higher event rate for heart failure hospitalisation.

9.3 Introduction

Heart failure is common in end stage kidney disease (ESKD) and has been reported to be present in 36% of patients undergoing dialysis (1). Patients with heart failure may have left ventricular (LV) mechanical dyssynchrony whereby there is discordance in the myocardial contractions between different LV myocardial segments (2). In the general population...
without renal impairment, LV dyssynchrony has been shown to be a predictor of hospitalisation for heart failure (3). Indeed, there is randomised trial evidence of improved survival and LV performance using cardiac resynchronisation therapy (CRT) for patients with symptomatic heart failure (LV ejection fraction, LVEF<35%), prolonged QRS duration (>120ms) and LV mechanical dyssynchrony (4–6).

LV mechanical dyssynchrony is traditionally determined using tissue Doppler imaging (TDI). This has good temporal resolution and ability to assess multiple LV segments simultaneously. However, it is limited by angle dependency so can only determine timing of motion in the longitudinal direction, not other planes of contraction, and is unable to reliably quantify apical LV wall motion. In addition, it has poor inter-operator reproducibility. In the last 10 years, real time three-dimensional, 3D, echocardiography (RT3DE) has been developed to detect mechanical dyssynchrony. Unlike TDI, it is independent of direction as so can measure dyssynchrony in all planes of movement. It also has the advantages of being reproducible, has good spatial resolution, and fast on-line analysis. It can capture the 3D dynamics of the entire LV simultaneously, including timing of wall motion. However, the use of RT3DE to measure LV mechanical dyssynchrony has not been performed in ESKD patients.

LV mechanical dyssynchrony can result from a delay in electrical conduction, coronary ischemia or infarction, and abnormal loading conditions (7). In haemodialysis (HD) patients, myocardial fibrosis is common, 38% have coronary artery disease, and abnormal loading will frequently occur due to having or missing dialysis sessions including the volume overload during inter-dialytic breaks. Therefore, it was hypothesized that many dialysis patients would have LV mechanical dyssynchrony independent of a diagnosis of heart failure. To our knowledge this has never been investigated in ESKD patients on a non-dialysis day, nor using RT3DE. There has been one study which has investigated the effect of LV dyssynchrony using speckle tracking strain imaging pre- and post- dialysis. It was reported that HD therapy improved radial LV dyssynchrony (7). If there was significant LV mechanical dyssynchrony in ESKD patients, this may highlight high risk groups who warrant thorough cardiac and dialysis therapy review.

This study aimed to characterise and quantify LV mechanical dyssynchrony measured using RT3DE in stable maintenance HD patients, determine the clinical and biochemical parameters associated with LV mechanical dyssynchrony, and the independent association
of mechanical dyssynchrony with clinical outcomes (mortality, major cardiac events, and heart failure hospitalisations).

9.4 Methods

9.4.1. Study Population

This was a prospective study carried out at Salford Royal Hospital NHS Foundation Trust, UK and its four satellite units. Recruitment of adult (≥18 years) maintenance HD patients occurred between March 2012 and 2014. All patients received standard thrice weekly, 3-4 hours HD with adequate dialysis clearance. Exclusion criteria were lack of capacity to consent, acute or chronic illness preventing attendance for echocardiography, and logistical barriers to suitable transportation for study visits (e.g. immobility requiring ambulance transportation). The study adhered to the Declaration of Helsinki and local ethical approval was obtained.

Clinical and demographic data were obtained from patient self-reported questionnaire on the day of assessment, and review of electronic medical records. Bloods were collected monthly from the HD circuit, immediately before the midweek session. The mean values for the blood results over the 3 months prior to echocardiography were used in the study. Parameters from dialysis prescription were also taken as a mean for the 3 months prior to echocardiography. A 12 lead electrocardiogram was performed on the same day as the echocardiogram.

All patients were followed up until renal transplantation, death, moving out of the country, or 16th November 2015. End point events were determined from patient self-reporting, detailed review of hospital electronic health records, and contact with patients’ primary care physicians. The primary outcome was all-cause mortality. The secondary outcomes were (i) number of patients sustaining a major adverse cardiac events (MACE) including cardiac death, myocardial infarction, coronary revascularisation, coronary artery bypass grafting, new onset angina, and hospitalisation due to heart failure, and (ii) heart failure hospitalisations. Cause of death and cardiac events were independently adjudicated by two assessors (DC and VK).
9.4.2. Three-dimensional Echocardiographic Dyssynchrony Data Analysis

Echocardiography was performed on a non-dialysis day after the short-break by an experienced technician (JS) or a consultant cardiologist (NA). All patients were positioned in the left lateral decubitus position for the echocardiographic examination. The echocardiographic equipment used was the Philips Medical Systems, Philips UK Ltd, United Kingdom, with a 1-3 MHz X3-1 matrix-array transducer (Philips, Andover, Massachusetts, USA).

Four real-time pyramidal wedge-shaped volumes (93-21º) during a single 5-7 seconds of breath hold in the apical view was acquired. This dataset was then used to build up a full single larger pyramidal LV volume (up to 90x90º). The acquisition of the LV sub-volumes are triggered by the occurrence of every R wave on the ECG of every cardiac cycle. Therefore, there was a total acquisition time of 4 heart beats. The images were analysed offline using the Xcelera R4.1 software, QLAB 9.0 (Philips, Andover, Massachusetts, USA). If there were 2 or more segments in the 3D images which could not be visualized, or if there were visible translation artefacts, these images were discarded and not included in subsequent analysis.

The image was optimised with magnification and gain settings. Analysis was based on the 2D approach, three apical views were identified (4-, 3-, 2-chamber) with apex seen (non-foreshortened). To trace the LV endocardial border, the end diastolic (first frame of sequence) and the end-systolic (frame with the smallest LV cavity and just ahead of the mitral valve closure) images were used. The papillary muscles and endocardial trabeculae were included in the tracing of the LV endocardium. Five points were applied to trace the endocardial border- mitral annulus at the anterior, inferior, lateral and septal annuli and the LV apex in the 2-chamber and 4-chamber view.

