Electrocardiography for cardiac risk stratification in haemodialysis patients

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3D - 3 dimensional

AF - Atrial fibrillation

AUC- Area under the Curve

BMI- Body Mass Index

BNP- Brain natriuretic peptide

CAD- Coronary Artery Disease

CI- Confidence Interval

CHB- Complete Heart Block

CKD- Chronic Kidney Disease

CMR- Cardiac Magnetic Resonance

CVA- Cerebrovascular Accident

CVD- Cerebrovascular Disease

ECG- Electrocardiogram

EDV- End Diastolic Volume

EDVTeich- End Diastolic Volume using the Teicholz equation

EF-Ejection Fraction

EGFR- Estimated Glomerular Filtration Rate

ESRD- End Stage Renal Disease

GLS- Global Longitudinal Strain

HAVcr-1- Hepatitis A Virus cellular receptor 1

HD- Haemodialysis

HF- High Frequency

HR- Hazard Ratio

HRV- Heart Rate Variability

IQR- Interquartile Range

KIM-1- Kidney Injury Molecule 1

LBBB- Left Bundle Branch Block

LF- Low Frequency

LVEF- Left Ventricular Ejection Fraction

LVH- Left Ventricular Hypertrophy

LVMI- Left Ventricular Mass Index

LVMIHt2.7- Left Ventricular Mass Indexed to Height2.7

MACE- Major Adverse Cardiac Events

NICE- the National Institute for Health and Care Excellence

NTproBNP- N- Terminal pro-hormone of Brain Natriuretic Peptide

NPV- Negative Predictive Value

NYHA- New York Heart Association

PD- Peritoneal Dialysis

PPV- Positive Predictive Value

PVD- Peripheral Vascular Disease

PWV- Pulse Wave Velocity

RBBB- Right Bundle Branch Block

ROC- Receiver Operating Characteristic

RRT- Renal Replacement Therapy

RT3DE- Real Time 3 Dimensional Echocardiogram

SBP- Systolic Blood Pressure

SCD- Sudden Cardiac Death

SDNN- Standard Deviation of Normal to Normal R – R intervals

SKS- Salford Kidney Study

TCRT- Total Cosine R to T

TIM-1- T-cell Immunoglobulin and Mucin domain 1

UF- Ultrafiltration

URR- Urea Reduction Ratio

VLF- Very Low Frequency

Abstract

Electrocardiography for cardiac risk stratification in haemodialysis

Sofia Skampardoni

For the degree of Doctor of Philosophy in the Faculty of Biology, Medicine and Health, University of Manchester, 2020

Cardiovascular disease is common in end stage renal disease. Emerging evidence suggests that repolarisation abnormalities play an important part in the pathogenesis of cardiac mortality. Risk stratification methods have traditionally relied on echocardiography. The ECG is in our opinion under-utilised for risk stratification in haemodialysis. The purpose of this thesis was to test the hypothesis that traditional and novel ECG parameters are independently predictive of cardiac outcomes in haemodialysis. Were this case, the ECG would have the potential to be used as a screening tool in haemodialysis patients.

In the first study (chapter 4) we examined the prognostic potential of QRS –T angle, an indicator of repolarisation heterogeneity that has been shown to be associated with cardiac outcomes in other populations. In our cohort of haemodialysis patients (n=171) we demonstrated that QRS–T angle carries independent prognostic value for MACE (follow up 2.3±1.1 years): hazard ratio 2.215, (1.188 - 4.131), p=0.012.

In the next study (chapter 5) we looked specifically at the presence of ECG strain and demonstrated that is associated with increased risk of MACE (HR 2.961, CI: 1.254 – 6.990, p= 0.013), but not all cause mortality, independently of LVH.

For the following study, we aimed to evaluate these biomarkers already used as diagnostic tests and assess whether an ECG based model can be reliably used as a diagnostic test for pending MACE in haemodialysis patients. We found that ECG biomarkers were overall very poor as a screening tool for cardiovascular outcomes and all cause mortality. This was evidenced by the very low sensitivity and very low AUC values on the ROC curves of all of them.

In chapter 6 we evaluated the diagnostic accuracy of electrocardiographic methods of calculating LVH compared to RT3DE and demonstrated that ECG methods for assessment of LVH that rely on voltage criteria have very low sensitivity and unreliable specificity compared to RT3DE and also conventional M –Mode echocardiography.

In the last study (chapter 7) we explored whether KIM-1 (a novel biomarker of cardiovascular risk in ESRD) exhibits any association or correlation with ECG recorded parameters of abnormal conduction and arrhythmia. Based on our data, we could not identify a correlation between KIM-1 and the above biomarkers.

Our results do not support the above hypotheses. Individual ECG markers demonstrate variable degrees of association with cardiac outcomes in particular group of patients, but this association was not reliably reproduced and often ceased to exist with the addition of other risk factors. A limitation of the findings is the possibility that the small sample size in some studies prevented conclusive results.

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

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First of all, I would like to thank the patients who took part in the Salford Kidney Study, as without that, none of the studies comprising this work would have been possible.

On professional level, I would like to thank my supervisory team; Professor Green, Professor Kalra and Dr Poulikakos. Professor Kalra gave me the opportunity to be involved in research in the first place and for that I am grateful. Additionally, his comments and suggestions proved extremely useful at crucial points of this process. Above all, I would like to thank my main supervisor, Professor Darren Green, whose support and guidance made the completion of this work possible and whose trust and constructive criticism enabled me to develop as a researcher and find my own voice.

August 2020

Thesis format

This thesis comprises a series of original research works covering the common theme of utilising ECG for diagnostic purposes in a haemodialysis patient cohort. Each chapter, save for those describing aims, methods and future studies, are formatted as manuscripts for submissions to peer reviewed medical journals. This includes the introduction chapter. Indeed, the introduction chapter, alongside the first results chapter are now both published articles.

This means that the thesis is presented as a journal format thesis. This may lead to some duplication within the introduction and methods sections of results chapters. For this reason the overall introduction and methods chapters (chapters 1 and 3) give a respective overview of the association of ECG parameters with outcomes in haemodialysis patients and broad descriptors of the Salford Kidney Study method applicable to all results chapters, with further specific detail described in each results chapter. Each results chapter also contains a short opening section titled "rationale" to provide extra context for the chapter within the overall aim of the thesis. These can be considered to form part of the chapter introduction.

The references for each chapter are collated in a single references section at the end of the thesis.

Contribution of the author to the research

The work undertaken in this thesis comprises studies which form part of a pre-existing longitudinal cardiovascular epidemiological study of chronic kidney disease. This study was devised by Professor Kalra, and the work expanded by Professor Green into a haemodialysis population. Dr Poulikakos provided expert supervision in respect of novel and emerging repolarisation ECG changes.

The role of the author in each chapter is detailed below. Additional contributions included the performing of biochemical assays by external laboratory partners, offline analysis of some novel echocardiographic parameters by cardiology peers, and ECG analysis of proprietary novel abnormalities by colleagues outside of the research group.

Chapter 1: All searches, collation of data and first draft manuscript preparation undertaken by SS.

Chapter 4: study design in conjunction with DP, development of collaboration with external partners for ECG analyses, collation of datasets from numerous SKS master files, full analysis design and undertaking, preparation of manuscript.

Chapter 5: study design in conjunction with DG, creation of dataset for analysis including further analysis of echocardiographic parameters to index left ventricular mass, all statistical analysis, manuscript preparation.

Chapter 6: study design in conjunction with PK, DG, creation of dataset and all analysis, manuscript preparation.

Chapter 7: study design, creation of dataset, all statistical analysis, manuscript preparation.

Chapter 8: study design in conjunction with DG, creation of dataset, all statistical analysis, manuscript preparation.

Chapter 1

Cardiovascular disease in Chronic and End Stage Renal Disease; an overview with particular focus on the use of electrocardiography

Rationale

This first part of chapter one will provide a brief overview of cardiac disease in chronic kidney disease (CKD) with particular focus on end stage renal disease (ESRD). This section will therefore concentrate on dialysis, looking at traditional and newly emerging risk factors and diagnostic strategies. Following this, a more detailed review of the use of electrocardiography in ESRD and haemodialysis, the main focus of this thesis, forms the second part of chapter one in the format of a published narrative review article.

Introduction

Chronic Kidney disease (CKD) is characterised by much higher cardiovascular mortality and morbidity compared to the general population. This risk increases exponentially in End Stage RenalDisease (ESRD)[1] US Renal registry data indicate that sudden death or fatal arrhythmia is the documented cause of death in approximately 26% of ESRD patients[2]. Whilst atherosclerotic disease is common in CKD and ESRD, evidence indicates that it only accounts for a small proportion of cardiovascular deaths in this population[3].Furthermore, extrapolating evidence from the general population for cardiac risk modification has proven to be of limited benefit in dialysis patients. Statin therapy for primary prevention does not reduce cardiac risk in dialysis patients[4] and also coronary revascularisation and implantable cardioverter defibrillators[5] based on current guidelines do not reduce arrhythmic mortality in these patients[3]. In the general population, most fatal arrhythmic events are triggered by underlying myocardial ischaemia, usually in

the presence of coronary artery disease[6] , and are mostly tachyarrhythmias. Although ischaemia is the commonest cause of arrhythmias in theses cases, in non- ischaemic causes, the commonest mechanism is fibrosis. In advanced CKD and ESRD, the mechanism, timeline, and specific rhythm of such events are not fully understood. Non- conventional cardiovascular risk factors such as electrolyte imbalances, volume shifts, and blood pressure changes have been implicated in the extremely high sudden death rates after the long dialytic interval[7] . The relative contributions of primary arrhythmic events, as well as the contribution of bradyarrhythmia rather than ventricular tachyarrhythmias in these deaths, remain unclear. Recent studies of prolonged implantable loop recording in five different HD cohorts have suggested that bradyarrhythmic events may be more common than ventricular arrhythmia in causing sudden cardiac deaths (SCDs)[8]. Although the underlying mechanisms are far from clear, 10% of the patients in these cohorts were noted to have heart block or other bradyarrhythmia that could be treated with permanent pacing systems, and this itself should make the case for more frequent use of standard electrocardiogram (ECG) in dialysis populations.

In recent years, data from experimental and population based studies have led to advances in our understanding of the underlying disease mechanisms. These focus on the dynamic interplay between myocardial structural changes, vascular changes, autonomic imbalance, inflammation and fluid and electrolyte shifts leading to arrhythmias[9]. The presumed high burden of arrhythmic deaths in dialysis patients has led to a renewed interest in the evaluation of electrocardiographic parameters as potential risk predictors. The 12-lead electrocardiogram is an easily accessible and inexpensive bedside test. Moreover, the implementation of advanced software in most modern electrocardiographic machines means that vectorcardiographic indices can be derived with accuracy from standard 12-lead electrocardiograms.

In the following sections, the identified risk factors and proposed mechanisms of pathogenesis of cardiovascular disease in CKD and ESRD will be discussed further. A particular focus will be given to electrocardiographic parameters and their role in risk prediction in haemodialysis as this is the main focus of this thesis.

Atherosclerotic cardiovascular disease

The incidence of atherosclerotic vascular disease, mainly expressed as coronary artery disease, cerebrovascular disease and peripheral vascular disease, is increased exponentially in end stage renal disease with studies reporting a prevalence of coronary artery disease of 40%, and cerebrovascular and peripheral vascular disease at about 20% in the haemodialysis population[10].

Atherosclerosis is considered an inflammatory process where injury to the endothelium leads to endothelial dysfunction characterised by expression of adhesion molecules, infiltration of mononuclear cells into the arterial wall and platelet activation[11].

It has been hypothesised that advanced renal disease and the process of haemodialysis itself represent a state of continuous low grade inflammation mediated by accumulation of a number of substances, collectively known as uraemic toxins, which are thought to induce enhancement of leukocyte oxidative activity.

This could offer an explanation as to why atherosclerotic risk factors whose importance has been well established in non CKD populations, such as smoking, hyperlipidaemia and hypertension are only partially accountable for the observed accelerated atherosclerosis in advanced renal disease. Additionally, data in advanced renal disease including CKD5 and End Stage Renal Disease indicate that interventions aimed at primary prevention of cardiovascular disease, and which have a proven beneficial effect in the general population, do not have a statistically significant effect in the majority of dialysis patients and in others they might be associated with adverse effects[12].

A limitation in the interpretation of these reports is that they derive from studies not specifically designed to assess cardiac outcomes in advanced CKD and which may therefore be underpowered.

There has been increasing focus on non traditional risk factors and their role in the pathogenesis of non-atherosclerotic vascular disease in advanced renal disease.

Some of the proposed risk factors are related to renal disease itself and others to the haemodialysis process. The main examples of the first category are anaemia and hyperparathyroidism. Examples of proposed risk factors related to the haemodialysis include exposure to impure dialysis fluid and use of non biocompatible membrane which potentiate inflammation and oxidative stress.

The overarching hypothesis is that inflammatory cytokines are linked to anaemia via a number of different mechanisms, including suppression of bone marrow erythropoiesis, their effect on iron metabolism and increased incidence of intestinal bleeding[13,14] There is increasing evidence that management of anaemia and intravenous iron supplementation is associated with reduction of adverse cardiovascular outcomes including coronary artery disease and myocardial infarctions[15]. In fact, as the PIVOTAL trial demonstrated high dose intravenous iron was superior to low dose iron[15]

Changes in the bone metabolism associated with chronic kidney disease (CKD mineral and bone disease, or CKD-MBD) have also been implicated in the pathogenesis of accelerated vascular disease observed in advanced CKD and end stage renal disease. Disordered phosphate metabolism and high levels of parathyroid hormone have been associated with increased medial calcification of the coronary arteries, which in turn is thought to be associated with increased risk of cardiovascular complications including myocardial infarction in chronic kidney disease[16].

There have been proposed associations between factors associated with the haemodialysis process itself and an increase in vascular disease observed in patients with end stage renal disease; these factors in particular relate to the composition of the dialysers and the purity of the water used and their effect is hypothesised to be mediated via increased inflammation. While increases in C-reactive protein (CRP) and inflammatory cytokines are observed in haemodialysis[17]patients, they have also been noted in patients receiving conservative management for end stage renal disease, and those with advanced CKD, making the presumed association between factors unique to the quality of dialysis water and dialyser membranes and vascular disease weaker[18].

Arrhythmic complications and sudden cardiac death

Recent data from cohort studies in haemodialysis patients investigated with implantable loop recorders (ILR), alongside registry data, suggest that arrhythmic complications and sudden cardiac death due to arrhythmias comprise a high proportion of the adverse cardiovascular outcomes in end stage renal disease. In addition, and perhaps surprisingly, data from ILR studies suggest that the majority of fatal or clinically significant arrhythmias in haemodialysis are bradyarrhythmias[19,20].

Specifically, Roy- Chaudhury et al in a sample of 66 patients recorded 1678 events in total, out of which 1461 were bradycardia and 14 asystole[19]. The CRASH –ILR study showed similar findings in a smaller cohort of 35 haemodialysis patients, but who had ILR monitoring for much longer, up to 3 years in some cases; marked bradycardia necessitating pacemaker insertion was found in 10% of this UK cohort[20].

These findings are in contrast to the general population where ventricular tachyarrhythmia is the predominant form of arrhythmia leading to sudden cardiac death. The low incidence of clinically significant tachyarrhythmias in haemodialysis patients is in keeping with data suggesting that implantable defibrillators do not consistently improve cardiovascular outcomes in this population[21].

Atrial fibrillation was also commonly detected in these studies, usually during a dialysis session, but it was not associated with significant clinical events or death. This may be partially explained by the relatively small sample sizes in these studies.

The pathogenesis of the increased arrhythmic risk in end stage renal disease and haemodialysis is far from clear.

Cardiomyopathy in end stage renal disease is characterised by left ventricular hypertrophy and extensive fibrosis, which create a myocardial substrate vulnerable to superimposed insults. A prominent hypothesis is that other factors

unique to haemodialysis, such as fluid and electrolyte shifts, destabilise this already vulnerable myocardium[22] and create the necessary conditions for development of arrhythmic complications.

The potential of electrocardiography for cardiac risk prediction in Chronic and End Stage Renal Disease

(A version of following has been published in *Nephrology Dialysis Transplantation*, Volume 34, Issue 7, July 2019, Pages 1089–1098)

The following segment will provide an overview of the use of electrocardiography for cardiac risk prediction in haemodialysis patients. It will be presented in the form of a review article, similar to the published paper.

Abstract

Cardiovascular mortality is very high in Chronic and End Stage Renal Disease, however risk stratification data are lacking.

The electrocardiogram is easily accessible in clinical practice. A number of studies have assessed the prognostic potential of various electrocardiographic parameters in the renal population.

For this review, we identified studies from the last decade that assessed conventional and novel electrocardiographic markers as risk predictors in Chronic and End Stage Renal Disease.

The literature indicates that conventional electrocardiographic markers are overall unreliable for risk stratification in the renal populations. Novel parameters have shown promising results in smaller studies, but further validation in larger ones is needed.

Aims of the review

The aim of this review was to provide an overview of studies which have assessed the use of selected electrocardiographic and vectorcardiographic parameters taken

from standard 12-lead and continuous Holter electrocardiography for the purpose of cardiac risk stratification in the CKD and ESRD populations.

Review methodology

I. Data sources and search strategy

MEDLINE through PubMed, Google Scholar and Cochrane Library were searched to identify potentially relevant articles and abstracts. Furthermore, we reviewed the bibliographies of the selected articles for additional relevant studies. The search terms are presented in table 1.1.

Table 1.1 Keywords used as Boolean operators or search terms

Renal Disease	Outcomes	Parameters
CKD	survival	LVH
HD	Death	QTc
PD	Mortality	PR
Chronic Kidney Disease	Cardiovascular Outcomes	QRS-T angle
Renal disease	Cardiac Outcomes	TCRT
Haemodialysis		HRV
Haemodialysis		Left Ventricular Hypertrophy
Peritoneal Dialysis		Heart Rate Variability
Dialysis		ECG
		electrocardiogram
		electrocardiographic

II. Eligibility of studies

Studies in any of the CKD, haemodialysis and peritoneal dialysis populations were considered eligible for inclusion if they met the following criteria: published between January 2007 and December 2016; had a study sample of at least fifty participants in the initial cohort; had a mean follow up time of at least a year; assessed at least one of death and cardiac outcomes as an endpoint; studied the

association of left ventricular hypertrophy, QTc interval, QRS complex, PR interval, QRS – T angle and / or heart rate variability with these endpoints. Cardiac outcomes included coronary events, arrhythmic events, cardiac failure or a combination of these. Death included all-cause mortality and, where possible, sudden death as defined by the authors.

Results

I. Electrocardiographic Left Ventricular Hypertrophy

Left ventricular hypertrophy (LVH) is a common finding in advanced CKD and ESRD[23]. Up to 75% of patients have LVH upon initiation of maintenance dialysis[23] and increased echocardiographic left ventricular mass index is associated with adverse cardiovascular outcomes and sudden cardiac death[24].