For determining the LV mass, both the endocardial and epicardial contours needed to be traced manually at end-diastole and the QLAB software computes the LV mass. The QLAB software automatically re-constructs the full LV volume throughout the cardiac cycle. Manual alterations can be applied to adjust the LV border, if necessary i.e. if the tracing is suboptimal. Once the 3D image is created, the LV can be visualised from any angle and it is divided into 17 segments (including the apical cap). Global and segmental volumes were generated. The following are automatically calculated: left ventricular end
diastolic volume (LVEDV), LV end systolic volume (LVESV), LV ejection fraction (LVEF) and volume-time curves for entire LV. The LV was divided into segments (Chapter 3, Figure 3-5) and standard deviation of time interval to the minimum systolic volume (Tmsv-SD) for 16 segments was automatically generated (six basal, six middle and four apical segments, excludes apical cap, segment 17). This was then adjusted for heart rate and presented as Tmsv 16-SD%.

There is no universally agreed pathophysiological cut off for LV mechanical dyssynchrony. In a comprehensive review of 12 published studies to date, attempting to define a normal value of LV dyssynchrony, most groups agree that a Tmsv-SD of 3% is considered normal (8). Therefore a cut-off of greater than 3% has been defined as abnormal for this study.

9.4.3. Statistical Analyses

Continuous variables were assessed for normality by the Kolmogorov-Smirnov test. If normally distributed this is presented as mean (standard deviation, SD), if non-normally distributed this is presented as median (25th-75th centile). Categorical variables are presented as frequencies and percentages. Comparison between groups of Tmsv-16 SD of ≤3% and >3% was using Fisher's exact test or Chi-squared test, where appropriate for categorical variables. For comparison between continuous variables unpaired t test was used for normally distributed variables and Mann-Whitney U test for non-parametric variables.

A sample size calculation was performed using all-cause mortality as an outcome measure, LV dyssynchrony (Tmsv-16 SD ≤3% versus >3%) as the predictor, α = 0.05, power = 0.80 (β 0.20). The expected incidence of death in patients without dyssynchrony was 10% (9) versus patients with LV dyssynchrony at 37% (10). With these presumptions, it was calculated that there needed to be at least 38 patients in each group to detect the desired level of difference in mortality.

Multivariable Cox regression analysis was performed to determine factors that were independently associated with mortality, MACE and heart failure hospital admissions. Variables were included in the multivariable model if factors were significant on univariate
analysis (P<0.05). Statistical analyses were performed in SPSS version 22.0 (SPSS, Inc, Chicago, IL). A two-sided P value of <0.05 was considered statistically significant.

9.4.4. Reproducibility

Inter-observer variability of echocardiographic measurements was carried out on the results for 20 randomly selected patients, by a third blinded assessor (JH). Intra-observer variability was performed by the same assessor (DC) repeating measurements two months after initial analysis. Bland-Altman analysis was used to assess agreement. The intra-observer variability for RT3DE measurements of LVEF and Tmsv-16 SD was good with a bias of 1.55±2.88, 95% LOA was -4.09-7.19 and -0.06±0.50, 95% LOA -1.04 to 0.92, respectively. Whilst the inter-observer variability for LVEF and Tmsv-16 SD had a bias of -1.36 ± 3.48, 95% LOA was -8.18 to 5.46 and -0.10±0.34, 95% LOA -0.76 to 0.57.

9.5 Results

9.5.1. Clinical and Echocardiographic Characteristics

Two hundred and nineteen patients consented to participate into the study and underwent echocardiographic assessment. However, due to the difficulties in obtaining adequate windows for interpretation, there were only 97 patients with adequate RT3DE LV mechanical dyssynchrony indices available for analysis. A flow diagram of inclusion and exclusion criteria is show in Figure 9-1. 67% of patients were male, the median age was 62.9 (50.0-71.6) years and coronary artery disease was present in 26 patients (26.8%). Ninety-one patients had a preserved LVEF >50% (93.8% of patients); for the 6 patients with reduced LVEF, the mean LVEF was 44.5 (3.0)%. Due to the difficulties in establishing endocardial border definition, it was only possible to obtain left ventricular mass indexed to height^{2.7} (LVMI/HT^{2.7}) measurements in 69 patients.
Figure 9-1. Flow-diagram of inclusion and exclusion criteria for the study. 97 RT3DE images were available for final analysis.

Overall, the mean Tmsv-16 SD was 3.35 (3.30)%, and 39 patients (40.2%) had Tmsv-16 SD >3%. In the 6 patients with reduced LVEF, there were 3 with LV dyssynchrony and 3 without. There was no difference between LV dyssynchrony groups in any clinical parameter, in particular LVEF (59.6% vs 63.3%, P=0.067) and LVMI/HT \(^2\) (58.7 g/m\(^2\) vs 54.1 g/m\(^2\), P=0.229), or QRS duration (98.7ms vs 95.8ms, P=0.540). A between group comparison of patients with LV dyssynchrony and those without is shown in Table 9-1.
Table 9-1. Clinical and echocardiographic parameters for the study group and comparison between patients above and below Tmsv-16 SD of 3%.

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Total</th>
<th>Tmsv-16 SD ≤ 3%</th>
<th>Tmsv-16 SD &gt; 3%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>97</td>
<td>58</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.9 (50.0-71.6)</td>
<td>64.6 (49.5-71.5)</td>
<td>60.4 (50.4-73.7)</td>
<td>0.941</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>65 (67)</td>
<td>38 (66)</td>
<td>27 (69)</td>
<td>0.826</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>81 (84)</td>
<td>46 (79)</td>
<td>35 (90)</td>
<td>0.265</td>
</tr>
<tr>
<td>Current or ex-smoker (%)</td>
<td>62 (64)</td>
<td>39 (67)</td>
<td>23 (59)</td>
<td>0.518</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 (5.2)</td>
<td>27.11 (5.33)</td>
<td>26.08 (4.95)</td>
<td>0.351</td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>14.5 (5.3-45.1)</td>
<td>17.1 (5.3-51.3)</td>
<td>12.1 (5.0-27.8)</td>
<td>0.163</td>
</tr>
<tr>
<td>URR (%)</td>
<td>69.2 (9.6)</td>
<td>69.9 (9.2)</td>
<td>68.2 (10.2)</td>
<td>0.419</td>
</tr>
<tr>
<td>UF volume (L)</td>
<td>2.21 (0.80)</td>
<td>2.20 (0.76)</td>
<td>2.23 (0.85)</td>
<td>0.878</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>144 (22)</td>
<td>147 (23)</td>
<td>143 (20)</td>
<td>0.354</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>31 (32)</td>
<td>20 (34)</td>
<td>11 (28)</td>
<td>0.658</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>26 (27)</td>
<td>13 (22)</td>
<td>13 (33)</td>
<td>0.252</td>
</tr>
</tbody>
</table>

Cardiovascular Medications

| ACEI/ARB (%)                   | 41 (42)        | 26 (45)         | 15 (38)         | 0.675 |
| B-Blocker (%)                  | 44 (45)        | 28 (48)         | 16 (41)         | 0.673 |
| CCB (%)                        | 47 (49)        | 28 (48)         | 19 (49)         | 1.000 |
| Statin (%)                     | 58 (60)        | 31 (53)         | 27 (69)         | 0.084 |

Serum Haematology and Biochemistry

| Haemoglobin (g/dL)             | 10.6 (0.02)    | 10.6 (1.3)      | 10.5 (1.2)      | 0.924 |
| Calcium (mmol/L)               | 2.36 (0.16)    | 2.36 (0.16)     | 2.37 (0.17)     | 0.891 |
| Phosphate (mmol/L)             | 1.59 (0.43)    | 1.54 (0.43)     | 1.68 (0.42)     | 0.115 |
| Albumin (g/L)                  | 39 (40)        | 39 (40)         | 39 (50)         | 0.957 |
| CRP (mg/dL)                    | 1.3 (1.2)      | 1.3 (1.4)       | 1.5 (2.6)       | 0.684 |

Echocardiographic Study

| LVEF (%)                       | 61.8 (10.0)    | 63.3 (10.5)     | 59.6 (8.8)      | 0.067 |
| Preserved LVEF (%)             | 91 (94)        | 56 (97)         | 35 (90)         | 0.708 |
| LVMI/HT².7 (g/m²)              | 29.1 (10.6)    | 54.1 (19.0)     | 58.7 (17.9)     | 0.229 |

ECG Findings

| QRS width (ms)                 | 97.0 (22.3)    | 95.8 (21.8)     | 98.7 (23.3)     | 0.540 |
| Heart Rate (bpm)               | 73 (13)        | 73 (13)         | 73 (14)         | 0.802 |
| LBBB (%)                       | 1 (1)          | 0 (0)           | 1 (2)           | 1.000 |

Data expressed as mean (standard deviation), median (25th-75th centile) and number (%). Abbreviations: BMI, body mass index; URR, urea reduction ratio; BP, blood pressure; ACEI/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker; CCB, calcium channel blocker; LVEF, left ventricular ejection fraction; LVMI/HT².7, left ventricular mass indexed to height².7; UF, ultrafiltration; LBBB, left bundle branch block; CRP, C-reactive protein.

Note: Coronary artery disease was defined as previous myocardial infarction, angina, revascularisation or coronary artery bypass grafting.
9.5.2. Prognostic Value of Left Ventricular Mechanical Dyssynchrony

Patients were followed up for a median of 27.6 months (17.9 - 30.8 months). During this time there were 25 deaths, of which 6 were from cardiac causes. There were 13 patients who had acute hospital admissions for heart failure and 21 had MACE (8 patients who did not have admissions for heart failure). No patients had cardiac resynchronization therapy nor revascularisation procedures during follow-up. There were more patients with LV mechanical dyssynchrony who died (38% vs 17%, P=0.032) and admitted for heart failure (5% vs 26%, P=0.006). A breakdown diagnostic of mortality and cardiac events can be found in Table 9-2.

Table 9-2. Outcomes of patients separated into above and below Tmsv-16 SD of 3%.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Tmsv-16 SD ≤ 3%</th>
<th>Tmsv-16 SD &gt;3%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-Cause mortality</td>
<td>97</td>
<td>58</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Cardiac deaths</td>
<td>6 (6)</td>
<td>3 (5)</td>
<td>3 (8)</td>
<td>0.682</td>
</tr>
<tr>
<td>Admissions due to heart failure</td>
<td>13 (13)</td>
<td>3 (5)</td>
<td>10 (26)</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>MACE</td>
<td>21 (22)</td>
<td>9 (16)</td>
<td>12 (31)</td>
<td>0.084</td>
</tr>
<tr>
<td>Renal transplant</td>
<td>16 (16)</td>
<td>8 (14)</td>
<td>8 (20)</td>
<td>0.414</td>
</tr>
</tbody>
</table>

Abbreviations: MACE, major adverse cardiac events (this includes cardiac death, myocardial infarction, coronary revascularisation, coronary artery bypass grafting, new onset angina and hospitalisation due to heart failure).