In CKD and dialysis, a number of studies have shown univariate association between the electrocardiographic diagnosis of LVH and cardiovascular outcomes, but in most of them LVH was not an independent risk factor. These studies are presented in table 1.2. Covic et al[25] evaluated the prognostic value of estimating LVH by twelve different sets of commonly used electrocardiographic criteria in a retrospective, observational, single-centre study which included prevalent haemodialysis and peritoneal dialysis patients[25]. Novacode, a method that does not use voltage criteria but incorporates repolarisation indices into an algorithm, was found to be predictive of cardiovascular mortality, whilst eleven other methods, including the more widely used Sokolow –Lyon and Cornell criteria, were not.

A Korean prospective observational study of incident haemodialysis patients[26] compared the prognostic value for cardiovascular mortality of commonly used ECG criteria for LVH, namely Sokolow – Lyon and Cornell with voltage duration product method which encompasses the QRS duration. The diagnosis of LVH using voltage duration product methods was an independent risk factor for cardiovascular outcomes, but LVH using fixed voltage Sokolow – Lyon and Cornell was not. Approximately half of the individuals with an echocardiographic diagnosis of LVH did not have a matching electrocardiographic one.

Krane et al[27], in a study of 1253 maintenance HD patients with diabetes, identified that ECG LVH with Sokolow– Lyon criteria was predictive of sudden death and

stroke [hazard ratio (HR) = 1.60, 95% confidence interval (CI) 1.05–2.44; p=0.027], but not of all-cause mortality, cardiac deaths and myocardial infarction, although a trend towards statistical significance for cardiovascular endpoints was observed. Cice et al.[28], in a prospective study of normotensive maintenance HD patients without coronary artery disease, found that the strain pattern on the ECG was associated with cardiovascular and sudden death.

There is a paucity of studies assessing the association between ECG diagnosis of LVH and mortality or cardiovascular outcomes in CKD. Agarwal and Light, in a cross-sectional study of 387 patients that included 243 patients with various degrees of CKD, found a statistically significant association between diagnosis of LVH with Sokolow–Lyon criteria and all-cause mortality. The LVH group had perhaps unsurprisingly higher baseline blood pressure readings, but the association between LVH and mortality still persisted even after adjustment for blood pressure[29]

Variable degrees of association between electrocardiographic diagnosis of LVH using voltage criteria and overall and cardiovascular mortality have been demonstrated in other studies of haemodialysis and CKD patients listed in Table 1.2. The electrocardiographic detection of LVH in patients with CKD correlates poorly with LVH detection using echocardiography. This observation is in line with findings in the general population[30], suggesting that changes in electrical remodelling depicted by ECG-LVH do not reflect anatomical structural changes depicted by echocardiogram and carry additional independent prognostic information. On the other hand, the predictive value of ECG-LVH with fixed voltage criteria is variable in dialysis patients and this may be the result of a variable and fluctuant impact of fluid status on the ECG waveform. Fluid removal immediately after dialysis leads to increase in ECG voltage due to impedance changes, an effect which is gradually attenuated as fluid accumulates until the next dialysis session[31]. As a result, an interdialytic ECG may obfuscate the presence of LVH in a patient with large interdialytic fluid gains which is in turn an independent risk factor for mortality[32].

Table 1.2 Studies evaluating the association of electrocardiographic left ventricular hypertrophy with clinical outcomes in chronic renal disease.

Study	Population	Sample size	Mean follow up	Results	Comments
Cice et al 2008	Prevalent HD	407	46 months	LVH with strain predictive of cardiovascular deaths ($p<0.05$) and sudden deaths ($p<0.01$)	Univariate analysis
Krane et al 2009	HD with Diabetes	1253	48 months	LVH with Sokolow -Lyon criteria was predictive of sudden death (HR 1.60, 95% CI 1.05-2.44, p=0.027)	A trend towards higher risk for cardiovascular endpoints was detected
Agarwal et al 2010	CKD, excluding ESRD	387	90 months (median)	LVH with Sokolow-Lyon criteria prognostic for all cause mortality (HR 2.84, 95% CI 1.50 – 5.37, $p< 0.001$)	Multivariate analysis including adjustment for blood pressure
Kim et al 2012	Incident HD	317	27.4 months	LVH by Sokolow-Lyon voltage duration product (HR 3.43, 95% CI 1.32-892, p= 0.011) and Cornell voltage duration product (HR 3.07, 95% CI 1.16-8.11, p=0.024) predictive of cardiovascular mortality	50% discordance between ECG and echocardiographic diagnosis of LVH
Covic et al 2013	Prevalent HD and PD	418	67 months	LVH by Novacode predictive of cardiovascular mortality(HR 3.04, 95% CI 1.11-8.28, $p<0.05$)	11 other methods not predictive

Key: HD = haemodialysis, CKD = chronic kidney disease, PD= peritoneal dialysis, LVH = left ventricular hypertrophy, ESRD = end stage renal disease, HR = hazard ratio.

II. QT interval

The electrocardiographic QT interval represents the time from the onset of ventricular depolarisation to the completion of repolarisation. QTc is the value of QT after correction for heart rate. The Bazett formula is the most commonly used method for QT correction[33]. Other formulae (Fridericia, Framingham, and Hodges) tend to provide similar estimations in resting heart rates between 60 – 90 bpm[34]. Regardless of the method used, prolongation of QTc is defined as QTc > 460 ms in women and QTc> 450ms in men[35].

Electrocardiographic QT duration reflects cardiac conduction and is influenced by electrolyte shifts, myocardial ischaemia, and structural heart disease. Fluid and electrolyte shifts may affect QT interval; fluid removal and potassium removal both contribute to QTc prolongation at the end of dialysis, whereas calcium changes are variable and can have a variable effect on the QTc. In the CKD population, several observational studies have identified a link between QTc duration and cardiovascular outcomes.

Hage et al[36] found that QT prolongation was an independent predictor of all cause mortality in a prospective cohort of both haemodialysis and peritoneal dialysis patients evaluated for renal transplantation (HR 1.008, 95% CI 1.001 -1.014, p= 0.016). This study did not show any difference in the proportion of QT prolongation between haemodialysis and peritoneal dialysis.

In another prospective study of both incident and prevalent dialysis patients evaluated for renal transplantation, Flueckiger et al[37] showed similar associations between the prolongation of the QT interval and all-cause mortality (HR 1.71, 95% CI 1.11-2.63, p=0.0158). This study did not show any difference in the proportion of patients with QT prolongation between HD and PD.

Genovesi et al[38] used 24hour Holter electrocardiography in a cohort of 122 prevalent haemodialysis patients. The mean QTc was estimated during dialysis treatment for four hours, during the 4 hours after dialysis treatment, and during the remaining 16 hours[38]. After a median follow up of 3.9 years, QTc prolongation was found to be independently associated with sudden cardiac death (HR 8.33, 95% CI 1.71-40.48, p=0.009). Interestingly, the mean QTc interval did not change significantly during or after dialysis.

In contrast to the previous observational studies, a large multicentre randomised controlled trial in diabetic HD patients, the German Diabetes and Dialysis study[27] (4D, Die Deutsche Diabetes Dialyse Studie), did not identify an association between the duration of the QTc interval and cardiovascular outcomes. The variable effect of fluid and electrolyte shifts on QTc may account for the variability in the reported predictive value.

In the CKD population, several observational studies have identified a link between QTc duration and cardiovascular outcomes[37,39,40].

Deo et al.[40], in a prospective study of almost 4000 CKD patients, found that prolongation of the QTc interval was associated with all-cause and cardiovascular mortality. This association, however, ceased to exist in sub-group analysis adjusted for LVMI and left ventricular ejection fraction.

Similarly, Dobre et al[39] in a study of mainly CKD3 patients demonstrated that QTc was associated with cardiovascular events.

Full details on both dialysis and CKD populations in table 1.3

Table 1.3 Studies evaluating the association of QTc with clinical outcomes in chronic renal disease.

Study	Population	Sample size	Mean follow up	Results	Comments
Krane et al 2009	HD with diabetes	1253	48 months	QT interval not associated with outcomes	
Hage et al 2010	HD and PD evaluated for transplantation	280	40 months	QTc independent predictor of survival (HR 1.008, 95% CI 1.001 -1.014, p= 0.016)	
Dobre et al 2012	CKD 3-5	1165	123.6 months	Prolonged QT was associated with 61% higher risk for cardiovascular events (HR 1.61, 95% CI 1.16 – 2.23)	Predominantly CKD 3 (95.6% of study population)
Genovesi et al 2013	HD	122	46.8 months (median)	Prolonged QTc independently associated with all cause mortality (HR 2.16, 95% CI 1.20-3.91, p=0.011) and sudden death (HR 8.33, 95% CI 1.71-40.48, p=0.009)	
Flueckiger et al 2014	CKD5 and ESRD evaluated for transplantation	930	37.2 months (median)	QTc>450msec associated with risk of death in adjusted analysis (HR 1.71, 95% CI 1.11-2.63, p=0.0158)	
Deo et al 2015	CKD	3939	90 months (median)	Prolonged QTc associated with all cause (HR 1.46, 1-16-1.84) and cardiovascular mortality (HR 1.72, 1.19-2.49)	Association with cardiovascular death ceased to exist in subgroup adjusted analysis that included LVMI and LVEF

Key: HD = haemodialysis, PD = peritoneal dialysis, CKD = chronic kidney disease, ESRD = end stage renal disease, HR = hazard ratio. LVMI = left ventricular mass index, LVEF = left ventricular ejection fraction.

In summary, fluid and electrolyte shifts may affect the QT interval; fluid and potassium removal both contribute to QTc prolongation at the end of the dialysis, whereas calcium changes are less consistent and can have a variable effect on the QTc[31]. Genovesi et al[41] have previously reported that low potassium and calcium dialysate are associated with prolongation of QTc interval towards the end of HD. Also, Bazett's correction, which has been used in many of the studies, is known to lead to artificially prolonged QTc values in the presence of increased heart rate. Although this is of little concern when dealing with singular QTc measurements in any given patient, it might represent a potential source of bias in statistical studies linking outcomes to QTc duration.

III. QRS complex

The electrocardiographic QRS complex represents the electrical activation of the ventricular system, from septal activation to full ventricular depolarization.

A broad QRS complex (>120ms) has been used as a marker of cardiac dyssynchrony in studies evaluating the incidence of sudden cardiac death in patients with heart failure[42], and is one of the criteria for resynchronisation therapy in congestive heart failure[43]. In the renal population, research suggests that the QRS interval increases with progression of CKD[44]. The amplitude of the QRS complex increases after haemodialysis. The latter is thought to be a result of the changes in body fluid volume. Fluid removal leads to a decrease in tissue conductivity which, as a result, affects the surface voltage of the electrocardiographic complexes[45]. Therefore, the change of the QRS amplitude with different fluid status is a result of different impedance and not electrophysiological changes.

A Spanish prospective study of 285 incident HD and PD patients with generally well-preserved left ventricular function did not show any independent association between QRS duration and SCD incidence[46]

In a prospective study of 3587 individuals with mainly early to moderate CKD [mean estimated glomerular filtration rate (eGFR) 50–60 mL/min/1.73 m2, median follow-up 7.5years), Deo et al. identified prolongation of the QRS interval as an independent risk predictor for cardiovascular death, even after adjustment for LVMI and ejection fraction. For QRS duration of 100–119ms, the HR was

1.64 (95% CI 1.20–2.25) and for QRS >120ms, the HR was 1.75 (95% CI 1.17–2.62) [40]

In summary, the use of the QRS complex for cardiovascular risk prediction is poorly investigated and appears to be unreliable as an independent marker based on what evidence is available.

IV. PR interval

The electrocardiographic PR interval represents the propagation of the myocardial electrical impulse between atrial depolarisation and the onset of ventricular depolarisation, and is normally between 120ms and 200ms. The PR interval is also affected by fluid and electrolyte shifts. In the general population, prolongation of the PR interval has been associated with increased risk of developing atrial fibrillation, requiring pacemaker implantation, and overall mortality[47].

Results from studies in dialysis and CKD populations are conflicting (Table 1.4). Flueckiger et al[37], in their study of 930 transplant candidates undergoing haemodialysis, demonstrated that prolonged PR interval was associated with all cause mortality in multivariate analysis (HR 1.97, 95% CI 1.18-3.29, p=0.090). Green et al[24], in a prospective observational study of 211 haemodialysis and a 112 peritoneal dialysis patients, identified a significant association between prolongation of the PR interval and cardiovascular outcomes in univariate, but not in multivariate analysis (mean follow up 3.6 years). Another prospective study of 116 HD patients by Badarau et al. evaluated that the PR interval derived from standard ECGs were acquired 5 min before and 30 min after a HD session. In this study, for the majority of patients, the PR interval decreased after dialysis and in multivariate Cox regression analysis, the difference between the pre- and post-dialysis PR interval duration was identified as an independent predictor of cardiovascular outcomes with longer PR having a lower risk (HR for log of change in PR = 0.387, 95% CI 0.251–0.597; P<0.001), but not of all-cause mortality[48]. A Brazilian prospective observational study aimed to evaluate the incidence of arrhythmias and their associations with ECG findings in a cohort of 100 HD patients using implantable loop recorders. During a follow-up period of 4246124 days, prolongation of the PR interval was found to be independently associated with the development of bradyarrhythmias[49]

In a prospective study of 3587 patients with different stages of pre-dialysis CKD, a prolonged PR interval was identified as an independent predictor of cardiovascular mortality (HR = 1.62, 95% CI 1.19–2.19)[40]. In contrast, Kestenbaum et al.[50] prospectively studied 600 individuals with a moderate degree of CKD (median eGFR 53 mL/min/1.73 m2) and did not observe any independent association between PR prolongation and incident cardiovascular events.
In conclusion, PR interval demonstrates variable association with mortality in CKD and ESRD that may be explained by fluid and electrolyte influences on PR interval. The link between prolonged PR interval and mortality is unclear but it may be related to mortality related to bradyarrhythmias or atrial fibrillation.

Table 1.4 Studies evaluating the association of PR interval with clinical outcomes in chronic kidney disease.

Study	Population	Sample size	Follow up	Results	Comments
Kestenbaum et al 2007	CKD	600	110.4 months (median)	No independent association between PR prolongation and incident cardiovascular events	
Flueckiger et al 2014	CKD5 and ESRD evaluated for renal transplantation	930	37.2 months (median)	PR interval was associated with all cause mortality (HR 1.97, 95% CI 1.18-3.29, p=0.090)	
Green et al 2014	HD and PD	323	43.2 months (mean)	No independent association between PR interval and cardiovascular outcomes in multivariate analysis	
Badarau, Siriopol et al 2015	HD	116	17.5 months (median)	Log pre-and post-dialysis difference in PR interval predicts cardiovascular events (HR 0.387, 95% CI 0.251-0.597, p< 0.001)	
Deo et al 2015	CKD	3939	90 months (median)	PR>200ms is associated with cardiovascular mortality (HR 1.62, 95% CI 1.19- 2.19)	
Silva et al 2015	HD	100	14 months (mean)	The duration of the PR interval was independently associated with bradyarrhythmias (OR 1.05, 95% CI 1.02-1.08, p< 0.001)	Candidates for renal transplantation

Key: HD = haemodialysis, PD = peritoneal dialysis, CKD = chronic kidney disease, ESRD = end stage renal disease, HR = hazard ratio.

V. QRS – T angle

In the last decade, there has been increasing interest in the spatial QRS–T angle that is defined as the angular difference between the orientation of the three-dimensional (3D) QRS and T vectorcardiographic loops that are either directly captured or calculated from the standard 12-lead recordings. This is because the angle has emerged as a novel marker for cardiac risk stratification[51] . A number of studies in different populations have demonstrated an association between a wide spatial QRS–T angle and cardiovascular and all-cause mortality[52]. The spatial QRS–T angle can easily be measured either on vectorcardiograms recorded using the Frank electrode positions[53] or by following orthogonal transformation from a digital 12-lead ECG using conversion systems such as Kors or inverse Dower matrices[54,55]. In these methods, the spatial orientation of the orthogonal XYZ leads is defined anatomically and is subject independent. A novel descriptor uses singular value decomposition to construct a mathematically derived subject dependent 3D space optimising the orthogonal leads in order to capture most of the ECG energy in each individual, and calculates the difference between the global direction of depolarisation and repolarisation expressed as an average cosine of the angles between the QRS and T vectors [total cosine R-to-T (TCRT)[56] Figure 1.1 depicts the TCRT. The definition and range of normal and abnormal QRS–T angles in healthy individuals depend on the method of estimation as well as on sex, age and underlying heart rate[57-60]. The spatial QRS–T angle may be calculated by several methods including using the peak angular difference between the QRS and T-vectors, their mean angular difference[61], the angle between the spatial mean QRS vector and spatial peak T-vector[62] and by using the average cosine of the angles between the QRS and T-vectors[63]. Therefore, 'absolute' values of the QRS–T angle should only be referenced in relation to the individual studies and methods they derive from.

Several studies evaluated the prognostic value of spatial QRS–T angle for all-cause and cardiovascular mortality in dialysis patients. These are presented in table 1.5.

In a retrospective study of 277 incident HD and PD patients, de Bie et al.[64] identified abnormal spatial QRS–T angle as an independent predictor of all-cause mortality (HR = 2.33, 95% CI: 1.46–3.70; P<0.01) and SCD (HR = 2.99, 95% CI

1.04–8.60; P<0.05) after multivariate analysis. An abnormal spatial QRS–T angle was defined as >130 degrees in men and >116 degrees in women in that study, and the length of follow-up was 2.1±1.7 years. In a pilot study of 81 prevalent HD patients, which used continuous Holter electrocardiographic recordings, Poulikakos et al.[65] reported higher TCRT values (expressed in degrees) in individuals who suffered major arrhythmic events (TCRT). Couderc et al. calculated the QRS–T angle from ECG Holter recordings in a study of 50 prevalent HD patients. They demonstrated a statistically significant greater average QRS–T angle in the first 6 h after initiation of the dialysis session compared with pre-dialysis that correlated with all-cause mortality[66]. A large prospective study of incident HD patients by Tereshchenko et al. evaluated the spatial QRS–T angle for risk stratification in a cohort of patients of predominantly African origin with overall normal LVEFs. The authors calculated the QRS–T angle as the angle between spatial mean QRS vector and spatial peak T-vector in averaged XYZ ECG from 5min signal-averaged ECGs. In multivariate adjusted analysis, a spatial QRS–T angle >75 degrees was independently associated with all cause (HR= 2.38, 95% CI 1.41–4.04; P = 0.001) and cardiovascular mortality (HR= 2.99, 95% CI 1.31–6.82; P = 0.01) [62]. There were no suitable studies in CKD patients at the time of this review.

In summary, the QRS–T angle has showed promising results for risk prediction in dialysis patients. However, there is a need for standardisation of the measurement[63] so that normal limits and clinically relevant risk stratification dichotomies can be established.