On univariate analyses, Tmsv-16 SD >3% was associated with all-cause mortality (HR 2.41, 95% CI 1.08-5.39, P=0.032), MACE (HR 2.44, 95% CI 1.02-5.81, P=0.044) and heart failure admissions (HR 6.01, 95% CI 1.65-21.89, P=0.007). Other univariate factors which were associated with all-cause mortality were age (HR 1.06, 95% CI 1.02-1.11, P=0.003) and history of diabetes mellitus (HR 2.48, 95% CI 1.12-5.49, P=0.025). For MACE, the significant factors were higher systolic blood pressure (HR 1.02, 95% 1.00-1.04, P=0.045), prevalent coronary artery disease (HR 2.53, 95% CI 1.07-5.99, P=0.034) and taking an angiotensin converting enzyme inhibitor or angiotensin receptor blocker [ACEI/ARB] (HR 3.10, 95% CI 1.20-7.98, P=0.019). In prediction of heart failure hospitalisation, systolic blood pressure was significant (HR 4.54, 95% CI 1.00-20.50, P=0.050) alongside Tmsv-16 SD >3%.
In a multivariable cox regression for all-cause mortality including Tmsv-16 SD >3%, age, and diabetes mellitus, only age remained significant for all-cause mortality (adjusted HR 1.05, 95% CI 1.01-1.10, P=0.019). The adjusted HR for diabetes was 1.61 (95% CI 0.70-3.71, P =0.262) and for Tmsv-16 SD >3% was 2.16 (95% CI 0.96-4.89, P=0.063).

For MACE, the multivariable model included systolic blood pressure and coronary artery disease (significant on univariate analysis) alongside LV dyssynchrony. ACEI/ARB use had also been significant on univariate analysis but because there was interaction between being on an ACEI/ARB and systolic blood pressure, it was decided to include only systolic blood pressure. In this model, the only significant factor associated with MACE was history of coronary artery disease (adjusted HR 2.54, 95% CI 1.06-6.08, P=0.036) and systolic blood pressure (adjusted HR 1.02, 95% CI 1.00-1.04, P=0.035). Tmsvd-16 SD >3% was not significant; adjusted HR 2.06, 95% CI 0.85-4.97, P=0.110).

In a multivariable model including systolic blood pressure and Tmsv-16 SD >3% for heart failure hospitalisation, both factors were significant (adjusted HR 5.61, 95% CI 1.53-20.58, P =0.009 and adjusted HR 1.03, 95% CI 1.00-1.06, P =0.046 respectively), Figure 9-2. As fluid overload is important in the context of heart failure admissions, the analysis was re-performed excluding patients with an inter-dialytic weight gain of greater than 3kg (N=17). In the same multi-variable model, LV dyssynchrony remained associated with heart failure admissions (adjusted HR 5.81, 95% CI 1.20-28.14, P=0.029).  

**Figure 9-2. Adjusted cumulative survival plot for time to admission for heart failure in patients with and without LV mechanical dyssynchrony.**
9.6 Discussion

To our knowledge, this is the first study to explore the LV mechanical dyssynchrony indices measured using RT3DE in a maintenance HD population. There was no difference in baseline characteristics including LVEF or LV mass in patients with and without LV mechanical dyssynchrony. In adjusted models, LV mechanical dyssynchrony was not predictive of all-cause mortality or major cardiac events. However, it was associated with heart failure hospitalisation.

In uraemic cardiomyopathy, there are known structural changes such as fibrosis and reduction in capillary density per myocardial unit (11). Fibrotic changes are a precursor to left ventricular hypertrophy and subsequent left ventricular systolic and diastolic dysfunction. Indeed up to 36% of prevalent haemo- and peritoneal dialysis patients have LV systolic functional impairment. We hypothesised that with the high prevalence of fibrosis there will also be a greater degree of LV mechanical dyssynchrony in HD patients. This was confirmed in the study with the finding that mechanical LV dyssynchrony indices were prolonged in ESKD. 40% of patients had Tmsvd-16 SD > 3% in this population with largely preserved LVEF. This is consistent with literature using TDI as a measure of LV dyssynchrony, defined as regional difference in time to peak systolic myocardial velocity >105ms. Using TDI there was LV dyssynchrony in HD patients prior to treatment (49% of HD patients)(12).

LV dyssynchrony can be detected with TDI, speckle strain analysis and RT3DE. However, it has been reported that TDI and RT3DE determinants of LV dyssynchrony are not directly comparable in terms of values and both provided different measures of ventricular timing(13). This is partly due to the fact that RT3DE takes into account of longitudinal and radial deformation whilst TDI only determines longitudinal deformation.

The intra and inter-observer reproducibility for Tmsv-SD was good in our study and was comparable with other study groups (14,15). It has been reported that reproducibility is dependent on image quality with better reproducibility with adequate compared with poor datasets (16). Conca et al. (17) compared measurements of LV dyssynchrony using TDI, RT3DE and speckle tracking in 120 healthy subjects. Forty datasets were sent to another center for analysis. It was reported that there was a large inter-institutional variability for
all measured parameters with the most reproducible parameters being RT3DE synchrony index and radial strain.

One of the limitations of our study is that we did not have a control group. However, when we compared our measurements with another published study group of 40 healthy volunteers, there were comparatively prolonged LV dyssynchrony indices in our HD population; in the published control group mean Tmsvd-16 SD was 1.11 (SD 0.39)% compared with our cohort where the mean Tmsvd-16 SD was 3.35 (SD 3.30)% (18). Certainly, this is only a crude comparison because the control group was different in respect of age and comorbidities. This control group, as with many others are in a younger cohort and their LV dyssynchrony indices may be very different compared with the older dialysis population. Nonetheless the indices of LV mechanical dyssynchrony found in our study seem to be more prolonged compared to studies in any other population (18,19).

Interestingly, the prolonged LV dyssynchrony indices has occurred in a population where there were not many patients with prolonged QRS (3/97 had QRS > 150ms) or LBBB (1/97) on electrocardiogram and majority of patients had preserved LVEF (94%). The commonest reason in the general population for LV mechanical dyssynchrony is conduction system disease manifested by a prolongation of the QRS complex. Since the indices of LV mechanical dyssynchrony is abnormal in the HD population, with relatively few QRS prolongation, it supports that other mechanisms are at play other than conduction delays. The additional pathological mechanisms may include chronic volume and pressure overload, uremic toxins, metabolic and hormonal imbalance such as chronic kidney disease-metabolic bone disease.

There was no association identified between LV dyssynchrony and parameters such as LVEF and LV mass. This may be because this study group consisted of patients with largely preserved LVEF and LV hypertrophy and few patients with QRS prolongation. In the general and dialysis populations, LV dyssynchrony has been described to be associated with LV mass (12,20), high LV end-diastolic pressure and reduced myocardial motion velocities (12). Therefore supporting that LV hypertrophy and LV dysfunction as part of the pathophysiological mechanisms involved.

The results show that LV dyssynchrony was not predictive of mortality or major cardiac events. However it was associated with hospitalisation for heart failure. In patients post
acute myocardial infarction, the presence of LV dyssynchrony detected on two
dimensional speckle tracking, after a mean follow-up of 40+/-17 months, is associated with
an increased risk for a composite end-point of all cause mortality and heart failure
hospitalisation episodes (adjusted HR 1.06, 95% CI 1.05-1.08, P <0.001, per 10ms
increase)(21). Similarly, in patients with heart failure, LV dyssynchrony at baseline prior
to CRT was predictive of cardiovascular death (HR 0.46, 95% CI 0.72-0.97)(22). These
cited studies have included mainly patients with reduced LVEF, whilst our study reports
the results in a group with largely preserved LVEF. To date, our study is the first to
demonstrate the prognostic potential of LV dyssynchrony in a preserved systolic function
group.

Our study has limitations. Firstly, there is no universally agreed definition of LV
dyssynchrony. Our study is lacking in not having a healthy control group to determine the
normal LV dyssynchrony value, nor an age and sex-matched control to compare the
differences in LV dyssynchrony indices. However, we have compared our findings with
other published control groups and have identified that there is a tendency to higher LV
dyssynchrony indices in HD patients (8).

Second, we were not able to adequately analyse all the scans that were performed. From
219 recruited patients, only 97 had adequate images. This is reflective of the difficulty of
performing RT3DE in HD patients and the need for adequate images for LV dyssynchrony
analysis using this modality.

Third, although our study consists of the largest group of HD patients with LV
dyssynchrony measured with RT3DE to date, the study cohort is still relatively small.
Despite this, our power calculation supports that the number of patients in each arm of
assessment is adequate to detect a large difference in mortality. Furthermore, our study
patients are representative of a prevalent dialysis population whom do have mainly
preserved LVEF.

In summary, abnormal LV dyssynchrony indices are common in patients undergoing
maintenance HD, despite them generally having a preserved LV function. No clinical
parameters were identified to be associated with LV dyssynchrony in our study group. LV
dyssynchrony was not associated with all-cause mortality or major cardiac events, but was
associated with heart failure hospitalisation.
9.7 References


15. Marsan NA, Bleeker GB, Ypenburg C, Ghio S, Van De Veire NR, Holman ER, et al. Real-time three-dimensional echocardiography permits quantification of left ventricular mechanical dyssynchrony and predicts acute response to cardiac...
Chapter 10

Conclusion- Evaluation of Findings, Discussion and Plans for Future Studies

10.1 Preface

This final chapter summarises the findings of all preceding chapters, and discuss how the results of the analyses have addressed the initial hypotheses. An overall summary of which of the explored novel techniques may be applied to clinical practice will be provided. Finally, there will be an outline of how the results of this thesis will lead to future investigations and projects.

10.2 Introduction

In chapter 1, it was identified that there are limitations to the use of standard two-dimensional (2D) transthoracic echocardiography in the assessment of patients undergoing haemodialysis therapy. Many current routine measurements are inaccurate or insensitive to identify high risk individuals in this high cardiovascular risk burdened population. As a result, this research study was designed to explore some novel 2D and three-dimensional (3D) echocardiographic techniques to assess their potential for predicting outcomes (mortality and cardiac events) in haemodialysis patients (described in chapter 3). The techniques investigated include: speckle tracking determined global longitudinal strain and tissue motion mitral annular displacement, 3D echocardiographic measurements of left ventricular mass and volume, and left ventricular mechanical dyssynchrony.

10.2.1. Evaluation of chapter 4: Non-recruitment to and selection bias in studies using echocardiography in haemodialysis patients

This analysis aimed to compare the study participants and non-participants. It was found that 44 patients were excluded. Inability to give consent was an exclusion criteria and the most common reason for this was due to a language barrier (36%). From 172 patients who declined consent, the majority (84%) was due to reluctance to attend for an extra hospital visit. Although there was no difference in Charleston co-morbidity index and social
deprivation scores between patients who were excluded versus recruited, patients who were excluded did have more abnormalities of blood biochemistry such as lower mean phosphate, albumin and higher C-reactive protein. After a median follow-up of 29.7 (25th-75th centile, 21.1-34.3) months, in a multivariable Cox regression model, patients who declined consent had an adjusted hazard ratio (HR) for all-cause mortality compared with recruited patients of 1.70, 95% confidence interval, CI, 1.10-2.52, P = 0.014. Excluded patients had an adjusted HR of 1.30, 95% CI 0.75-2.25, P=0.347.

Several important points are raised from these findings. First, in the design of future research studies, there should be an inclusion of costs for interpreters in grant applications because language barrier was a greater logistical hurdle to participation than had been anticipated. Our study may have been biased against patients where English was not a spoken language. Second, not all patients gave an in-depth reason for reluctance to attend for an extra study visit. We speculate that this may be because of work, child care arrangements and many patients may have needed to attend multiple out-patient appointments for co-existing medical conditions. There should be specific discussions with patient focus groups to explore the reasons for non-participation. An understanding of this may help in the design of future studies to help recruitment. Third, patients who declined consent to participation to the study had a worse survival compared with patients who were recruited. The reasons for this were not entirely apparent. It is hypothesised whether patients with end-stage kidney disease may have other psychosocial factors such as depression that lead to lack of inclination to participate in studies, in turn, this may be related to poor survival. Similarly, if patients are non-adherent to treatment, they may also be less eager to participate in studies and non-adherence to treatment may be associated with early demise. There may also be other co-morbidity burdens that were not reflected in the data which was collected and adjusted for.