Figure 1.1. TCRT

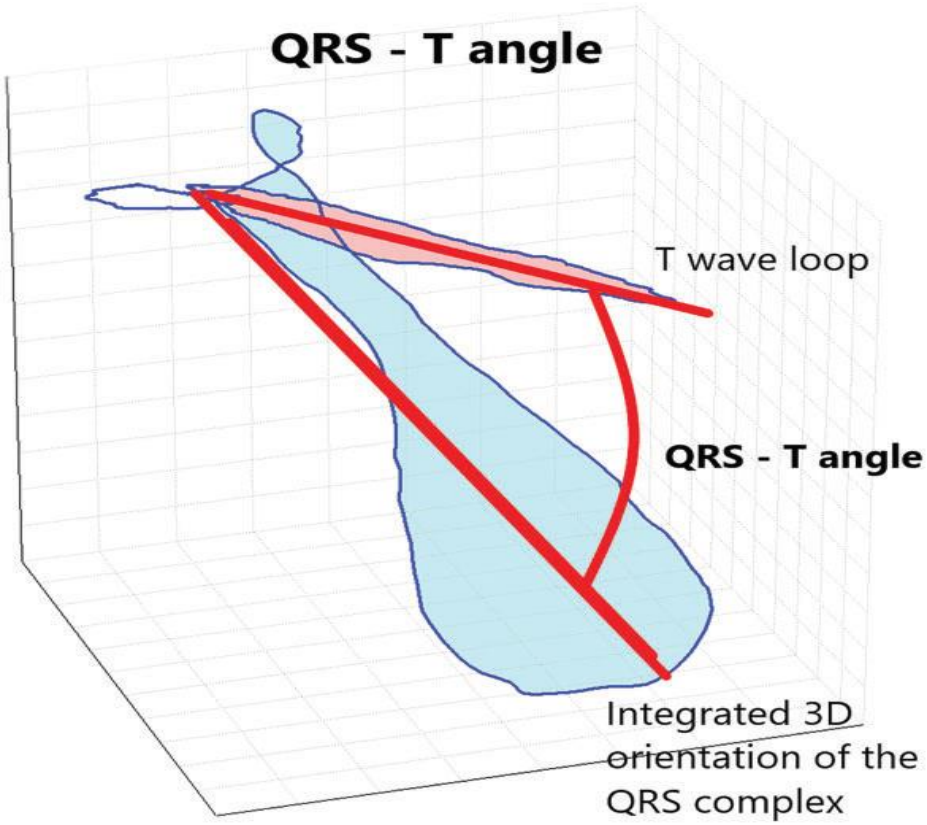


Table 1.5 Studies evaluating the association of spatial QRS-T angle and outcomes in end stage renal disease.

Study	Population	Sample size	Follow up	Results	Comments
Couderc et al 2011	HD patients above the age of forty with history of diabetes or hypertension	50	13 months	Statistically significant increase of the QRS-T angle after the dialysis session in the non –survivor group (p< 0.05)	Holter
De Bie et al 2013	HD and PD	277	25.2 months (mean)	QRS-T angle independent predictor of all cause mortality (HR 2.33, 95% CI 1.46-3.70, p <0.01) and sudden cardiac death (HR 2.99, 95% CI 1.04 – 8.60, p < 0.05)	Single surface ECG
Poulikakos et al 2014	HD	81	18 months	Extremely high TCRTs in patients who experienced arrhythmic events	Holter
Tereschenko et al 2016	Incident HD	358	864.6 person years	Spatial QRS –T angle > 75 degrees was independently associated with all cause (HR 2.38, 95% CI 1.41 – 4.04, p 0.001) and cardiovascular mortality (HR 2.99, 95% CI 1.31 – 6.82, p 0.01)	5 minute baseline and 12 – lead surface ECG

Key: HD = haemodialysis, PD = peritoneal dialysis, HR = hazard ratio

VI. LBBB versus RBBB QRS morphology

There are few data available in comparing left bundle branch block (LBBB) versus right bundle branch block (RBBB). In the study of diabetic patients on HD by Krane et al.[27], neither RBBB nor LBBB showed any association with mortality or cardiovascular outcomes in multivariate analysis adjusting for co-morbidities and demographics. The presence of LBBB may obscure the electrocardiographic diagnosis of LVH as they both cause conduction delays and as a result the inclusion of LBBB as a separate variable in a model that includes electrocardiographic LVH is not without problems[67]. Covic et al.[25], in a study of HD patients that compared different electrocardiographic methods of LVH estimation and their association with outcomes, also noted that LBBB was associated with all-cause mortality in univariate analysis. However, they suggested caution while using LBBB and ECG LVH in the same model.

VII. Heart Rate Variability

HRV gained popularity, among other ECG parameters, because of its importance for cardiovascular risk prediction[68,69]. HRV measurement is based on different assessments of the oscillations of the intervals between consecutive cardiac beats. It has been used as a surrogate method of assessing the sympathetic and parasympathetic cardiac autonomic modulation[70]. Reduced HRV has been associated with increased mortality in different populations including healthy individuals and patients post-myocardial infarction[71,72]. HRV can be measured using time- and frequency domain methods as well as employing nonlinear dynamics analyses. Standards of HRV assessment are available[73,74] and are followed in most of the risk assessment studies. A summary of studies detailed below is found in Table 1.6.

In a study of 383 incident and prevalent HD patients, Oikawa et al.[75] reported an independent association between reduced overall HRV and all-cause (HR = 2.181, 95% CI 1.530–3.108; P<0.001) and cardiovascular mortality (HR=2.114, 95% CI 1.200–3.725; P=0.01) [65]. A study of 81 PD patients also reported on the prognostic value of spectral HRV assessment for all-cause mortality during 4 years of follow-up[76]. In a prospective study of 281 prevalent HD patients, Suzuki et al. evaluated different HRV measures. Time and spectral assessment of short-

term HRV indices predicted mortality but after adjusting for age, LVEF, serum albumin, C-reactive protein and calcium x phosphate product, only one of the nonlinear dynamics parameters was an independent mortality predictor (HR = 1.46, 95% CI 1.16–1.85; P=0.001)[77]. Badarau et al.[48] reported an association between very low frequency HRV and all-cause mortality in a study of 116 HD patients (HR= 1.741, 95% CI 1.047–2.895; P=0.033), but did not find such an association with the other spectral HRV components. In the latter two studies, the 24-h Holter ECG was recorded during the interdialytic interval, whereas in other studies, it took place on a dialysis day that included the dialysis session. In CKD, a multicentre prospective study of 305 patients with CKD stages 3–5 demonstrated a strong association between decreased spectral HRV parameters and the cumulative probability of adverse cardiovascular events[78].

In summary, the variable results of studies using out-of-hospital 24-h Holter ECGs can be explained by the difficulty in standardising the environmental factors that influence HRV assessment, including HD[79], during the recording. Indeed, total 24-h R–R interval variability analysis of recordings in truly ambulating out-of-hospital patients is of little prognostic value because of the differences in the environmental challenges to which the autonomic system responds, and is no longer recommended as a favoured approach for autonomic nervous system assessment[73,80]. It has also been reported that high phosphate and parathyroid hormone levels[81] and fluid overload[82] are associated with reduced HRV in HD patients, whereas daily HD[83] and haemofiltration[84] are associated with less pronounced reductions in HRV compared with standard HD.

Table 1.6 Studies evaluating the association of heart rate variability and outcomes in chronic kidney disease.

Study	Population	Sample size	Follow up	Results	Comments
Oikawa et al 2009	HD	383	2110 ± 903 days	Independent association between reduced SDNN and all cause (HR 2.181, 95% CI 1.530 – 3.108, p< 0.001) and cardiovascular mortality (HR 2.114, 95% CI 1.200 – 3.725, p=0.01)	
Chandra et al 2011	CKD 3-5	305	2.7 years (median)	A LF/HF ratio below the median was associated with a significantly increased risk of cardiac events (HR 2.52, p=0.002)	
Suzuki et al 2012	HD	281	87 months (median)	The scaling component α_1 was independently associated with all cause mortality (HR 1.46, 95% CI 1.16 – 1.85, p = 0.001)	None of the traditional HRV parameters were associated with mortality
Badarau, Siriopol et al 2015	HD	116	17.5 months (mean)	Association between VLF component and all cause mortality (log VLF, HR 1.741 95% CI 1.047 – 2.895, p= 0.033)	
Pei et al 2015	PD	81	43.78±14.77 months	LF/HF below the median significantly associated with all cause mortality (p=0.012)	

Key: HD = haemodialysis, PD = peritoneal dialysis, CKD = chronic kidney disease, HR = hazard ratio, HRV = heart rate variability, SDNN= standard deviation of normal to normal R – R intervals, VLF = very low frequency, LF= low frequency, HF = high frequency

Discussion

A number of electrocardiographic parameters have been used as potential risk predictors in advanced renal disease and dialysis with variable results. For conventional ECG parameters, a major limitation on their use in dialysis patients is the effect that fluid and electrolyte shifts have on their measurements. Inconsistency and lack of reproducibility makes them unreliable as independent biomarkers. In the case of the PR interval prolongation in particular, the link between abnormal PR and mortality might reflect the mortality risk associated with bradyarrhythmias or atrial fibrillation.

In the case of electrocardiographic LVH, the use of Novacode has shown promising results. Novacode has the advantage that it does not rely on voltage criteria, but has the disadvantage of requiring software interpretation of EGC waveform and so, unlike conventional methods such as Sokolow-Lyon, LVH cannot be determined by manual observation. Novel markers, as the QRS-T angle have shown promising results in haemodialysis cohorts. However, the definitions of abnormal QRS – T angle vary significantly depending on the method of calculation used. Further work into standardisation of the measurements is required. Moreover, the prognostic value of QRS – T angle needs to be evaluated in larger studies and different patient groups. In general, there is a paucity of studies assessing electrocardiographic markers as risk prediction tools in peritoneal dialysis compared to haemodialysis.

In summary, larger studies are needed and also studies assessing the evolution of electrocardiographic changes from CKD to HD and PD and the relation of these changes to cardiac mortality. Risk stratification models that incorporate echocardiographic, electrocardiographic and laboratory parameters together will likely lead to more sensitive and specific risk prediction.

Chapter 2

Aims and Objectives

Introduction

As discussed previously, cardiovascular disease is very common in ESRD and dialysis. Sudden cardiac death accounts for up to 26% of all mortality in ESRD and it is generally agreed to be arrhythmic in origin. Emerging evidence suggests that repolarisation abnormalities play an important part in the pathogenesis of cardiac mortality in ESRD and haemodialysis. However, risk stratification methods have traditionally relied on echocardiography given the high prevalence of left ventricular hypertrophy in this population. The ECG is an easily accessible and inexpensive test which can be hypothesised that is under-utilised for risk stratification in haemodialysis. The purpose of this thesis was to test the hypothesis that traditional and novel ECG parameters are independently predictive of cardiac outcomes in haemodialysis. The particular aims and objectives are detailed below.

Aims

1. Assess the prognostic value of electrocardiographic markers of heterogeneity of repolarisation in haemodialysis patients.

2. Assess whether the combined use of electrocardiography and echocardiography in assessing LVH in ESRD can provide improved risk stratification by concurrently evaluating both the presence of increased LV mass and whether there is a demonstrable effect on cardiac conduction pathways.

3. Assess whether the presence of ECG strain is associated with cardiac risk independently of LVH in haemodialysis.

Objectives

1. Describe the study methodology; in particular, patient selection, ECG acquisition process and study endpoints.

2. Investigate whether the spatial QRS – T angle (as a surrogate marker for repolarisation heterogeneity) derived from standard 12-lead ECGs carries independent prognostic value for cardiac outcomes and all cause mortality in haemodialysis

3. Investigate whether the spatial QRS- T angle is associated with electrocardiographic and echocardiographic parameters in a haemodialysis population.

4. Compare cardiac and all cause mortality outcomes in haemodialysis patients with no LVH, LVH but normal ECG, and LVH with strain pattern repolarisation on ECG.

5. Assess the sensitivity and specificity of different methods of diagnosing ECG LVH; Compare these with 3D echocardiography.

6. Assess the predictive value of prevalent and incident ECG abnormalities using a scoring system based on individual and combined abnormalities.

Chapter 3

Methodology

Rationale

This chapter briefly outlines the overall study protocol for the work undertaken in this thesis so as to avoid excessive duplication through this thesis. The full details of the methodology including cardiovascular assessment as well as the statistical methodologies specific to each result chapter in this thesis are provided in the relevant chapter itself.

Introduction

This work was part of a sub-study of the Salford Kidney Study (SKS). Patients included into this analysis are from the sub-group of SKS previously known as the CRISIS-HD cohort. This chapter outlines the SKS study protocol with regard to patient selection, definition of endpoints, follow up and general assessments relevant to the work presented in this thesis. The SKS has led to more than 40 original work publications in peer reviewed journals and so the general study method is well described elsewhere.

SKS recruited more than 3300 patients on first presentation of CKD or on initiation of renal replacement therapy. SKS was formerly known as the CRISIS study (Chronic renal insufficiency standards implementation study) and has been recruiting since 2002. In this thesis we only included patients from the haemodialysis arm of SKS, previously known as CRISIS – HD. CRISIS-HD was initially funded by a Kidney Research UK Project Grant and recruited patients from 2011 (project Grant ID RP35/2011).

Study design

I. Inclusion and exclusion

Adult maintenance haemodialysis patients at Salford Royal NHS Foundation Trust, including its satellite units, were considered for inclusion into the CRISIS-HD sub-study of the SKS. The four satellite units are located in Rochdale, Oldham, Bolton and Wigan. There were very few exclusion criteria. Patients could not be enrolled if they are aged under 18 years, on home rather than unit haemodialysis, and if they underwent non-standard dialysis hours (i.e. a programme other than 4 hours three times per week). The eligibility criteria were deliberately broad as this study was designed to identify and describe possible risk prediction patterns.

Patients were given a patient information sheet and consented for inclusion approximately one to two weeks after. They were eligible if they were able to consent to participate in the study and also if they are able and willing to attend for the study assessments. Patient transport was offered for mobile patients. In all cases, written informed consent was obtained before any study visits were planned.

II. Study visits

Patients who did participate in the study underwent detailed cardiovascular assessment including SKS study specific extended protocol echocardiography and electrocardiography. An overview of these is provided below with greater detail found in the methods section of each chapter in this thesis. These were performed on a non-dialysis day during a short, mid-week interdialytic break. Patients also underwent blood sampling and data collection regarding co-morbid conditions, dialysis prescription and medication. These phenotype data were collected using self-reported questionnaires and by referring to the Hospital's electronic patient records.

III. Ethics and Information Governance

The study complies with the declaration of Helsinki and local ethical approval has been obtained (current UK REC reference 15/NW/0818). No patient identifiable information was used in the formation of datasets relating to this

study. Unique study identification numbers were used for participants. A datafile linking patient hospital number to SKS study number was kept on a secure server within the Hospital and not accessed or stored in any other way.

Frozen plasma samples sent to external sites for analysis were given unique barcode identifiers and data sharing agreements created with partners before distribution. A similar approach was used for xml format ECG data files analysed externally. These were sent without any patient identifiers in accordance with the ethical approval for the study.

IV. Endpoints

Patients were followed up on a longitudinal basis with formal annual study visits and also remote evaluation of mortality and major events using electronic patient records.

The primary outcomes were all cause mortality and major cardiac adverse events (MACE). MACE comprised arrhythmia, myocardial infarction, acute coronary syndrome, coronary revascularisation or bypass procedure and new diagnosis of heart failure. The definitions of the above outcomes for the purposes of our study are as follows:

Arrhythmia: Arrhythmic or syncopal event requiring hospital admission or medical intervention.

Myocardial infarction (as per NICE guidance): A rise in cardiac biomarkers (preferably cardiac troponin) with at least 1 value above the 99th percentile of the upper reference limit and/or a fall in cardiac biomarkers, together with at least 1 of the following:

- symptoms of ischaemia
- new or presumed new significant ST-segment-T wave changes or new left bundle branch block
- pathological Q wave changes in the ECG
- imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
- Identification of an intracoronary thrombus by angiography.

Acute Coronary syndromes include myocardial infarction and unstable angina

Coronary revascularisation only includes non elective procedures

Data collection and follow up

Demographics and information on co-morbidities were obtained either from the Trust's electronic medical notes, from general practitioners' records or self reported by the patients. Prescription records were obtained from patients or from the Greater Manchester Health Records for each patient that are integrated into the hospital electronic patient records.

Dialysis characteristics such as ultrafiltration, blood pressure and urea reduction ratio and blood results were obtained from the electronic medical notes. We elected to use the three monthly average values in order to minimise the confounding effect that day to day variations of these parameters might have had. The patients are assessed annually for mortality and disease progression. Death and cause of death data were obtained from the Office of National Statistics. Other event data were obtained from the Trust's electronic patient records, and from integrated primary care records. All patients were followed up until death, transplantation, or study end date (September 2016).

Electrocardiographic and echocardiographic assessments

Patients who participated in the study underwent detailed cardiovascular assessment including SKS study specific extended protocol echocardiography and electrocardiography on a non-dialysis day during a short, mid-week interdialytic break. The first study protocol echocardiogram and ECG performed on participants after enrolment into SKS were selected for baseline measurements, and further investigations were performed annually thereafter.

All 12 lead ECGs were obtained on single visits. They were performed with the patients in a recumbent position and with standard lead placement using a Philips Pagewriter TC 20 device for ECG acquisition at a sampling rate of 500

Hz. Automated measurements by Philips measurement algorithm of heart rate and ECG intervals for QRS and QTc were used in the analysis. QTc was calculated using the Bazett's formula. Electrocardiographic strain was defined as a down slopping convex ST segment with inverted T waves in leads V5 and / or V6 . Left bundle branch block (LBBB), Right bundle branch block (RBBB) and complete heart block (CHB) definitions were based on established criteria. The angle between the principal orientations of the QRS complex and T wave loop (QRS – T angle) was calculated using singular value decomposition with the aid of custom developed software as TCRT and was expressed in degrees.

Transthoracic echocardiography was performed immediately after ECG. All patients were assessed in the left lateral decubitus position, using Philips echocardiography equipment with 3.5 MHz transducers. Measurements were obtained according to published guidelines by the European Society of Echocardiography. Ejection fraction (EF) was calculated using the Biplane method as recommended by the ESC. LV mass was calculated using 2D linear measurements and the Devereux formula, and indexed to body surface area. LVH was determined if LVMI >116g/m^2 for male patients, and >100g/m^2 for female patients. Full detail of the echocardiographic methodology is detailed in later chapters where echocardiography forms a major part of the analyses.

Laboratory parameters

Standard laboratory parameters used in this study were eGFR, urea, C-reactive protein, haemoglobin, serum albumin, parathyroid hormone, phosphate, calcium, sodium, and potassium. These were taken from pre-dialysis sampling from the dialysis unit visit closest to the date of the ECG and echocardiography visit.

Other assays were measured at the Biochemistry Unit of the University Hospital of Geneva. These were measured from samples taken at the same time as the standard laboratory measurements but which were then stored at -

80^{o}C immediately thereafter until the point of analysis. These biomarkers included KIM-1, NT-proBNP, and troponin.