In terms of the thesis, this analysis has highlighted that the study population which was recruited did have a survival advantage over patients who were not included. As such, it is not entirely representative of the whole haemodialysis population. This needs to be borne in mind when interpreting the results. In this respect, patients who refuse to attend for an extra visit for an echocardiogram may be seen as a biomarker for risk of death, which is probably as useful as actual imaging parameters such as left ventricular mass index. We would recommend that it should be more common practice for the research community, as
a whole, to report the characteristics and survival of both patients who are recruited and excluded from research studies.

10.2.2. Evaluation of chapter 5: Novel approach to cardiovascular outcome prediction in haemodialysis patients

The first novel 2D echocardiographic parameter investigated was speckle tracking determined global longitudinal strain (GLS), to address the hypothesis that GLS is a better prognostic indicator compared to standard echocardiographic parameters and pulse wave velocity (PWV). It was found that in a separate univariate model both PWV and GLS were associated with all-cause mortality. However, in a combined Cox regression model adjusting for factors associated with mortality, only left ventricular mass indexed height\(^{2.7}\) [LVM/IHT\(^{2.7}\)] (adjusted HR 1.02, 95% CI 1.00-1.04) and PWV (adjusted HR 1.23, 95% CI 1.03-1.47) were significant. GLS was independently associated with cardiac death (adjusted HR 1.24, 95% CI 1.00-1.54) and cardiac events (adjusted HR 1.13, 95% CI 1.03-1.25). It is hypothesised that patients undergoing dialysis may die of vascular causes in addition to cardiac modes of death. PWV and left ventricular hypertrophy may be more sensitive to predict all-cause mortality whilst GLS is more specific to cardiac death and events. Left ventricular hypertrophy may be a consequence of hypertension and hence its association with vascular death. Similarly, PWV is reflective of aortic stiffness which may be related more to vascular deaths. In clinical practice, it may be important to be carrying out PWV routinely to risk stratify haemodialysis patients.

10.2.3. Evaluation of chapter 6: Cardiovascular risk assessment in patients with preserved left ventricular ejection fraction and left ventricular hypertrophy.

The previous chapter's analysis was in a relatively 'unselected' haemodialysis population. This chapter aimed to assess whether GLS was predictive of outcome in specific subgroups of patients based on echocardiographic phenotype. The sub-groups chosen were those patients with left ventricular hypertrophy and those with preserved LVEF. A similar analysis was performed in these groups as per chapter 5 to test whether GLS was still not associated with all-cause mortality when accounting for echocardiographic parameters and PWV. It was found that in the sub-group with left ventricular hypertrophy, GLS was not predictive of prognosis. Only age (HR 1.07, 95% CI 1.01-1.13) was predictive of mortality. In contrast, in the sub-group with preserved LVEF, in a combined model
adjusting for age, smoker, coronary artery disease and diabetes mellitus, PWV (HR 1.23, 95% CI 1.04-1.45) and GLS (HR 1.16, 95% CI 1.01-1.33) were both predictive of mortality. For cardiac deaths and cardiac events the sample size was too small to perform any survival analysis in the separate sub-groups. It is concluded that the prognostic value of GLS is different in different sub-groups. It is likely that in a variable LVEF population, GLS is too insensitive for prediction of mortality unlike in preserved left ventricular ejection fraction. Considering that the majority of haemodialysis patients have a preserved LVEF, GLS may still be a useful marker of outcome, and future risk stratification methods should encompass GLS, and indeed PWV, as well as standard echocardiographic parameters such as LVMI/HT²/² and LVEF.

10.2.4. Evaluation of chapter 7: Speckle tracking determination of tissue motion annular displacement: comparison with strain, ejection fraction and association with outcome in haemodialysis patients.

The purpose of the investigation in this chapter was to determine whether another novel marker, speckle tracking determined tissue motion mitral annular displacement (TMAD), was of similar or better prognostic value than GLS if included in a survival analysis instead of GLS. This is because TMAD, like GLS, is a measure of strain but has the advantage over GLS of being quicker to measure and also less user dependent. It would therefore have the potential to translate more easily into front line clinical practice. In this analysis, there was a strong negative correlation of TMAD with GLS (r=-0.614, P<0.001). In a multivariable Cox regression analysis which did not include GLS, TMAD was not associated with mortality (HR 1.04, 95% CI 0.91-1.19), cardiac death (HR 1.03 (95% CI 0.80-1.32) or cardiac events (HR 0.91 (95% CI 0.80-1.02). These findings did not support the use of TMAD as an alternative measure to GLS when attempting to use abnormal strain as a prognostic marker in haemodialysis patients.

10.2.5. Evaluation of chapter 8: Comparison of two-dimensional and three-dimensional echocardiographic parameters and association with mortality in haemodialysis patients

Moving on from 2D echocardiography, another new technology is the use of real time 3D echocardiography (RT3DE). This can be performed with the same echocardiography machine as 2D echocardiography but with a different transducer. This technology is rapidly becoming available in clinical practice, yet the literature on its use in patients with
end stage kidney disease is limited. Furthermore, it is unknown whether its measurement of left ventricular mass and volume is predictive of mortality in the same way as those measurements when taken with 2D echocardiography. From the analysis in this chapter, it was found that there was good correlation between 2D echocardiographic and RT3DE measures of left ventricular volumes, however, the measurement of left ventricular mass was significantly higher in 2D echocardiography compared with RT3DE. Two-dimensional echocardiographic measure of a lower LVEF was associated with mortality, adjusted HR 0.94, 95% CI 0.89-0.99, whilst 2DE measure of LVMI/HT$^{2.7}$ was not associated with death (adjusted HR 1.02, 95% CI 0.99-1.04, P=0.205). RT3DE measures of LVMI/HT$^{2.7}$ and LVEF were not (HR 1.03, 95% CI 0.98-1.09 and HR 0.95, 95% CI 0.90-1.00, respectively). This analysis has also highlighted some of the particular challenges with obtaining RT3DE images (97 of 219 patients had adequate imaging for LVEF, but only 69 of 219 had adequate images for LVMI/HT$^{2.7}$). Although RT3DE may produce more accurate measurements, akin to cardiac magnetic resonance imaging in published studies, the prognostic value of measures of mass and volume are limited in a end stage renal disease population. Therefore the applicability in routine everyday practice may not be practicable.

10.2.6. Evaluation of chapter 9: 3D echocardiographic left ventricular dyssynchrony indices in end stage kidney disease: associations and outcomes

The last hypothesis tested was whether left ventricular dyssynchrony indices determined by RT3DE were associated with adverse outcomes. It is also unknown whether left ventricular dyssynchrony measured by RT3DE is different in an end stage kidney disease population compared to the general population and, if so, whether this would be a useful marker of prognosis. It was found that left ventricular dyssynchrony indices were more prolonged compared to a previously published healthy control group. A limitation of the thesis study design was that a control group was not included and this would be necessary in future studies to confirm the findings. When using a cut off of standard deviation of time to minimum systolic volume (Tmsv-16 SD) of >3% for left ventricular dyssynchrony, this was not predictive of all-cause mortality (adjusted HR 2.16, 95% CI 0.96-4.89, P =0.063), cardiac events (adjusted HR 2.06, 95% CI 0.85-4.97, P=0.110). However, it was associated with heart failure hospitalisation (adjusted HR 1.03, 95% CI 1.00-1.06, P=0.046). At present, routine left ventricular dyssynchrony measurements may not be immediately useful in practice.
10.3 Overall Summary

From the echocardiographic parameters investigated, the measurements which seem to be useful for prognostication in haemodialysis patients are GLS (in patients with preserved LVEF), and pulse wave velocity (in an unselected group). Speckle tracking determined TMAD may be a rapid alternative to GLS but was not of prognostic value. 2D echocardiographic measurements of LV mass gives higher measured values than RT3DE. There was good correlation between 2DE and RT3DE measurements of left ventricular volumes, with larger measured values with RT3DE. However, the difficulties in obtaining adequate images using RT3DE in end stage renal failure patients largely preclude from use in everyday clinical practice at present. RT3DE determined left ventricular dyssynchrony indices are prolonged in haemodialysis patients compared to the general population, but their prognostic significance remains unclear because of the small sample size having adequate RT3DE images in this study. Although it may be a promising prognostic indicator of future heart failure hospitalisation episodes.

10.4 Future Studies

There are 3 future studies which are underway as a result of this thesis.

1. Measuring longitudinal changes in cardiac structure and function, and aortic stiffness in haemodialysis patients, and determining the association of these longitudinal changes with prognosis.

This thesis has examined single time point echocardiographic measurements in a single centre cohort of patients undergoing haemodialysis. It is unknown whether longitudinal changes, exploring the same echocardiographic parameters using 2D and 3D echocardiography, pulse wave velocity, and also electrocardiographic findings will produce more powerful predictors of outcome (mortality and cardiac events) than baseline values alone. There have been some previous reports stating that changes in some echocardiographic parameters are predictive of outcome. For example, in a study of 123 patients undergoing haemodialysis, a 1 gram increase in left ventricular mass index was associated with an adjusted HR of 1.03, 95% CI 1.02-1.05, P=0.001, for sudden cardiac death over a 10 year follow-up period (1). In addition, the rate of increase in left
ventricular mass index was associated with incidence of death. If the change in left ventricular mass was greater than the 75% percentile, this resulted in a HR 2.56, 95% CI 1.94-3.31 for all-cause mortality which was greater than if the mass increased greater than 25th percentile (2). Other parameters such as an increase in left ventricular atrial volume from 10.5±5.0 to 11.6±5.6ml/min \(^{2.7}\); P<0.001 has also been reported to be a predictor of cardiovascular events, independent of left ventricular mass (3). It is unknown, however, whether progressive changes in global longitudinal strain or left ventricular dyssynchrony are associated with mortality and cardiac events.

To address this aim, the patients in the Salford Kidney Study have undergone annual echocardiographic assessments with the same protocol, as described in the chapter 3. To date (8th February 2016), there has been 162 patients who have had year 2 assessments (19 patients died before their echocardiogram, 4 patients weren't invited back because of inadequate baseline scans, 2 patients were too unwell to have assessments, 2 patients did not have first year echocardiograms, but came for 2nd year scan, 3 patients moved out of the region and 27 patients refused to re-attend for a follow-up assessment). 66 patients have had year 3 scans, 10 patients have had year 4 scans. All surviving patients will have 4 scans in total and ethical approval allows for follow-up thereafter until 2026. Data collection and analysis of longitudinal data will be carried out at intervals during follow-up to assess the relationship with future outcomes.

2. Changes in cardiac structure and function, and aortic stiffness, before and after renal transplantation.

There were 44 patients who had been transplanted during the course of this thesis (until 16th November 2015). At present, there has not been a study which has carried out echocardiographic assessments with pulse wave velocity measurements before and after transplantation, in particular with repeated assessments post-renal transplantation. It is well established that survival with a kidney transplant is longer than continuing to receive dialysis therapy (4). This is likely to be multi-factorial, of which it is plausible that improvements in cardiac function may play a part. There have been studies published reporting the changes in 2D echocardiographic parameters after kidney transplantation. However, the literature is conflicting. Some authors report improvement in left ventricular ejection fraction 3 months after kidney transplantation (5) with reduction in left ventricular hypertrophy and amelioration of diastolic dysfunction (6), whilst others report that
parameters such as diastolic function worsen after renal transplant (7). No study has investigated whether there are changes in sub-clinical echocardiographic parameters such as global longitudinal strain or left ventricular dyssynchrony. In addition, published studies have not included pulse wave analysis in the multivariable models. Arterial stiffness has been shown not to progress over one year post renal transplant (8), but repeated measures after one year have not been studied.