Statistical analysis

Throughout the thesis, continuous descriptive variables were expressed as mean ± standard deviation for normally distributed data and median ± interquartile range for non-normally distributed data. Normality was assessed graphically with Q-Q plots and was subsequently tested with the Kolmogorov –Smirnov test. Categorical variables were expressed as frequencies and comparisons between categorical variables performed using Chi Square tests. Statistical significance was set at $p< 0.05$ for all analyses.

The specific statistical methodology applied to analyses within each results chapter are described in detail therein. For example, for time dependent outcomes, Cox regression analysis was used to determine associations between the primary endpoints and demographic, clinical, electrocardiographic, echocardiographic and laboratory data.

Other methods used were correlation using Pearson and Spearman co-efficient, diagnostic test evaluation using area under curve of receiver operator characteristic curves, and sensitivity, specificity, negative predictive and positive predictive value calculations. All analyses were performed using IBM SPSS Version 22.

Chapter 4

Repolarisation heterogeneity and cardiac risk in prevalent haemodialysis patients

A version of this chapter has been published as "*QRS-T Angle Predicts Cardiac Risk and Correlates with Global Longitudinal Strain in Prevalent Haemodialysis Patients"* in Front. Physiol., 25 February 2019.
In the published version a third endpoint of cardiac death was included in addition to the endpoints of all cause mortality and major adverse cardiac events.

Rationale

In this chapter we examined the prognostic potential of a novel ECG biomarker of aberrant conduction, in a haemodialysis population. This biomarker (QRS –T angle) is also an indicator of repolarisation heterogeneity and has been shown to be associated with cardiac outcomes in other populations in previous studies.

Abstract

Background and objective: Cardiovascular disease is the commonest cause of death in End Stage Renal Disease (ESRD), but accurate risk prediction is lacking. Arrhythmias are believed to contribute significantly to the burden of cardiac disease, although the pathogenic mechanism is not fully understood. The spatial QRS – T angle is a marker of repolarisation heterogeneity which has been associated with increased cardiac risk in cardiac patients and in the general population. The aim of this study was to assess the prognostic value of spatial QRS-T angle derived from standard 12 lead electrocardiograms and its associations with conventional electrocardiographic, echocardiographic parameters in haemodialysis patients.

Methods. A prospective single centre cohort study of prevalent haemodialysis patients. Electrocardiography and echocardiography were performed on a mid-week non- dialysis day in clinically stable patients on an annual basis for two years. The QRS – T angle was calculated from the 10 second ECG as the total cosine R to T (TCRT) expressed in degrees using singular value decomposition with the aid of custom written software. An abnormal TCRT was defined as greater than 100 degrees. End points were death and major cardiac events (MACE: acute coronary syndrome, coronary revascularisation, hospitalization due to heart failure or arrhythmia, cardiac or sudden cardiac death).

Results 171 patients were included in the final analysis. Mean age 62.3 ± 13.6 years, 72.5% males, mean time on dialysis was 2.8±3.2 years, 25.9% had history of coronary artery disease (CAD) and 28.7% of diabetes mellitus. After a mean follow up of 2.3±1.1 years, there were 48 deaths and 43 MACE. By Cox regression analysis, TCRT > 100 was associated with increase in MACE, but not in all cause mortality. The hazard ratio (HR) for MACE was 2.215, (1.188 -4.131), p=0.012 and for death 0.901, (0.454 – 1.789), p=0.786.

Conclusion TCRT is independently associated with adverse cardiac outcomes in haemodialysis patients and it may be used for risk stratification in this population.

Introduction

Chronic Kidney disease (CKD) is characterised by much higher cardiovascular mortality and morbidity compared to the general population. This risk increases exponentially in End Stage Renal Disease (ESRD)[1] Renal registry data indicate that sudden death or fatal arrhythmia is the documented cause of death in approximately 26% of ESRD patients[2]. Whilst atherosclerotic disease is common in CKD and ESRD, evidence indicates that it only accounts for a small proportion of cardiovascular deaths in this population[3]. The pathogenesis of cardiac disease in CKD is not well understood and it is speculated that the pathogenic mechanism in CKD and ESRD differs compared to the general population[85]· Coronary revascularisation in dialysis patients does not improve cardiac mortality rates compared to the general population[3], and there is very limited, or even contested, survival benefit from implanted cardioverter defibrillators in patients with advanced CKD and ESRD who have ischaemic left ventricular dysfunction[5]. This latter example demonstrates how standard guidelines and indications for interventions do not always translate into use in the advanced CKD population. As a result, conventional risk stratification is largely ineffective in this population. This means that CKD and ESRD specific risk identification strategies with the ultimate aim to improve outcomes are also required, but presently lacking.

So far, risk stratification models in haemodialysis have focused mainly on echocardiographic markers. Left Ventricular Mass Index (LVMI) and Global longitudinal strain (GLS) have been shown to have predictive value for mortality[86,87] and cardiovascular disease outcomes[88]. In addition, markers of arterial stiffness, such as Pulse Wave Velocity (PWV), have been utilised as potential risk predictors with positive results[89]

In the last decade there has been increasing interest in the spatial QRS – T angle as a marker of repolarisation heterogeneity. QRS- T angle is defined as the angular difference between the three dimensional QRS and T vectors in the planes where they form. Traditionally, the spatial QRS - T angle has been measured either on vectorcardiograms recorded using the Frank electrode

positions[53] or following orthogonal transformation from a digital 12-lead electrocardiogram using conversion matrices[54,55]. In these methods, the spatial orientation of the orthogonal XYZ leads is defined anatomically and is subject independent. TCRT(average cosine of the angles between the QRS and T vectors[90]), on the other hand, measures the difference between the global direction of depolarisation and repolarisation and is calculated with the use of singular value decomposition in order to construct a mathematically derived subject-dependent three-dimensional (3-D) space, this way capturing most of the ECG energy[63].

The definition and range of normal and abnormal QRS - T angle in healthy individuals varies depending on the method of estimation used and also on sex and age[57,58]. Methods of spatial QRS- T angle calculation include using the peak angular difference between QRS and T vectors, their mean angular difference[61], the angle between spatial mean QRS vector and spatial peak T vector[62], and also the average cosine of the angles between the QRS and T vectors[90]. Therefore, "absolute" values of the QRS-T angle should only be referenced in relation to the individual studies and methods they derive from.

De Bie et al identified the abnormal spatial QRS – T angle as an independent predictor of all cause mortality and sudden cardiac death in a study that included haemodialysis patients. In this study the QRS- T angle was derived from standard 10 second ECGs and calculated as the mean angular difference between the QRS and T vectors[64]. Tereshchenko et al demonstrated an independent association between QRS- T angle and all cause and cardiovascular mortality in a cohort of incident haemodialysis patients[62].

The aim of this study was to assess the prognostic value of the QRS-T angle calculated from the standard 12 lead ECG, and its associations with conventional echocardiographic and electrocardiographic indices in a cohort of haemodialysis patients.

Methods

I. Patient population and protocols

This was a sub-study of the haemodialysis sub-study [89]of the Salford Kidney Study (SKS, previously known as CRISIS – HD), a prospectively collected longitudinal study of > 3300 patients recruited on first presentation of CKD or on renal replacement therapy. The ethical permission extends to facilitating annual sequential ECG recordings and echocardiography as well as full clinical assessment and serum, plasma and whole blood sampling. The patients are assessed annually for mortality and disease progression. For this analysis we excluded patients without an available baseline TCRT.

Average values of haemoglobin, albumin, troponin and brain natriuretic peptide (BNP) were calculated using values from routine blood tests performed in the three months preceding the study visit. Arterial stiffness was measured by aortic Pulse Wave Velocity (PWV) using the Vicorder device (Skidmore Medical Ltd., Bristol, UK).

Prevalent coronary artery disease (CAD), heart failure, diabetes, peripheral vascular disease (PVD), cerebrovascular disease (CVD), hypertension and medication lists were extracted from medical records. CAD was defined as previous myocardial infarction, coronary revascularisation or medically managed angina. Heart failure was diagnosed in patients with NYHA Class II – IV symptoms in the absence of alternative aetiology. The definition of PVD included history of previous revascularisation, claudication symptoms and ischaemic ulcers. Cerebrovascular disease was defined as history of clinical stroke or transient ischaemic attack. The study endpoints were all-cause mortality and major cardiac events (MACE). MACE encapsulated acute coronary syndrome, coronary revascularisation, hospitalisation due to heart failure or arrhythmia and cardiac[1] or sudden cardiac death. Sudden cardiac death was defined as natural death due to cardiac causes within an hour of onset of acute symptoms in a patient not known to have fatal illness[91]. The study subjects were followed up until death or transplantation.

II. Electrocardiographic acquisition and analysis

All 12 lead ECGs were performed with the patients in recumbent position and with standard lead placement using a Philips Pagewriter TC 20 device for ECG acquisition at a speed of 25mm/sec and voltage gain of 10mm/mV. The first study protocol echocardiogram and ECG performed on participants after enrolment into SKS were selected for baseline measurements, and further investigations were performed annually thereafter. ECG and echocardiography were performed at a single visit on a non-dialysis day. Software conversion routines were programmed allowing the signal to be exported from the XML Schiller format to an open format suitable for further computer processing. Subsequently, the ECG signals were filtered to remove baseline wander and high frequency noise. The QTc was calculated using the Bazett's formula[33] and it was defined as abnormal if > 460ms in women and > 450 ms in men. Left bundle branch block (LBBB), Right bundle branch block (RBBB) and complete heart block (CHB) definitions were based on established criteria. The angle between the principal orientations of the QRS complex and the T wave loop (QRS –T angle) was calculated using singular value decomposition with the aid of custom developed software as the total cosine R to T^{90} and expressed in degrees. An abnormal TCRT was defined as > 100 degrees based on previously published cut–off values for sudden death stratification in a population study that included 5618 adults (cosine -0.21, corresponding to 102.1 degrees angle had specificity of 85.0%)[92,93]. Previous studies have utilised a variety of cut off points, ranging from 75 degrees to 135 degrees, depending on the method of calculation of the QRS-T angle used.

III. Echocardiographic analysis

Echocardiograms were performed on eligible, clinically stable patients on a non-dialysis day. Ejection fraction (EF) was calculated using the biplane Simpson method, end diastolic volume was estimated using the Teicholz equation from M– mode images (EDVTeich) and left ventricular mass was adjusted for height ($LVMIht^{2.7}$) based on the previously demonstrated

superiority of $LVMIht^{2.7}$ in predicting cardiovascular events compared to LVMI indexed to body surface in dialysis patients[94].

IV. Pulse wave analysis

The patients were examined in a semi – recumbent position and had a neck and a femoral cuff applied and inflated to 65mmHg.The aortic path length was defined as the distance between the patient's supra-sternal notch and the midpoint of the thigh cuff. The signal from each cuff was digitally analysed in order to accurately record the pulse time delay and the corresponding pulse wave velocity.

V. Statistical analysis

Analyses were performed with IBM SPSS Statistics version 22.0. Continuous variables were expressed as mean ± standard deviation. Categorical variables were expressed as frequencies. Cox regression analysis was used to determine associations between the primary endpoints and demographic, clinical, electrocardiographic, echocardiographic and laboratory data. Statistical significance was set at $p< 0.05$. TCRT and all other statistically significant variables in the univariate analysis were included in the multivariate in a forward stepwise model for each of the outcomes, (MACE and all–cause mortality). With the exception of TCRT no other parameter was included a priori in the multivariate analysis. Secondary analysis using t –test for continuous variables and chi squared test for categorical variables was performed in order to compare the co-variables between the TCRT < 100 degrees and TCRT > 100 degrees groups. For the purpose of the longitudinal analysis, the annualised change in the relevant parameters was calculated and expressed as d – value. We used days as time periods for the annualization of the data. For simplification of the conversion we used 360 days in a year.

Results

I. Baseline characteristics

220 haemodialysis patients were considered for inclusion in this study. 49 did not have TCRT data and were therefore excluded. Of the 171 patients

included in the final baseline analysis, the majority were white (134, 78.4%), 124 (72.5%) were men and 47 (27.5%) women. Mean age was 62.3 years and mean time on dialysis was 2.8 years. Of the total number of patients, 28.7% were diabetic, 62.7% were smokers and 25.9% had history of CAD. 10% of the study participants had atrial fibrillation and 38% had bundle branch or complete heart block. The mean TCRT was 90.9 degrees (SD 39.9 degrees). The mean PWV was 8.7ms (SD 2.1ms) and the mean biplane ejection fraction was 60.7% (SD 12%). Baseline characteristics of the study participants are presented in Table 4.1. The mean annualised changes of the electrocardiographic, echocardiographic and laboratory parameters are presented in table 4.2.

II. Outcomes

MACE

During a mean follow up period of 2.3 years (SD 1.1), 43 MACE occurred. On univariate Cox regression analysis the factors associated with MACE were TCRT greater than 100 degrees (HR 2.215, p= 0.012., CI: 1.188 -4.131), history of coronary artery disease (HR 2.250, p= 0.010 CI: 1.219– 4.152), duration of the PR interval (HR: 1.008, p= 0.048 CI: 1.000- 1.017) and greater values of PWV (HR 1.147, p = 0.015 CI: 1.027 – 1.281). On multivariate Cox regression analysis for cardiac outcomes, TCRT greater than 100 degrees maintained a statistically significant association with MACE (HR 2.022, p=0.036, CI: 1.047 – 3.905) and so did increases in pulse wave velocity (HR: 1.133, p= 0.038, CI: 1.007 -1.274). The result of the multivariate analysis for all cardiac outcomes is presented in table 4.3. Figure 4.1 shows the Kaplan - Meir curve.

Table 4.1 Baseline characteristics of study participants

		Overall	TCRT>100°	TCRT<100°	
	N	n=171	n=75	n=96	p value
Demographic and clinical characteristics					
Ethnicity					0.059
Asian (%)	171	19	24	15	
White (%)	171	78	71	84	
Male (%)	171	73	83	65	0.009
Age (SD), years	171	62.13 ± 13.6	61.4 ± 12.9	63.1 ± 14.2	0.422
Smokers n (%)	169	106 (62.7)	49 (66.2)	57 (60)	0.407
BMI (kg/m2)	166	28.1 ± 6.2	28.6 ± 6.2	27.7 ± 6.3	0.458
Diabetes (%)	171	49 (28.7)	23 (30.7)	26 (27.1)	0.607
History f DCA (%)	170	44 (25.9)	22 (29.3)	22 (23.2)	0.334
History of PCD (%)	170	18 (10.6)	7 (9.3)	11 (11.6)	0.658
CVA / stroke (%)	170	15 (8.8)	8 (10.7)	7 (7.4)	0.435
SBP (SD), mmHg	167	138.9 (25.2)	142.5 (24.4)	136.1 (25.5)	0.101
Dialysis vintage (years)	165	2.8 ± 3.2	2.8 ± 2.4	2.9 ± 3.7	0.843
Electrocardiographic and echocardiographic					
AF n (%)	170	17 (10)	8 (10/8)	9 (9.4)	0.494
HR (SD), bpm	171	73.5 ± 13.8	74.5 ± 15.5	72.7 ± 12.4	0.415
PR interval (ms)	152	176.1 ± 35.3	173.8 ± 31.5	178.0 ± 38.1	0.462
QRS axis (°)	171	13.4 ± 41.2	5.6 ± 43.6	19.6 ± 38.4	0.027
T axis (°)	171	54.9 ± 46.9	65.0 ± 56.2	47.1 ± 36.7	0.013
QRS - T axis (°)	171	57.4 ± 41.6	76.9 ± 45.0	42.2 ± 31.4	<0.001
QTc (SD), ms	171	407.3 ± 38.4	407.6 ± 40.3	407.0 ± 37.1	0.909
Block					0.154
LBBB n (%)	171	56 ± 32.7	29 ± 38.7	27 ± 28.1	
RBBB n (%)	171	8 ± 4.7	2 ± 2.7	6 ± 6.3	
CHB n (%)	171	1 ± 0.6	1 ± 1.3	0 ± 0.0	
Paced n (%)	171	1 ± .0.6	0 ± 0.0	1 ± 1.0	
Biplane EF (%)	153	60.7 ± 12.0	58.3 ± 13.2	62.6 ± 10.7	0.057
LVMI/Ht$^{2.7}$ (SD), g/m$^{2.7}$	144	5.4 ± 2.4	5.9 ± 2.5	4.9 ± 2.3	0.017
EDV Teich (SD), ml	168	109.3 ± 37.1	118.2 ± 42.4	102.4 ± 31.0	0.006
PWV (SD), ms	165	8.7 ± 2.1	8.8 ± 2.2	8.6 ± 2.0	0.696
Laboratory findings					
Troponin (ng/L)	164	123.8 ± 539.7	202.1 ± 741.3	59.5 ± 271.2	0.099
BNP (pg/ml)	161	382.3 ± 579.1	425.1 ± 676.0	346.7 ± 485.5	0.349
Albumin (g/L)	153	37.7 ± 4.0	37.3 ± 4.8	38.1 ± 3.2	0.21
Haemoglobin (g/L)	153	106.5 ± 13.0	107.1 ± 13.5	106.0 ± 12.7	0.58

BMI: Body Mass Index, CAD: Coronary artery disease, PVD: Peripheral Vascular Disease, CVA: Cerebrovascular accident, SBP: Systolic Blood Pressure, AF: Atrial Fibrillation, HR: Heart Rate, LBBB: Left Bundle Branch Block, RBBB: Right Bundle Branch Block, CHB: Complete Heart Block, EF: Ejection Fraction, LVMI: Left Ventricular Mass Index, EDV: End Diastolic Volume, PWV: Pulse Wave Velocity, BNP: Brain Natriuretic Peptide, CI: confidence interval. Figures are displayed as mean ± standard deviation or percent.

Table 4.2 Longitudinal change in electrocardiographic, echocardiographic and laboratory parameters

Characteristic	N	Mean annualised change	Standard Deviation
TCRT	102	5.19	28.72
PR interval (ms)	106	4.74	18.9
QTc interval (ms)	115	47.4	108.86
QRS axis (°)	116	-4	9.74
T axis (°)	116	1.95	36.17
QRS –T axis difference (°)	116	60.39	39.59
Heart rate (bpm)	118	-1.17	8.41
LVMIHt2.7 (g/m2.7)	104	-0.19	4.86
EDVTeich (ml)	133	2.53	27.88
Biplane EF	126	2.42	15.65
BNP (pg/ml)	132	209.47	1435.55
Troponin (ng/l)	149	164.21	441.04

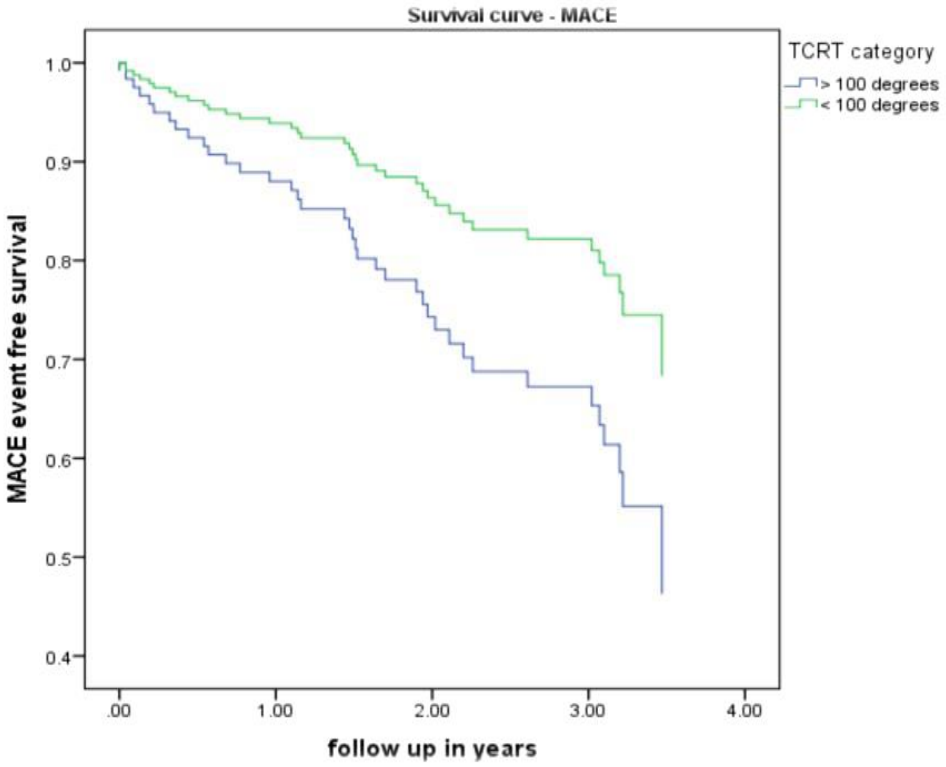
TCRT: total cosine R to T, LVMI: Left Ventricular Mass Index, EDV: End Diastolic Volume, EF: Ejection Fraction, BNP: Brain Natriuretic Peptide

Table 4.3 Multivariate Cox regression analysis for Major Adverse Cardiac Events at baseline

Variable	Hazard Ratio	Significance (p)	95% Confidence Interval
TCRT>100	2.022	0.0036	1.047 – 3.905
Hx of CAD	2.831	0.002	1.466-5.467
PR interval	1.004	0.338	0.995-1.014
PWV	1.133	0.038	1.007-1.274

TCRT: Total cosine R to T, CAD: coronary artery disease, PWV: pulse wave velocity

Figure 4.1. MACE free survival curve with regard to TCRT category



Longitudinal changes in TCRT, heart rate and biplane ejection fraction were associated with cardiac outcomes in univariate Cox regression analysis in our sample (HR: 1.014, p=0.041- CI: 1.001 – 1.028, HR: 1.057, p=0.013- CI: 1.012- 1.104 and HR: 0.969, p= 0.037, CI: 0.941 – 0.998, respectively). In a multivariate Cox regression model that included all the variables that were statistically significant in univariate analysis only longitudinal changes in the biplane ejection fraction remained associated with MACE (HR: 0.962, p= 0.018, CI: 0.932 – 0.994).

Survival

During a mean follow up period of 2.3 years (SD 1.1), 48 deaths occurred. On univariate Cox regression analysis the factors associated with increased all cause mortality were: male sex (HR 1.838, p = 0.042, CI: 1.023 – 3.301), age (HR 1.033, p = 0.008, CI: 1.009 -1.059), PR interval (HR: 1.013, p= 0.001, CI: 1.005 -1.021) and higher values of

PWV (HR: 1.219, p=< 0.001, CI: 1.111-1.338). Higher values of BNP were also very modestly associated with increased mortality (HR: 1.001 p= 0.015, CI: 1.000 – 1.001). Increases in the dialysis vintage in years were associated with reduced mortality risk (HR 0.856, p=0.031, CI: 0.743 – 0.986). On multivariate analysis only age, duration of the PR interval and PWV remained associated with all cause mortality (HR 1.029, p=0.033- CI: 1.002 -1.056, HR: 1.014. p=0.003-CI: 1.005-1.023 and HR: 1.266, p< 0.001, CI: 1.117 – 1.434 respectively) (Table 4.4).

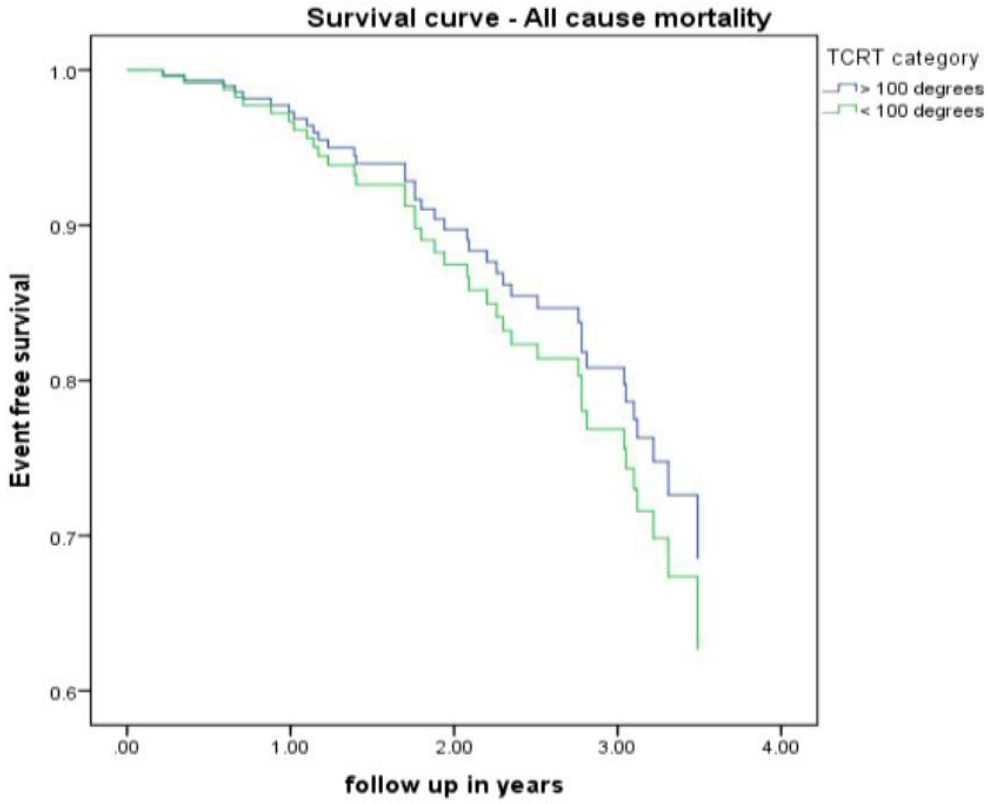
Figure 4.2 presents the Kaplan Meir curve for survival.

Table 4.4 Multivariate Cox regression survival analysis for all cause mortality at baseline

Variable	Hazard Ratio	Significance (p)	95% Confidence Interval
Sex	1.739	0.123	0.861-3.514
Age	1.026	0.051	1.000-1.054
Dialysis vintage (years)	0.834	0.054	0.693-1.003
PR interval	**1.013**	**0.004**	**1.004-1.023**
BNP	1.000	0.044	1.000 – 1.001
PWV	**1.275**	**<0.001**	**1.121-1.451**
TCRT	0.809	0.559	0.398-1.645

BNP: brain natriuretic peptide, PWV: pulse wave velocity, TCRT: Total cosine R to T

Figure 4.2 Survival curve with regard to TCRT category



None of the longitudinal changes of the electrocardiographic, echocardiographic or laboratory parameters was statistically significant for all cause mortality in Cox regression analysis in our cohort of haemodialysis patients

Discussion

In our cohort of prevalent haemodialysis patients we demonstrated that the QRS – T angle, expressed as TCRT, carries independent prognostic value for major adverse cardiac events. This is in keeping with previously published findings from other studies in dialysis patients[62,64]

The association of PWV with cardiovascular and all cause mortality has been widely reported in haemodialysis patients and the general population[89,95-97]. The results from our cohort are in keeping with previous reports. This is possibly a reflection of the role of arterial stiffness and calcification in the development and progression of cardiac disease.

We did not find an association between higher values of TCRT and all cause mortality. This finding might suggest that distinct mechanisms, unique to haemodialysis, are involved in the pathogenesis of cardiac mortality and morbidity in this population and perhaps TCRT reflects the phenotype of uraemic cardiomyopathy. In contrast to our findings, other studies in haemodialysis populations have found associations between the spatial QRS – T angle and all cause mortality[62,64]. The acquisition method of the QRS T angle and the definition of normal and abnormal values differ between these studies and ours. Tereshchenko et al[62] utilised 5 minute signal averaged ECGs, defined the QRS –T angle as the angle between the mean QRS vector and the peak T vector and used a cut off value of normal QRS – T angle of 75 degrees. De Bie[64] et al defined the QRS – T angle as the angle between the mean vectors of the QRs and T, acquired it from standard 10 second ECGS and calculated it using the Dower conversion matrix. In this study, values greater that 130 degrees in males and 116 degrees in females were considered abnormal.

In our study we used TCRT (total cosine of R to T) as an expression of the QRS – T angle. In a previous comparative study between different methods of calculation the QRS T angle in cardiac patients and the healthy individuals TCRT was found to be more reliable and statistically more powerful with regard to mortality prediction compared to other methods of QRS –T angle calculation[63]. TCRT is based on singular value decomposition to construct a mathematically derived subject-dependent three-dimensional (3-D) space optimising the orthogonal leads in order to capture most of the ECG energy in each individual. This method deals with the problem that, unlike the T vector that is considered to have a defined spatial orientation, the QRS vector is wide and curved; therefore no single vector can represent its orientation[63].

Another advantage of our methodology is that the QRS – T angle was derived from standard 10 second surface ECGs, as opposed to 5 minute signal averaged ECGs that are not widely used in clinical practice.

Our study has some limitations. Firstly, our sample consisted of prevalent, not incident, haemodialysis patients, therefore survival bias cannot be ruled out. In addition to this, previous research in haemodialysis populations have demonstrated improved survival rates for recruiters versus non recruiters in studies[98]. This could be another potential source of bias. Moreover, our sample consisted largely of white and male patients and the results cannot be necessarily extrapolated to other patient groups. Additionally, we relied on cross sectional data for the diagnosis of atrial fibrillation and the calculation of the electrocardiographic intervals, therefore a distinction between paroxysmal or temporary and persistent changes could not be made. Lastly, although analysis of the digital ECG recordings was performed in blinded manner using purpose designed software we did not undertake any additional calculations for margins of error in TCRT measurement.

In conclusion, our study demonstrated that TCRT, a novel electrocardiographic marker of repolarisation heterogeneity that can be derived from standard 10 second ECGs, carries independent prognostic significance for cardiac risk in a haemodialysis cohort. Further validation in larger cohorts of patients with the aim to generate larger databases of conventionally acquired digital ECGs is needed. Standardisation of the methods for QRS – T angle calculation is another area where further research is warranted.

Chapter 5

Strain repolarisation on ECG, not left ventricular mass, associates with cardiovascular outcome in a prospective cohort of haemodialysis patients

Rationale

In the previous chapter we assessed the prognostic value of a novel biomarker in a cohort of haemodialysis patients. In this chapter we will continue the exploration of the prognostic value of electrocardiographic markers of heterogeneity of repolarisation by focusing on ECG strain. Although a well established abnormality of ECG, it is not routinely used as a risk maker in the general population. Given that arrhythmia, sudden death and left ventricular hypertrophy (the main cause of ECG repolarisation strain) are all much more common in haemodialysis patients than the general population there is a rationale for why this may be a more useful tool in this group.

Abstract

Introduction. Cardiovascular disease is common in chronic and end stage kidney disease. Left ventricular hypertrophy (LVH) has been identified as contributor to cardiovascular risk in this population. The aim of the study was to assess whether the combined use of electrocardiography and echocardiography in assessing LVH in a haemodialysis population can provide improved risk stratification.

Methods. Prospective study of 192 prevalent maintenance haemodialysis (HD) patients. 12 lead ECGs were performed on a mid week non –dialysis day. Electrocardiographic strain was defined as a down sloping convex ST segment with inverted T waves in leads V5 and / or V6. Transthoracic echocardiographic was performed immediately after ECG .LV mass was indexed to body surface area (LVMIBSA). LVH was present if LVMI was >116g/m^2 for male patients, and >100g/m^2 for female patients. The primary study endpoint was major cardiac events (MACE). A secondary endpoint was all cause mortality.

Results. 192 patients were included in the final analysis, 137 (71.4%) male. The mean ejection fraction (EF) was 60.6± 11.1 % and the mean LVMI (BSA) was 115.0± 36.8 g/m$^{2.}$ During a mean follow up period of 2.4 ± 1.0 years, 50 patients reached a MACE end point and 62 patients died. On univariate Cox regression analysis, the factors associated with MACE were the presence of ECG strain (HR 2.961, CI: 1.254 – 6.990, p= 0.013)) URR (HR 0.968, CI: 0.942 – 0.994, p=0.015) and history of CAD (HR: 2.397 CI: 1.363 -4.2515, p= 0.002). In multivariate Cox regression analysis adjusting for baseline cardiovascular phenotype and dialysis parameters ECG strain remained significantly associated with MACE.

Conclusion. The presence of electrocardiographic strain increases the risk for MACE independently of LVH in haemodialysis patients. ECG strain has potential to be a simple bedside prognostic biomarker and even therapeutic target in haemodialysis patients.

Introduction

Cardiovascular disease is common in CKD and ESRD, and cardiovascular mortality is very high. Up to 15% of dialysis patients die every year and more than half of these are likely to be due to cardiovascular complications. Although traditional risk factors such as coronary artery disease account for a proportion of this[1], new studies have identified factors such as bradyarrhythmia and left ventricular hypertrophy (LVH) as significant contributors to cardiovascular risk in this population. Emerging risk stratification models in haemodialysis patients predominantly rely on echocardiographic parameters. LVH is common in advanced and End Stage Renal Disease (ESRD) and its prognostic value has been established in these populations[23,99].

LVH is also associated with arrhythmia in ESRD, the mechanism proposed to be via fibrotic remodelling causing aberrant conduction through the myocardium. However, echocardiography cannot directly measure abnormalities of conduction. Electrocardiography is inexpensive and easily accessible. However it is not commonly utilised for risk stratification in renal populations. Some electrocardiographic parameters, such as the QTc and PR intervals can be dynamically affected by the patients' fluid volume and electrolyte status, potentially making them unreliable as prognostic biomarkers using single time point recordings as a screening tool. Additionally, fluid removal during dialysis leads to an increase in the ECG voltage which is gradually attenuated as fluid accumulates. This makes the electrocardiographic diagnosis of LVH in dialysis patients with large interdialytic gains less reliable[31]. Electrocardiographic LVH correlates poorly with echocardiographic LVH[30] suggesting that changes in electrical remodelling depicted by ECG-LVH do not mirror anatomical structural changes depicted by echocardiogram. This means that ECG and echocardiographic LVH may carry independent prognostic value. Electrocardiographic strain pattern as a marker of LVH severity has been shown to add prognostic information to risk stratification models in CKD[100].

The aim of this study was to evaluate whether the combined use of echocardiography and echocardiography in assessing LVH in ESRD can provide improved risk stratification by concurrently evaluating both the presence of increased LV mass and whether there is a demonstrable effect on cardiac conduction pathways. We undertook this by comparing outcomes in patients with no LVH, LVH but normal ECG, and LVH with strain pattern repolarisation on ECG. This has the potential to provide improved risk stratification in these patients.

Methods

This was a sub-study of the Salford Kidney Study (SKS), a prospectively collected longitudinal study of > 3000 patients recruited on first presentation of CKD or on renal replacement therapy. Patients included into this analysis are from the sub-group of SKS previously known as the CRISIS-HD cohort, and described in detail elsewhere[89].

In short, adult maintenance haemodialysis patients at Salford Royal NHS Foundation Trust, UK or one of its satellite units are considered for inclusion. Exclusion criteria were age under 18 years, home haemodialysis, and non-standard dialysis hours (4 hours three times per week).

Patients who eventually participate in the study undergo detailed cardiovascular assessment including SKS study specific extended protocol echocardiography and electrocardiography on a non-dialysis day during a short, mid-week interdialytic break. Patients also undergo blood sampling and data collection regarding co-morbid conditions, dialysis prescription and medication using self-reported questionnaires and Hospital electronic patient records. The patients are assessed annually for mortality and disease progression. Death and cause of death data were obtained from the Office of National Statistics. Other event data were obtained from the Trust's electronic patient records, and from integrated primary care records. The study complies with the declaration of Helsinki and local ethical approval has been obtained (current UK REC reference 15/NW/0818).

I. Cardiovascular assessment

All 12 lead ECGs were obtained on single visits during a mid-week non-dialysis day. They were performed with the patients in a recumbent position and with standard lead placement using a Philips Pagewriter TC 20 device for ECG acquisition at a sampling rate of 500 Hz. Automated measurements by Philips measurement algorithm of heart rate and ECG intervals for QRS and QTc were used in the analysis. QTc was calculated using the Bazett's formula. Electrocardiographic strain was defined as a down slopping convex ST segment with inverted T waves in leads V5 and / or V6[101].

Transthoracic echocardiography was performed immediately after ECG. All patients were assessed in the left lateral decubitus position, using Philips echocardiography equipment with 3.5 MHz transducers. Measurements were obtained according to published guidelines by the European Society of Echocardiography. Ejection fraction (EF) was calculated using the Biplane method as recommended by the ESC. LV mass was calculated using 2D linear measurements and the Devereux formula, and indexed to body surface area. LVH was determined if LVMI >116g/m^2 for male patients, and >100g/m^2 for female patients.

Values of haemoglobin, albumin, potassium and brain natriuretic peptide (BNP) were expressed as the mean value of results from standard, routine sampling taken during the three months prior to echocardiography. Urea reduction ratio was used (URR) as a measure of dialysis adequacy. URR is the percent reduction in the blood urea concentration during a single dialysis treatment. The urea reduction ratio is calculated from blood urea levels measured five minutes after the end of dialysis and the pre dialysis blood urea.

II. Co-morbidity and end point definitions

Coronary artery disease (CAD) was defined as previous myocardial infarction, coronary revascularisation or medically managed angina. Heart failure was diagnosed in patients with NYHA Class II – IV symptoms and echocardiographic changes consistent with heart failure. The definition of

peripheral vascular disease (PVD) included history of previous revascularisation, claudication symptoms and ischaemic ulcers. Cerebrovascular disease was defined as history of clinical stroke or transient ischaemic attack. The primary study endpoint was major cardiac events (MACE). MACE included acute coronary syndromes, coronary revascularisation, hospitalisation due to heart failure or arrhythmia, and cardiac or sudden cardiac death. A secondary end point was all-cause mortality. Patients' follow up was censored at transplantation.