Therefore, Salford Kidney Study patients will continue to have annual assessments, as described in the longitudinal study above, after renal transplantation. Using the echocardiographic and pulse wave velocity measurements, the aim would be to characterise the cardiac changes that occur post renal transplantation with repeated measures. In addition, it would be explored how changes are related to outcome.

3. Changes in cardiac structure and function, and aortic stiffness in transitioning from pre-dialysis to dialysis therapy.

The baseline echocardiographic and strain results, described in chapter 4 and 5, have been used for a research grant application. Similar to kidney transplantation, there is rationale that patients starting dialysis treatment (from pre-dialysis to being established on treatment) may have improved cardiac structure and function. This may be due to a combination of improvement in fluid and electrolyte balance with amelioration of the uraemic milieu. In a seminal paper by Foley et al. 227 patients starting haemodialysis therapy had echocardiograms at inception and then repeated after 1 year. Almost half of all patients had reductions in left ventricular mass and increased systolic function as evidenced by improved fractional shortening. Patients who had improved echocardiographic parameters were less likely to develop new onset of cardiac failure (9). This study has been published over a decade ago and now there are novel techniques that can be applied with greater accuracy and detection of subclinical changes such as 3D echocardiography and speckle tracking echocardiography. It is unknown whether there are changes in these parameters soon after starting dialysis.

Furthermore, it has been reported that patients undergoing peritoneal dialysis have a higher left ventricular mass index compared with patients on haemodialysis therapy (139±19 g/m² vs 104± 22 g/m², P=0.004)(10). It is proposed that this is due to better fluid and metabolic control with haemodialysis therapy. The follow on to Salford Kidney Study will
investigate whether there are cardiac functional and structural differences between patients starting peritoneal versus haemodialysis and, in addition, whether the longitudinal rates of change of these parameters differs between dialysis modalities.

To address these hypotheses, it is proposed that patients who attend the low clearance clinic at Salford Royal NHS Foundation Trust will be approached for recruitment, and undergo cardiovascular assessment when they are 12 months from the expected date of starting dialysis. They will have repeat analysis at the inception of dialysis, and finally again at 12 months after starting dialysis. The rate of change of parameters before and after starting both dialysis modalities will be compared using patients as their own control, and the rate of change of parameters after starting peritoneal dialysis will be compared with haemodialysis.

10.5 References

7. Dudziak M, Debska-Sлизен, A, Rutkowski B. Cardiovascular effects of successful


Appendix 1.

Publications and abstracts arising from this thesis

A 1 Publications included in the thesis


A 2 Related publications not contained in thesis


A 3 Manuscripts submitted to journals pending decision

Non-recruitment to and selection bias in studies using echocardiography in haemodialysis patients. Submitted to *American Heart Journal*, has been sent for peer review.

Cardiovascular risk assessment in haemodialysis patients with preserved left ventricular ejection fraction and left ventricular hypertrophy. Submitted to the *Journal of Cardiology*.

Speckle tracking determination of tissue motion annular displacement: comparison with strain and ejection fraction, and association with outcomes in haemodialysis patients. Submitted to *Nephrology*.

Comparison of two-dimensional and three-dimensional echocardiographic parameters and association with mortality in haemodialysis patients. Submitted to *European Heart Journal: Cardiovascular Imaging*. 
3D echocardiographic left ventricular dyssynchrony indices in end stage kidney disease: associations and outcomes. Submitted to Journal of American Society of Echocardiography and is undergoing peer review.

A 4 Related abstracts presented at international meetings

American Society of Nephrology Kidney Week, 2015 (Awarded 'Top Oral Abstract by Trainee')
[SA-OR035] Abnormal global longitudinal strain is associated with all-cause mortality in haemodialysis patients.

World Congress of Nephrology, 2015
[SAT-236] Reduced systolic longitudinal strain in haemodialysis patients with preserved ejection fraction is independent of symptomatic coronary artery disease.

European Society of Cardiology - Euro-Echo Imaging, 2014
[90603] Tissue motion annular valve displacement, measured using two-dimensional speckle tracking, correlates with global longitudinal strain in haemodialysis patients with preserved ejection fraction.

A 5 Related abstracts presented at national meetings

Renal Association Meeting, 2016 (Accepted for oral presentation).
Non-recruitment to and selection bias in studies using echocardiography in haemodialysis patients

British Cardiovascular Society Annual Conference, 2016.
[1894] Abnormal global longitudinal strain is associated with all-cause mortality in haemodialysis patients.

Renal Association Meeting, 2014
[P264] Prescribing patterns of cardio-protective medications in chronic kidney disease and end stage kidney disease

The University of Manchester, Summer Postgraduate Showcase, 2014
The Tissue motion annular valve displacement, measured using two-dimensional speckle tracking, correlates with global longitudinal strain in haemodialysis patients with preserved ejection fraction.
A 6  Grants and Bursaries

2013  American Society of Nephrology Travel Grant- Geriatric Nephrology (800 USD)
2013  Renal Association Amgen Bursary (£500)
2014  American Society of Nephrology Travel Grant - Innovation Course (800 USD)
2015  American Society of Nephrology Travel Grant - Women through the Decades (800 USD)
2015  University of Manchester, Faculty of Medical and Human Sciences Travel Grant (£500)
2015  British Kidney Association Travel Grant (£1500)

A 7  Awards and Prizes

Hyponatraemia is associated with all cause mortality. Best Abstract Prize for Institute of Population Health (IPH), The University of Manchester, poster presented at the IPH Faculty Showcase, 2013.

Hyperuricaemia is associated with cardiovascular deaths. Best Poster Prize at the Renal Association Meeting, 2014.