III. Statistical analysis

We categorised patients into those with and without echocardiographic LVH, and further divided those with LVH in to those with and without ECG strain pattern repolarisation. We then compared baseline phenotype, including echocardiographic assessment, between these three groups. Continuous variables were expressed as mean ± standard deviation for normally distributed data and median ± interquartile range for non-normally distributed data. Normality was assessed graphically with Q-Q plots and was subsequently tested with the Kolmogorov – Smirnov test. Categorical variables were expressed as frequencies. One way ANOVA and Kruskal Wallis test were used for comparisons between groups. Chi square test was used for categorical variables.

A comparison of outcomes between patients without LVH, with LVH but normal ECG, and LVH with strain pattern repolarisation on ECG was then performed using a multivariate Cox proportional hazard model. Co-variates entered into the model were determined a priori. In the first model assessing LVH category against cardiovascular co-morbidities, age, sex, diabetes and previous cardiovascular events were included in the model. In a second model assessing LVH category against dialysis parameters, urea reduction ratio (URR), ultrafiltration volume, serum potassium levels and dialysis vintage were included as co-variables. Statistical significance was set at $p< 0.05$. Analyses were performed with IBM SPSS Statistics Version 22.0.

Results

I. Baseline characteristics

205 haemodialysis patients were considered for inclusion in this study. Of those, 9 were excluded as they had echocardiography but no ECG data, and 4 were lost to follow up. Of the 192 patients included in the final analysis, 137 (71.4%) were male. Diabetes was present in 56 (29.2%), CAD in 47 (24.5%), PVD in 18 (9.4%), atrial fibrillation (AF) in 16 (8.3%), and cerebrovascular disease in 16 (8.3%). The mean age was 61.6 ± 13.9 years, mean time on dialysis was 4.8 ± 5.4 years. 93 patients (48.4%) did not have LVH, 85 (44.3%) had LVH without evidence of ECG strain and 14 (7.3%) had LVH with ECG strain. The mean heart rate (HR) was 74.1 ± 13.4 bpm. The mean potassium was 4.7 ± 0.6 mmol/L and the mean ultrafiltration volume achieved on each dialysis session was 2.1± 0.7 litres. The median URR was 70 (IQR: 10).

Comparisons between the three groups (no LVH, LVH without strain and LVH with strain) revealed statistically significant differences in systolic Blood Pressure (SBP), Urea Reduction Ratio (URR), Brain Natriuretic Peptide (BNP) values, PR interval duration, T axis. EF, LVMI (BSA) and Global Longitudinal Strain (GLS). Specifically, the mean SBP in the non LVH group was 136±19 mmHg, in the LVH without strain group was 149±19 mmHg and in the LVH with strain group was 160±20mmHg ($p=0.026$). The mean EF in the no LVH group was 63.0±10.0%, in the LVH without ECG strain group was 58.8±10%, and in the LVH with strain group was 56.9±15% ($p<0.001$). The mean LVMI (BSA) in the non-LVH group was 87.0±21.3g/m^2, in the LVH without strain group was 139.9±28.0g/m^2, and in the LVH with strain group was 148.2±25.0g/m^2 ($p<0.001$). Full details of baseline characteristics of the study population divided into each group are presented in table 5.1.

Table 5. 1. Baseline characteristics.

Data are presented as mean ± standard deviation for parametrically distributed parameters, median (interquartile range) for non-parametrically distributed parameters, and percent for categorical variables.

	Overall	No LVH	LVH no strain	LVH with strain	p
N	192	93	85	14	-
Systolic BP (mmHg)	142±20	136±19	149±19	160±20	<0.001
Heart rate (bpm)	74±13	75±12	*75*±12	71±18	0.427
QTc (ms)	417± 49	416±38	422±38	412±35	0.741
Haemoglobin (g/dL)	106±12	107±12	107±12	100±11	0.161
Potassium mEq/L)	4.7±0.6	4.7±0.6	4.7±0.6	4.5±0.4	0.534
UF volume (litres)	2.1±0.7	2.1±0.6	2.3±0.7	2.1±0.6	0.303
PWV (m/s)	8.7±2.1	8.6±2.2	9.8±2.3	8.2±2.5	0.489
Male	137	71	54	12	0.078
Diabetes	29.2	21	31	4	0.126
CAD	24.4	21	20	6	0.249
PVD	9.4	11	6	1	0.528
AF	8.3	8	7	1	0.982
CVA	8.3	6	10	0	0.222
Age (years)	63(21)	64(19)	60(21)	64(18)	0.986
Dialysis vintage (years)	3.5(4.2)	3.9(3.7)	2.1(3.6)	1.5(6.6)	0.166
URR	70(10)	71(11)	68(9)	70(11)	0.009
Albumin (g/L)	39(3)	39(3)	39(3)	37(5)	0.703
Ferritin (ug/L)	452(463)	558(566)	361(415)	475(219)	0.199
CRP (mg/L)	9.4(17)	9(19)	9(11)	12(35)	0.986
Troponin (ng/L)	14(22)	14(21)	14(21)	14(25)	0.736
BNP (pg/ml)	143(447)	136(292)	173(662)	519(515)	0.025
PR interval (ms)	172(45)	164(53)	184(39)	184(50)	0.007
QRS axis (degrees)	14(51)	14(51)	3(57)	12(59)	0.346
T axis (degrees)	56(59)	42(45)	68(61)	134(210)	0.017
QRS vs T axis (degrees)	47(58)	40(62)	56(40)	100(80)	0.005
EF (%)	60.6(11.1)	063.0(10.8)	58.8(10.3)	56.9(15.1)	0.026
LVMI (BSA) g/m^2	115.0(36.8)	87.0(21.3)	139.9(28.0)	148.2(25.0)	<0.001
GLS	-12.8(3.7)	-13.7(3.8)	-12.5(3.0)	-9.0(3.6)	<0.001

UF – ultrafiltration, PWV- pulse wave velocity, CAD: coronary artery disease, PVD: peripheral vascular disease, AF: atrial fibrillation, CVA: cerebrovascular accident, URR: Urea Reduction Ratio, BNP: brain natriuretic peptide, EF: Ejection fraction, LVMI(BSA) : Left ventricular mass indexed to body surface area, GLS: Global longitudinal strain.

Following this, we performed pair-wise comparisons for the above variables. We identified statistically significant mean differences in the echocardiographically determined LV mass between the non LVH and both LVH groups (x^2= -53.53, p< 0.001 and x^2= -61.44, p< 0.001), but not between the LVH groups with and without strain (x^2 = -7.913, p=0.888). There was a significant difference in the LV ejection fraction between the no LVH and LVH without strain groups. There was a statistically significant difference in SBP between patients without and with LVH regardless of strain (standardised $x2^{:}$ - 3.049, p: 0.007, x^2: -3.870, p< 0.001, respectively). This observation is in keeping with the known association between blood pressure and LVH. No statistically significant difference was found between the strain and no strain groups (x2: -36.2, p: 0.71). The PR interval was longer in patients with LVH and strain compared to the other two groups (x2: 2.621, p: 0.026). GLS was lower in the LVH and strain group compared to the other two (x2: -3.795, p: 0.002 compared to the LVH without ECG strain group, and x2: -5.210, p: < 0.001 compared to the non LVH group). Our hypothesis is that GLS in the strain group probably reflects the more advanced degree of myocardial remodelling / fibrosis in these patients; in the same patients the presence of ECG strain might be a reflection of conduction abnormalities which could be associated with fibrosis. Full pairwise comparisons are presented in table 5.2.

II. Outcomes

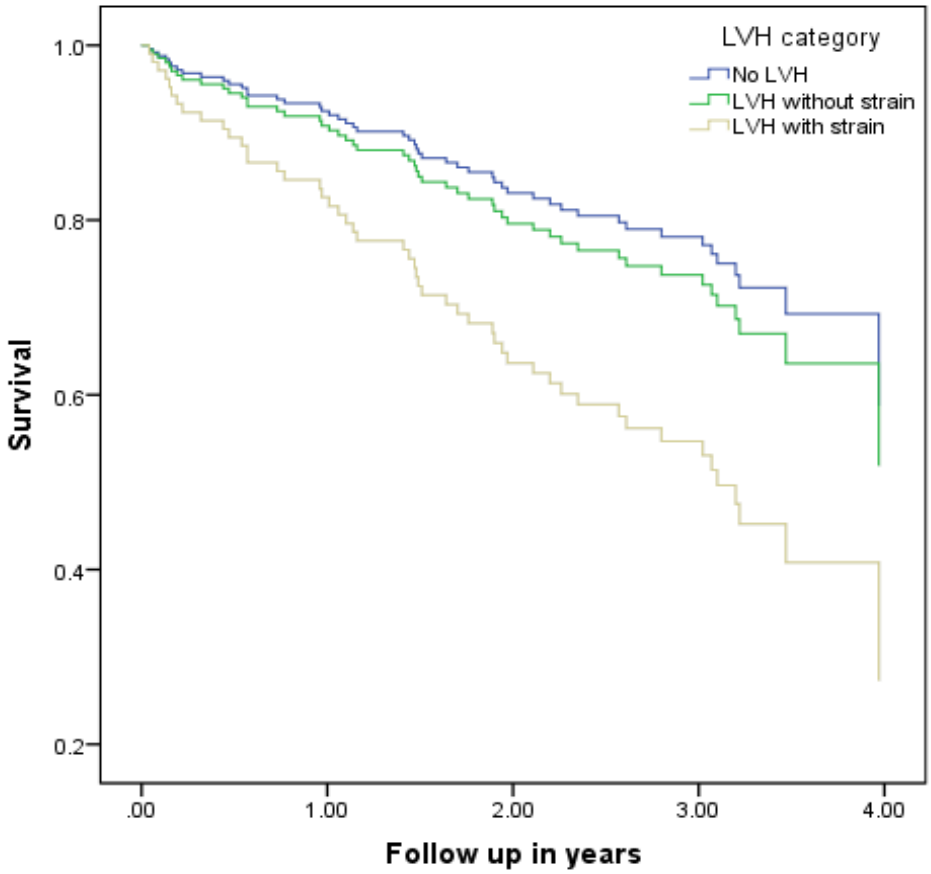
During a mean follow up period of 2.4 ± 1.0 years, 50 patients reached a MACE end point. On multivariate survival analysis adjusting for baseline cardiovascular phenotype (age, sex, presence of diabetes and history of prior cardiovascular disease), there was no difference in MACE events between patients without LVH and those with LVH but no ECG strain (HR 1.232, CI: 0.667 -2.276, p=0.505). However, patients with LVH who showed strain pattern repolarisation on ECG had a HR for MACE compared to the no LVH group of 2.439 (CI: 1.004 – 5.924, p=0.049). The Kaplan Meir curves for the three groups are found in figure 5.1. The only other parameter which was significant in this model was history of coronary artery disease (HR 2.340. CI: 1.301 – 4.210, p= 0.005).

Table 5.2. Pair wise in group comparisons of baseline parameters.

Variable	Group Comparison	Standardised test statistic	P value
SBP	No LVH – LVH without strain	-3.049	0.007
	No LVH – LVH with strain	-3.870	<0.001
	LVH without strain – LVH with strain	-2.260	0.071
PR interval	No LVH – LVH without strain	-2.329	0.060
	No LVH – LVH with strain	-2.621	0.026
	LVH without strain – LVH with strain	-1.385	0.498
T axis	No LVH – LVH without strain	-0.776	1.000
	No LVH – LVH with strain	-2.852	0.013
	LVH without strain – LVH with strain	-2.431	0.045
QRS v T	No LVH – LVH without strain	-1.677	0.281
	No LVH – LVH with strain	-3.113	0.006
	LVH without strain – LVH with strain	-2.222	0.079
URR	No LVH – LVH without strain	0.998	0.954
	No LVH – LVH with strain	2.351	0.056
	LVH without strain – LVH with strain	2.503	0.037
BNP	No LVH – LVH without strain	-0.357	1.000
	No LVH – LVH with strain	-2.682	0.022
	LVH without strain – LVH with strain	-2.478	0.040
LVMI	No LVH – LVH without strain	-53.535	<0.001
	No LVH – LVH with strain	-61.448	<0.001
	LVH without strain – LVH with strain	-7.913	0.888
GLS	No LVH – LVH without strain	-1.415	0.105
	No LVH – LVH with strain	-5.210	<0.001
	LVH without strain – LVH with strain	-3.795	0.002
EF	No LVH – LVH without strain	4.153	0.050
	No LVH – LVH with strain	6.274	0.181
	LVH without strain – LVH with strain	2.047	1.000

SBP: Systolic Blood Pressure, URR: Urea Reduction Ratio, BNP: Brain Natriuretic Peptide, LVMI: Left Ventricular mass indexed to body surface area, GLS: Global longitudinal strain, EF: Ejection fraction.

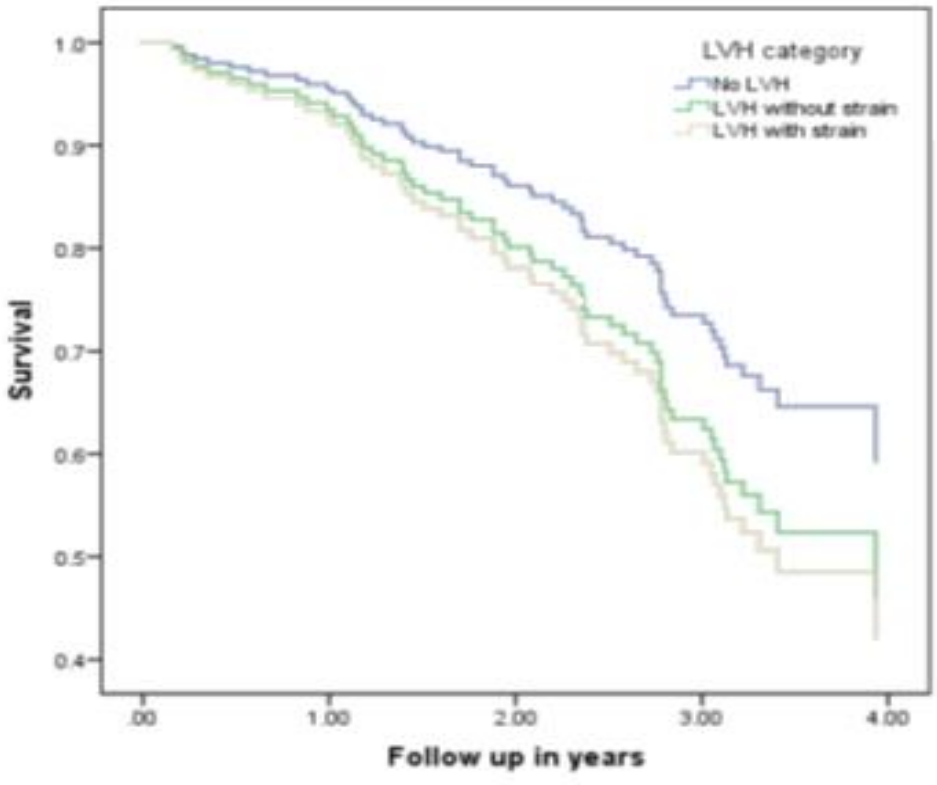
Figure 5.1. Survival curve for major cardiac events, comparing patients without left ventricular hypertrophy (LVH) against those with LVH but no strain pattern abnormalities on ECG, and those who have LVH with strain.



On multivariate survival analysis adjusting for baseline dialysis parameters, (URR, UF volume, serum potassium levels, dialysis vintage), there was no difference in MACE events between patients without LVH and those with LVH but no ECG strain (HR: 0.888, CI: 0.456 -1.730, p= 0.727). However, patients with LVH who showed strain pattern repolarisation on ECG had a HR for MACE compared to the no LVH group of 2.546, CI: 1.002 – 6.469, p= 0.049). The only other parameter which was significant in this model was URR (HR: 0.968, CI: 0.94- 0.997, p=0.032).

During the same follow up period, 62 deaths occurred in total. The commonest reported causes of death were myocardial infarctions (11 patients) and infections (11 patients) followed by sudden death (9 patients). On multivariate survival analysis adjusting for baseline cardiovascular phenotype (age, sex, presence of diabetes and history of prior cardiovascular disease), there was no difference in all cause mortality between patients without LVH and those with LVH but no ECG strain (HR 1.480, CI: 0.862 – 2.542, p= 0.155). Similarly, patients with LVH who showed strain pattern repolarisation on ECG had a HR for all cause mortality compared to the no LVH group of 1.652 (CI 0.662 – 4.125, p= 0.282). Out of the 14 patients with LVH and ECG strain, 6 died; the commonest reported cause of death was sudden death (3 patients). The Kaplan Meir curves for the three groups are found in figure 5.2.

Figure 5.2. Survival curve for all-cause mortality, comparing patients without left ventricular hypertrophy (LVH) against those with LVH but no strain pattern abnormalities on ECG, and those who have LVH with strain.



On multivariate survival analysis adjusting for baseline dialysis parameters, (URR, UF volume, serum potassium levels, dialysis vintage), there was no difference in all-cause mortality between patients without LVH and those with LVH but no ECG changes (HR 1.546, CI 0.867 – 2.758, p=0.140). Similarly, patients with LVH who showed strain pattern repolarisation on ECG had a HR for all cause mortality of 1.974, (CI: 0.789 – 4.941, p= 0.146) compared to the no LVH group.

Discussion

In our cohort of prevalent haemodialysis patients we have demonstrated that it is the presence of ECG strain in association with LVH that is independently associated with adverse cardiovascular outcome, rather than the presence of LVH itself. Patients with LVH and strain showed no statistically greater LV mass than those without strain and so this supports the hypothesis that a mechanism by which LVH leads to adverse outcomes in dialysis patients is by way of aberrant conduction because of fibrotic myocardial remodelling. Alongside this, patients with strain had higher BNP and systolic blood pressure values than those with LVH without strain, but were not more likely to have coronary artery disease and did not have higher troponin levels. These factors again support that markers of the nature of remodelling may be a better target future prognostic biomarker rather than simply LV mass itself.

This association has previously been shown by Cordeiro et al[100] in a cohort of CKD patients, but to our knowledge this has never been reported in haemodialysis patients. In the general population studies involving hypertensive patients, electrocardiographic strain has also been associated with poor prognosis independent of severity of LVH[102].

In recent years, data from experimental and population based studies have led to advances in our understanding of the underlying cardiovascular disease mechanisms in advanced kidney disease. This led to focussing on the dynamic

interplay between myocardial structural changes, vascular changes, autonomic imbalance, inflammation and fluid and electrolyte shifts that can lead to arrhythmias[9] and ultimately sudden death. In addition, recent studies of prolonged ILR recordings in 5 different haemodialysis cohorts have suggested that bradyarrhythmic events contribute significantly to cardiovascular mortality[8]. Although the underlying mechanisms are far from clear, approximately 10% of the patients in these cohorts were noted to have heart block or other bradyarrhythmia. This is also in keeping with the hypothesis that cardiac mortality in ESKD is propagated by aberrant conduction.

LVH is also the most frequent abnormality found in haemodialysis patients in respect of standard changes on non-invasive bedside assessments using echocardiography or ECG. In this study, more than half of patients exhibited LVH on echocardiography (52%) and in other studies the prevalence has been up to 70%. Whilst LVH is associated with worse outcome and may therefore be a viable therapeutic target (e.g. for blood pressure control, optimisation of fluid balance), the presence of strain on ECG may provide us with the ability to be more targeted in understanding which patients should be prioritised for aggressive intervention. Using strain as a target may also mean that monitoring response is easier because regular bedside ECG is far easier to perform than repeated echocardiography.

The main limitation of our study is that due to the small number of cardiac arrests and arrhythmic deaths it did not have enough power to assess associations between ECG stain and different categories or cardiac mortality separately, specifically sudden cardiac death.

In conclusion, in our cohort of prevalent haemodialysis patients we demonstrated that the presence of electrocardiographic strain increases the risk for MACE independently of LVH. This adds to a series of other studies that have demonstrated the relation between conduction abnormalities and cardiovascular outcomes in haemodialysis populations. Whether strain improves or worsens over time with changes in LV mass associated with dialysis, volume status and blood pressure control would be further valuable information as, this being the case, ECG strain has potential to be a simple

bedside prognostic biomarker and even therapeutic target in haemodialysis patients.

Chapter 6

The use of ECG parameters for diagnostic test evaluation of future cardiac events in haemodialysis patients

Rationale

Previous chapters have demonstrated variable degrees of association between ECG biomarkers and outcomes (major atherosclerotic events [MACE] and all-cause mortality). This chapter aims to evaluate these biomarkers collectively as a single diagnostic test and assess whether, beyond any individual statistical significance, an ECG based model can be reliably used as a screening test for pending adverse cardiovascular outcomes in haemodialysis patients. The overarching aim of this is to examine whether the experimental findings of previous chapters may translate into a clinically useful bedside tool that could reliably improve the clinician's predictive capacity for determining cardiovascular risk.

Abstract

Background and Objectives: As demonstrated in previous chapters, any given ECG abnormality does not appear to have significant predictive ability in order to be useful as a diagnostic test. However, collectively they may do. This study aims to explore this hypothesis

Methods: The study population consists of the haemodialysis arm of the Salford Kidney Study (SKS). 12 lead ECGs were obtained during a mid-week non-dialysis day. Diagnostic test evaluation was performed using sensitivity and specificity for ECG biomarkers with binary outcomes (presence/ absence of strain, presence/absence of block) and using ROC curve analysis for continuous outcomes. In addition to the individual biomarkers we included a scoring system where one point was allocated to each abnormal result.

Results: 215 patients were included in the final analysis. The majority (156, 72.6%) were men. The mean age was 67.5 ±13.9 years. Atrial fibrillation was found in 10%. The mean heart rate was 74±13 bpm, mean PR interval 176± 38 ms; mean QTc 418± 39 ms. 66 patients (30.7%) had a history of diabetes and 95 (44.2%) were current or former smokers. 99 patients (46%) were on a beta blocker. Out of the 215 subjects, 9 had left bundle branch block (LBBB), 2 right bundle branch block (RBBB) and 2 complete heart block (CHB). 21 patients had strain pattern on their ECG.

The sensitivity of all the ECG biomarkers examined was very low for detecting both mortality and pending MACE events. The same was the case for positive predictive value (PPV), with the exception of ECG changes that were rare in our population (tachycardia, RBBB, CHB). The opposite trend was observed for specificity, where for each one of the prevalent changes examined it was >75% for MACE and >80% for all cause mortality. For incident changes the specificity was > 80% for MACE and all cause mortality and for de novo incident changes it was > 88% for both MACE and all-cause mortality.

Conclusion: In our cohort of haemodialysis patients ECG biomarkers were overall very poor as a screening tool for cardiovascular outcomes and all cause mortality. This was evidenced by the very low sensitivity and very low AUC values on the ROC curves of all of them.

Introduction

Chronic kidney disease (CKD) carries a significant cardiovascular risk which increases with advancing CKD. This risk is particularly high in End Stage Renal Disease (ESRD) where up to a fourth of the patients may die as a result of an arrhythmia according to data from the renal registry[1]. Further to this, abnormalities of cardiac conduction and prevalent supraventricular arrhythmia are highly prevalent in haemodialysis patients, as set out in earlier chapters.

Whilst coronary artery disease accounts for a number of these deaths, as would be the case in the majority of sudden cardiac death in the general population, evidence suggests that non –atheromatous risk factors such as chronic inflammation, autonomic dysfunction, increased arterial stiffness, and changes in the left ventricular mass and function may be implicated to a greater degree compared to the general population[3]. Each of these may be associated with high risk of aberrant conduction.

Both conduction abnormalities and left ventricular hypertrophy can be detected on a standard 12 lead ECG. In addition, the 12 lead ECG is an inexpensive, readily available in most healthcare settings test that carries low risk for the individual. All medical practitioners are trained on ECG interpretation making this a universally implementable test. These factors mean that the 12 lead ECG has the potential to be a useful diagnostic tool in the haemodialysis population.

Previous chapters, and indeed the bulk of previous studies as detailed in chapter 1, demonstrate occasions where specific ECG changes show a statistical association with cardiovascular end points. Abnormalities of atrial conduction (prolonged PR interval), ventricular conduction (prolonged QRS duration and the presence of bundle branch block), and repolarisation changes including QTc and TCR are all well described in haemodialysis populations in association with adverse outcome (chapter 1). This is also true for prevalent rhythm abnormalities, particularly atrial fibrillation.

However, although these associations indicate greater risk, they lack the ability to be used for a specific diagnostic utility in a novel way for the haemodialysis population.

The hypothesis that underpins this chapter is that because there is a high level of heterogeneity in both the type of ECG changes seen in haemodialysis patients and also the mode of cardiovascular events they suffer, a collective assessment of ECG abnormalities may provide a better ability to predict overall future risk than each individual abnormality in isolation. This study aims to explore this hypothesis.

A secondary aim was to describe the prevalence and, more importantly, the incidence of these abnormalities within a dialysis patient population.

Methods

The study population consists of the haemodialysis arm of the Salford Kidney Study (SKS). This is the same population used in all the studies included in this thesis. The full methodology has been presented separately in the methods chapter, but in summary SKS comprises prevalent and incident haemodialysis patients who receive thrice weekly treatment either in Salford Royal NHS Foundation Trust or one of its satellite units. Patients undergo annual cardiovascular assessment and detailed evaluation of all-cause and cardiovascular outcomes during follow up.

Amongst the evaluation tools, ECG is performed. All 12 lead ECGs were obtained during a mid-week non-dialysis day. They were performed with the patients in a recumbent position and with standard lead placement using a Philips Pagewriter TC 20 device for ECG acquisition at a sampling rate of 50 Hz. Eligible subjects had annual follow up which included 12 lead ECGs. Automated measurements by Philips measurement algorithm of heart rate and ECG intervals for QTc and PR were used in the analysis. QTc was calculated using the Bazett's formula. Electrocardiographic strain was defined as a down slopping convex ST segment with inverted T waves in leads V5 and / or V6. Normal PR interval was defined as 120 – 200 ms, QTc prolongation was

defined as QTc> 450ms in men and QTc > 460 ms in women[35] and normal heart rate range was defined as heart rate between 60 -100 bpm. Left Bundle Branch Block (LBBB), Right Bundle Branch Block (RBBB) and Complete Heart Block (CHB) were diagnosed based on established criteria[103].

Patients were followed up on an annual basis and outcomes recorded, with definitions set out in chapter 2. The outcomes examined within this chapter are Major Adverse Cardiac Events (MACE) and all-cause mortality. MACE comprises acute coronary syndrome, coronary revascularisation, hospitalisation due to heart failure or arrhythmia, and cardiac or sudden cardiac death. The definitions of these are described in chapter 2.

Analyses were performed with IBM SPSS Statistics version 22.0. Continuous variables were expressed as means ± standard deviation. Categorical variables were expressed as frequencies. Diagnostic test evaluation was performed using sensitivity and specificity for biomarkers with binary outcomes (presence/ absence of strain, presence/absence of block) and using ROC curve analysis for continuous outcomes. In addition to the individual biomarkers we included a scoring system where one point was allocated to each abnormal (positive) result.

The sensitivity of a test refers to its ability to correctly identify the patients with the disease. The specificity refers to its ability to identify patients without the disease. An ideal test would be 100% sensitive and 100% specific.

Sensitivity was calculated as:

$$Sensitivity = \frac{True\ positives}{True\ positive\ +\ False\ negative}$$

Specificity was calculated as

$$Specificity\ = \frac{Tue\ negatives}{True\ negatives + False\ positives}$$

Positive predictive value was calculated as:

$$PPV = \frac{True\ positives}{True\ positives + False\ positives}$$

Negative predictive value was calculated as:

$$NPV = \frac{True\ negatives}{True\ negatives + False\ negatives}$$

We examined ECG abnormalities present at the first point of contact with the study subjects (prevalent) and also changes present at the first follow up point (12 months) that were not present at baseline (incident). For incident changes we examined whether they happened de novo or as a result of clinical causes (myocardial infarction, heart failure, drugs).

For the purposes of this analysis a positive test was defined as an ECG parameter whose value was outside the widely accepted range of normal for this particular parameter. If we take the PR interval as an example, the test was considered positive if the duration of the PR interval was either below 120ms or above 200 ms.

New biomarkers are traditionally assessed on their ability to correctly identify individuals with a particular condition whilst excluding "healthy" individuals. In a diagnostic test with dichotomous outcomes (positive / negative) the comparison of sensitivity and specificity against the gold standard is the conventional approach. In the case of continuous outcomes the improvement of the area under the curve (AUC) of the receiver operating characteristics (ROC), which is a plot of the sensitivity versus 1 – specificity, has been used as the main criterion in evaluating the diagnostic ability of new biomarkers in cardiovascular epidemiology[104]. In the case of continuous outcomes the use of single specificities and sensitivities as a marker of accuracy can be problematic as it depends on a cut –off point for positivity which can be arbitrary. Using AUC from ROC partially overcomes this problem and also it can reduce bias as by being dependent on both sensitivity and specificity is not directly affected by the prevalence of the condition in question in the examined

population. For these reasons and also because you can simultaneously test different parameters, ROC is widely used in medical epidemiology in the evaluation of new diagnostic markers.

Results

I. Baseline characteristics

215 patients were included in the final analysis. The majority (156, 72.6%) were men. The mean age was 67.5 ±13.9 years. The mean heart rate was 74±13 bpm, mean PR interval 176± 38 ms; mean QTc 418± 39 ms. 66 patients (30.7%) had a history of diabetes and 95 (44.2%) were current or former smokers. 99 patients (46%) were on a beta blocker. Out of the 215 subjects, 9 had LBBB, 2 RBBB and 2 complete heart block (CHB). 21 patients had strain pattern on their ECG. Full descriptive statistics for prevalent abnormalities are presented in table 6.1.

Table 6.1.Prevalent ECG abnormalities at study enrolment

ECG parameter	Frequency (%)
ECG strain pattern	21 (9.8%)
LBBB	9 (4.2%)
RBBB	2 (0.9%)
CHB	2 (0.9%)
Tachycardia	4 (1.9%)
Bradycardia	34 (15.8%)
Long QTc	40 (18.6%)
AF	21 (9.8%)
Score	
1	68 (31.6%)
2	26 (12.1%)
3	5 (2.3%)
4	4 (1.9%)

LBBB: Left Bundle Branch Block, RBBB: Right Bundle Branch Block, CHB: Complete Heart Block, AF: Atrial Fibrillation

II. Prevalence and incidence of ECG abnormalities

The commonest ECG abnormality at baseline was prolonged QTc interval (18.6%), followed by bradycardia (15.8%). The same trend was observed in the case of incident and de novo changes. Only one ECG abnormality was present in 31.6% of patients, followed by presence of two abnormalities in 12.1%. The combination of three or four abnormalities in the same ECG was rare in our cohort (2.9% and 1.9% for three and four abnormalities respectively). In our study sample, there was never more than one incident ECG abnormality present per patient. Descriptive statistics for incident and de novo changes are presented in table 6.2.

Table 6.2. Incident and de novo ECG changes at 12 months from enrolment.

LBBB: Left Bundle Branch Block, CHB: Complete Heart Block, AF: Atrial Fibrillation

ECG parameters	Incident changes %	De novo %
LBBB	13 (8.4%)	11 (7.1%)
CHB	1 (0.6%)	0 (0%)
Bradycardia	22 (14.3%)	20 (13%)
Long QTc	29 (18.8%)	15 (9.7%)
AF	5 (3.2%)	3 (1.9%)

End points were common during a mean follow up of 2.4±1.0 years. There were 73 patient deaths. 57 patients suffered a MACE event during follow up.

III. Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) analyses

In our cohort of haemodialysis patients, the sensitivity of all the ECG biomarkers examined was very low for detecting both mortality and pending MACE events. For MACE in particular, in the case of prevalent ECG abnormalities, the presence of more than one ECG abnormality had the highest sensitivity at 51.67%. Out of the individual abnormalities, abnormal PR interval (including short and long PR interval) had the highest sensitivity at 27.7%. This was followed by PR prolongation with sensitivity of 25% and bradycardia with sensitivity of 24.56%.For all cause mortality the presence of more than one ECG abnormality had a sensitivity of 50%, followed by prolonged PR interval at 60% and long QTc at 50%. The same was the case for PPV, with the exception of mainly ECG changes that were rare in our population (RBBB, CHB). With regard to MACE the PPV for RBBB was 100%, followed by short PR interval at 60% and CHB at 50%. In the case of all cause mortality the abnormalities with the highest PPV were CHB and RBBB with 100% and LBBB with PPV of 67%.

The opposite trend was observed for specificity, where for each one of the prevalent changes examined it was > 75% for MACE and >80% for all cause mortality. With regard to MACE the highest specificity was found in RBBB and tachycardia (100%) followed by CHB (99.37%), short PR interval (98.57%) and ECG strain (92.4%). In the case of all cause mortality the prevalent abnormalities with the highest specificity were RBBB and CHB (100%), tachycardia (99%) and LBBB (98%).

For incident changes similar patterns were observed. The sensitivity of all the biomarkers we examined was low for both MACE and all cause mortality. With regard to MACE, the two best performing parameters were new onset LBBB with sensitivity of 25% and new onset QTc prolongation with sensitivity of 17.6%.The specificity for these two parameters was 93.38% and 83.54% respectively. For all cause mortality the incident changes with the highest sensitivity were QTc prolongation and bradycardia (30.7% for both). For these two parameters the specificity was 82.27% and 85.37% respectively.

The findings for de novo incident changes were similar with all of the parameters showing low sensitivity.

The ROC curves for the non- dichotomous parameters are presented in figures 6.1-6.3. The Area under the curve (AUC) was 0.567 for the duration of the PR interval, 0.527 for the duration of the QTc interval and 0.381 for heart rate.

Full results for prevalent, incident and de novo changes are presented in tables 6.3-6.10.

Figure 6.1. ROC curve for duration of PR interval with regard to Major Adverse Cardiac Events. Prevalent parameters

AUC: 0.567

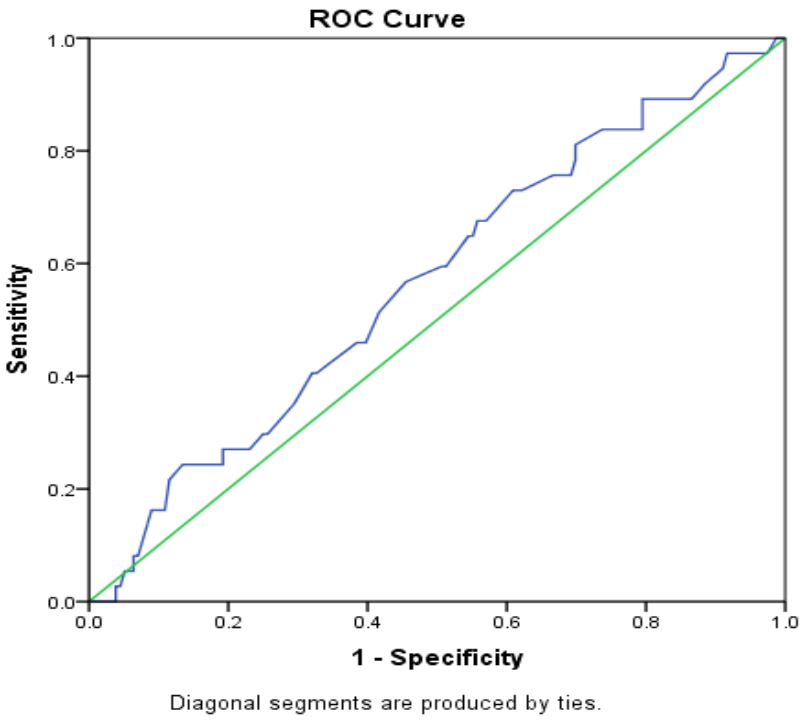


Figure 6.2: ROC curve for QTc duration with regard to Major Adverse Cardiac Events. Prevalent parameters

AUC: 0.527

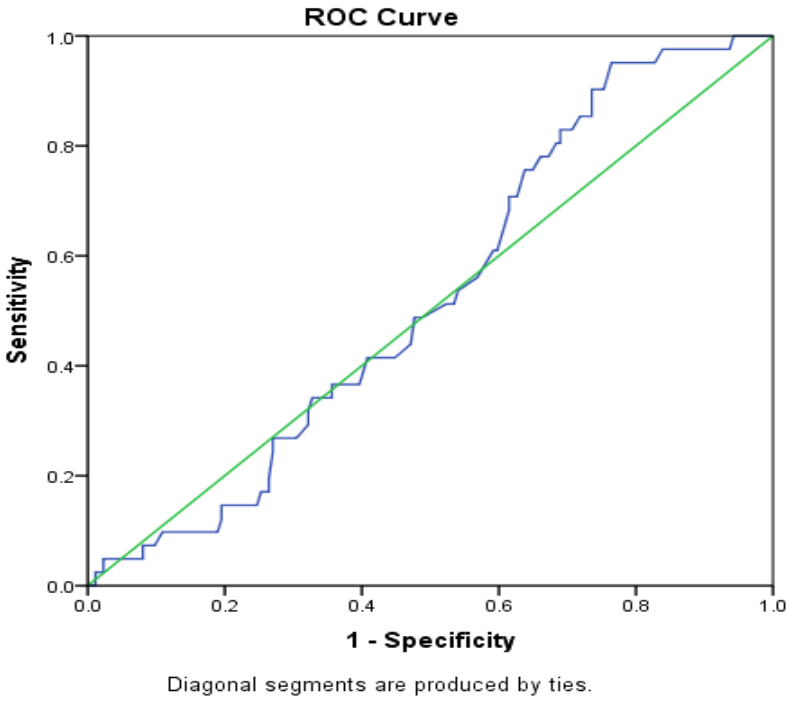


Figure 6.3. ROC curve for heart rate with regard to Major Adverse Cardiac Events. Prevalent parameters.

AUC: 0.381

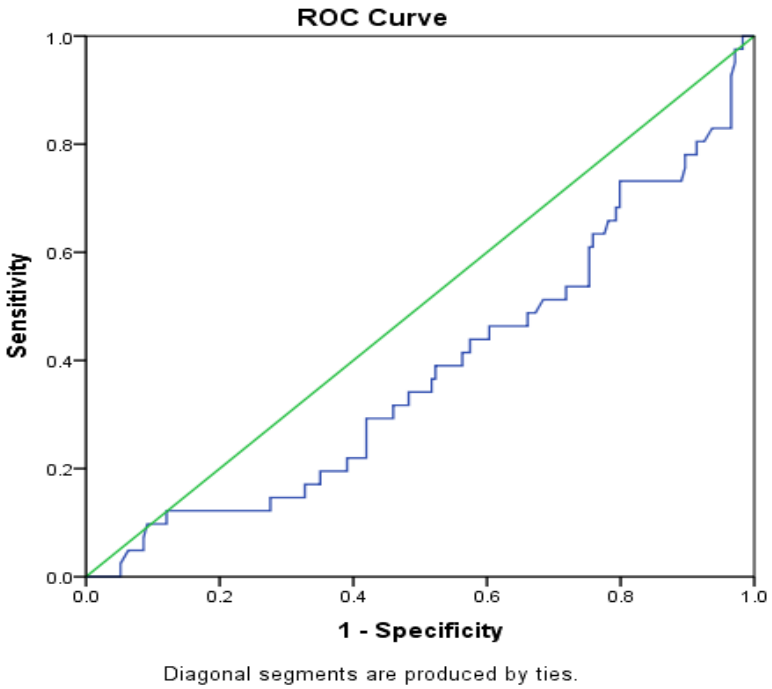


Table 6.3. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of prevalent ECG biomarkers for Major Adverse Cardiac Events.

	MACE			
	Sensitivity (%)	*Specificity (%)*	*PPV (%)*	*NPV (%)*
Abnormal PR	28	75	28	75
Short PR	6	**99**	60	74
Long PR	25	81	33	75
ECG strain	16	**92**	43	76
Long QTc	14	80	20	72
AF	9	**90**	24	73
Bradycardia	25	87	41	76
Tachycardia	0	**100**	n/a	72
LBBB	4	**96**	22	73
RBBB	3	**100**	**100**	74
CHB	2	**99**	50	74
Score ≥1	52	53	24	79

AF: Atrial Fibrillation, LBBB: Left Bundle Branch Block, RBBB: Right Bundle Branch Block, CHB: Complete Heart Block

Table 6.4 Sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of prevalent ECG biomarkers for all cause mortality

All cause mortality				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Short PR	2	**97**	28	67
Long PR	32	85	50	72
ECG strain	15	**92**	50	68
Long QTc	22	83	40	67
AF	14	**92**	48	68
Bradycardia	19	87	44	65
Tachycardia	3	**99**	50	68
LBBB	8	**98**	n/a	67
RBBB	3	**100**	**100**	67
CHB	3	**100**	**100**	67
Score ≥1	53	55	38	70

AF: Atrial Fibrillation, LBBB: Left Bundle Branch Block, RBBB: Right Bundle Branch Block, CHB: Complete Heart Block

Table 6.5 Sensitivity, Specificity. Positive predictive Value and Negative Predictive Value of incident ECG biomarkers for Major Adverse Cardiac Events (MACE)

MACE				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Long QTc	18	84	28	**90**
AF	6	**97**	20	89
Bradycardia	13	83	9	88
LBBB	25	**93**	25	**93**
CHB	0	**100**	n/a	89

AF: Atrial Fibrillation, LBBB: Left Bundle Branch Block, CHB: Complete Heart Block

Table 6.6. Sensitivity, Specificity. Positive Predictive Value and Negative Predictive Value of incident changes of ECG biomarkers for all cause mortality

All cause mortality				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)

Long QTc	31	82	28	**93**
AF	0	**97**	0	**91**
Bradycardia	31	85	18	**92**
LBBB	8	**91**	8	**91**
CHB	0	**99**	0	**92**

AF: Atrial Fibrillation, LBBB: Left Bundle Branch Block, CHB: Complete Heart Block

Table 6.7. Sensitivity, specificity, PPV and NPV of "de novo" changes in ECG biomarkers for MACE

	MACE			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
LBBB	6	**92**	28	79
Bradycardia	13	88	22	80
Long QTc	13	**91**	27	81
AF	3	**98**	33	80

AF: Atrial Fibrillation, LBBB: Left Bundle Branch Block

Table 6.8. Sensitivity, specificity, PPV and NPV of "de novo" changes in ECG biomarkers for all cause mortality

	All cause mortality			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
LBBB	8	**93**	28	**92**
Bradycardia	31	89	20	**93**
Long QTc	15	**91**	13	**92**
AF	0	**98**	0	**91**

AF: Atrial Fibrillation, LBBB: Left Bundle Branch Block

Discussion

In our cohort of haemodialysis patients ECG biomarkers were overall very poor as a screening tool for cardiovascular outcomes and all cause mortality. This was evidenced by the very low sensitivity and very low AUC values on the ROC curves of all of them. This finding is in keeping with results in previous chapters where models based on ECG biomarkers were found to be poor fit for mortality and MACE risk prediction.

Conversely, the specificity of the ECG parameters was high, especially in the case of incident and de novo changes. This could theoretically indicate that ECG parameters may have some use as a "rule out" test if used in conjunction with another diagnostic test with high sensitivity. A perfect test would be one with almost 100% sensitivity and specificity. This is rarely achieved in reality. By combining one test with high sensitivity with one with high specificity it is on occasions possible to gain diagnostic value[105]. An example where this principle has been used successfully in diagnostic screening in the general population is screening programmes for cervical cancer, where cervical cytology smears (high sensitivity, but low specificity) are used in conjunction with testing for high risk human papillomavirus (low sensitivity, high specificity).

It is noteworthy that although nearly half of the study population exhibited at least one common ECG abnormality, just 5% of patients had more than 2 abnormalities. It is possible that patients who develop severe or multiple abnormalities do not survive to enrol in studies such as this. This will potentially mean the highest risk patients and those that would be most likely to benefit from a scoring system such as that described here are also those least likely to be captured by the study design. This can create a survivor bias favouring fewer ECG changes.

One of the limitations of this study is the small sample size and number of events. In addition, for the purposes of sensitivity/ specificity analysis we

defined MACE and all cause mortality as “disease”; these are, however, a combination of different conditions that may have distinct aetiologies and as a result this necessary assumption may potentially be a source of errors[106]. Additionally, patient survival past the initial 12 months of follow up was a prerequisite in order for a study subject to be included in the incident changes analysis for MACE and all –cause mortality. As a result, immortal time bias cannot be excluded.

A further limitation is the acknowledgement that ROC analyses are a better fit for a diagnostic test in an acute presentation setting. The exploratory nature of the work presented here combined with the very high rate of sudden death due to cardiac disease in dialysis patients (i.e. where an in situ diagnostic test would not be viable) means that the approach taken here provides us with useful, if not optimal results.

The MACE event rate may also be understated in these results. Due to change in the way the Office of National Statistics formed partnerships with research teams to provide cause of death data, cause of death data for those patients who died between December 2013 and the study end date in 2016 were not available to us. Some of these deaths are likely to have been of a cardiovascular cause and so event rate is likely to be higher.

Examining the ECG parameters as diagnostic tests has the advantage that it is a pragmatic approach that relates directly to clinical practice and this is the main advantage of this study. In our cohort of haemodialysis patients we demonstrated that the ECG is a very poor diagnostic test with regard to all-cause mortality and MACE; this was the case for both prevalent and incident ECG changes. It has the potential, though, to be used as a “rule out” test ideally in combination with another test with high sensitivity.

Chapter 7

Diagnostic accuracy of electrocardiographic methods of estimation of Left Ventricular Hypertrophy in a haemodialysis population.

Rationale

In this chapter we looked at different ECG methods of estimating left ventricular hypertrophy (LVH) and compared these to more established methods, namely 2D and 3D echocardiography. LVH estimated by echocardiographic methods has been consistently demonstrated to be associated with adverse cardiac outcomes in haemodialysis. In addition, there is some emerging evidence its regression is associated with improvement of outcomes. The ECG is an inexpensive and readily available test. Therefore if an ECG method of LVH estimation was proven to be accurate and reliable that could make it a very valuable screening test.

Abstract

Introduction: Left ventricular hypertrophy is common in end stage renal disease (ESRD) and haemodialysis. Its association with cardiovascular outcomes has been demonstrated.

In this study we evaluated the diagnostic accuracy of electrocardiographic methods of calculating LVH compared to Real Time 3- Dimensional Echocardiogram (RT3DE) which we used as a surrogate gold standard test.

Methods: This study was performed as a post-hoc analysis of a sub-group of patients enrolled into the Salford Kidney Study (SKS). We examined the sensitivity, specificity, positive predictive value and negative predictive value of Sokolow – Lyon and Romhilt –Estes methods in comparison to RT3DE.

Results: The final sample comprised 44 patients. The vast majority were Caucasian (39 patients). The mean age of the patients was 62 ± 13 years and mean time on dialysis 5.1 ± 2.9 years. The sensitivity of both ECG methods for diagnosis of LVH was very low. This was the case for the whole sample and also for individual groups. Romhilt – Estes was marginally better than Sokolow – Lyon and that was especially the case for male patients.

Discussion: Our study shows that ECG methods for assessment of LVH that rely on voltage criteria have very low sensitivity and unreliable specificity compared to RT3DE and also conventional M –Mode echocardiography. As a result they could not be reliably used as a quick and inexpensive method of LVH estimation in clinical practice in the case of haemodialysis patients.

Introduction

Left ventricular hypertrophy (LVH) is common in end stage renal disease (ESRD) and haemodialysis (HD) patients[23]. Up to 70% of dialysis patient have LVH at the initiation of RRT and it is likely to represent the most common cardiac abnormality in this population. Likely pathogenic factors include hypertension, anaemia, over-activation of the renin-angiotensin system, and chronic inflammation. There is emerging evidence of a role of factors associated with CKD-MBD. For example, intravenous injections of FGF-23 into wild type mice result in the development of LVH.

LVH is associated with worse cardiovascular outcomes, demonstrated extensively and consistently in observational studies in ESRD and CKD populations[86,107]. This includes an association with SCD and also all-cause mortality. Importantly, one study has shown an association between regression of LVH over time and improvement in outcome in a dialysis population. This means that LV mass may prove to be a viable treatment target in the future.

For this to happen, a dependable and cost effective method of monitoring LVH is required. There is considerable heterogeneity, however, in the calculation of left ventricular mass and the subsequent cut off values for LVH depending on the imaging method used.

In the haemodialysis population LVH has been traditionally diagnosed using M mode 2D echocardiography, as is typically the case in the general population. Echocardiography is accessible, non-invasive and largely risk free. The main shortcoming of this approach is that inter user variability can be very high meaning that long term monitoring of changes in mass may be imprecise. Electrocardiography (ECG) is the simplest bedside test which can assess for LVH. However, it is both poorly sensitive and poorly specific for LVH detection and there is a significant degree of discordance between ECG and echocardiographic diagnoses of LVH in haemodialysis patients. This means that its use as a basic screening tool for LVH may be limited in this population.

In general, the gold standard for calculation of left ventricular mass is considered to be cardiac magnetic resonance imaging (CMR). Recent studies have, however, demonstrated non inferiority of 3D echocardiography for the assessment of LV volume and mass compared to CMR[108,109]. In addition, echocardiography is a simpler and more easily accessible test. Real time 3-dimensional echocardiography (RT3DE) measures the LV volume directly, without geometric assumptions about the LV shape and hypertrophy distribution (as is the case for standard M mode 2D echocardiography described above). RT3DE has also been shown to have higher reproducibility of results than standard echocardiography[110]. However, up to now there has been a relative paucity of 3D echocardiographic studies assessing LV mass cut off points. This means that there is no official recommendation on how to define LVH using this modality[111]. Alongside this, no previous studies to this date have evaluated ECG and M mode 2D echocardiography in comparison to RT3DE echocardiography in the haemodialysis population.

In this study we evaluated the diagnostic accuracy of electrocardiographic and conventional (M mode) echocardiographic methods of calculating LVH in a haemodialysis population, compared to RT3DE which we used as a surrogate gold standard test. We also compared whether the relative accuracy differed between sub-groups of patients based on phenotypic characteristics such as age, sex and body mass.

Methods

This study was performed as a post-hoc analysis of a sub-group of patients enrolled into the Salford Kidney Study (SKS). Patients included into this analysis are from the sub-group of SKS previously known as the CRISIS-HD cohort, and this is described in detail elsewhere.

As discussed in previous chapters, adult maintenance haemodialysis patients at Salford Royal NHS Foundation Trust, UK or one of its satellite units are considered for inclusion into CRISIS-HD. Exclusion criteria were age under 18 years, home haemodialysis, and non-standard dialysis hours (standard

therapy is 4 hours three times per week). Patients are enrolled into the study if written informed consent is obtained.

Patients who eventually participate in the study undergo detailed cardiovascular assessment including SKS study specific extended protocol echocardiography and electrocardiography on a non-dialysis day during a short, mid-week interdialytic break. Patients also undergo blood sampling and data collection regarding co-morbid conditions, dialysis prescription and medication using self-reported questionnaires and by review of hospital electronic patient records by the study doctors.

The aim of the study was to evaluate the diagnostic accuracy of 2 commonly used ECG methods of diagnosis of LVH in comparison to RT3DE echocardiography. The ECG methods we used were the Sokolow – Lyon and the Romhilt- Estes. These methods were chosen as they can be performed at the bedside and do not need computerised analysis of the ECG as is the case for some ECG based diagnostic criteria such as Novacode. The criteria for the Sokolow –Lyon method are S wave depth in V1 + tallest R wave height in V5-V6 > 35 mm. Romhilt –Estes is a point based method. The criteria are as follows:

- Amplitude of largest R or S in limb leads ≥ 20 mm = 3 points
- Amplitude of S in V1 or V2 ≥ 30 mm = 3 points
- Amplitude of R in V5 or V6 ≥ 30 mm = 3 points
- ST and T wave changes opposite QRS without digitalis = 3 points
- ST and T wave changes opposite QRS with digitalis = 1 point
- Left Atrial Enlargement = 3 points
- Left Axis Deviation = 2 points
- QRS duration ≥ 90 ms = 1 point
- Intrinsicoid deflection in V5 or V6 > 50 ms = 1 point

If the score equals 4, LVH is present with 30% to 54% sensitivity. If the score is greater than 5, LVH is present with 83% to 97% specificity[112].

The method to acquire 2D echocardiographic assessment of left ventricular mass and to determine LVH is set out in the methods chapter (chapter 3).

The gold standard echocardiographic assessment of left ventricular mass using 3DTE was undertaken using a Phillips iE33 (Philips, Andover, Massachusetts, USA) with a 1-3 MHz X3-1 matrix-array transducer. The transducer acquired 4 sets of real-time pyramidal wedge-shaped volumes over 5 seconds during a single breath hold. The apical view was used. These 4 volumes were then used to build up a full single larger pyramidal left ventricular volume. A higher frame rate than with 2D echocardiography was also required (>20frames per second).

I. Statistical analysis

We examined the sensitivity, specificity, positive predictive value and negative predictive value of Sokolow – Lyon and Romhilt –Estes in comparison to RT3DE. Cut off values for LVH using RT3DE were taken from previously published 95% centile upper limits of normal in the general population (n>5000) using cardiac magnetic resonance imaging These values were used in lieu of agreed limits for RT3DE and the published equivalence found between these two gold standard modalities[113].

The sensitivity of a test refers to its ability to correctly identify the patients with the disease. The specificity refers to its ability to identify patients without the disease. An ideal test would be 100% sensitive and 100% specific.

Sensitivity was calculated as:

$$Sensitivity = \frac{True\ positives}{True\ positive\ +\ False\ negative}$$

Specificity was calculated as

$$Specificity = \frac{Tue\ negatives}{True\ negatives + False\ positives}$$

Positive predictive value was calculated as:

$$PPV = \frac{True\ positives}{True\ positives + False\ positives}$$

Negative predictive value was calculated as:

$$NPV = \frac{True\ negatives}{True\ negatives + False\ negatives}$$

The predictive value of ECG LVH, especially with regard to methods that rely on voltage criteria, is influenced by changes in volume status[31]. Additionally, there are sex differences in the electrocardiographic diagnosis of LVH, possibly related to body size and also differences in QRS duration and voltage[114]. For these reasons, we assessed the diagnostic accuracy of ECG LVH methods both in the whole cohort of patients and also in sub-groups of patients. We categorised the patients according to sex (male – female), age (younger and older than 70 years of age), BMI (greater and lower than 25kg/m^2 respectively).

In addition, we then assessed the sensitivity, specificity, positive predictive value and negative predictive value of a conventional echocardiographic method commonly used in the haemodialysis population in order to compare these results with the ones using the ECG methods for our patient sample.

Continuous variables were expressed as mean ± standard deviation for normally distributed data and median plus interquartile range (IQR) for non-normally distributed data. Normality was assessed graphically with Q-Q plots and was subsequently tested with the Shapiro – Wilk test. Out of the variables tested, only haemoglobin and albumin were non-normally distributed.

Secondary analysis using student's t-test was performed in order to compare the means of the LVH versus the non – LVH group. Statistical significance was set at $p< 0.05$. Analyses were performed with IBM SPSS Statistics Version 22.0.

Results

Of the total number of haemodialysis patients who eventually participated in the CRISIS-HD study, 69 had undergone RT3DE echocardiography. Of these 69 patients, 21 did not have an ECG at the same time as the echo and as a result they were excluded. 4 ECG files were corrupted and could not be read; therefore these patients were also excluded. The final sample comprised 44 patients. The vast majority were Caucasian (39 patients). Other ethnic groups seen were Asian = 4 patients and black = 1 patient. Of all the patients, 18 were women and 26 men; 11 had a history of diabetes and 22 a diagnosis of hypertension according to established criteria[115]. The mean age of the patients was 62 ± 13 years and mean time on dialysis 5.1 ± 2.9 years. The mean BMI of the patients was 25 ± 4 kg/m^2 and the mean ultrafiltration volume achieved on each dialysis session was 2.0 ± 0.8 litres. The mean systolic BP was 143 ± 29 mmHg.

The true prevalence of LVH based on RT3DE as a gold standard was 64.4% overall, and 51.8% in males, 83.3% in females, 73.3% in older patients aged >70 years and 60.0% in younger patients.

A comparison of the characteristics of the LVH and non – LVH groups was performed using student t-test for continuous variables and chi square test for independence for categorical variables. There were no statistically significant differences in the characteristics between the two groups when using Sokolow –Lyon method for diagnosis of LVH. There was a statistically significant in-group difference with regard to sex ($x^2 = 6.0$, $p= 0.014$) when using the Romhilt- Estes point based method for diagnosis of LVH and a statistically significant difference with regard to sex ($x^2 = 4.671$, $p= 0.031$) and ethnic group ($x^2= 8.341$, $p= 0.015$) when using RT3DE for diagnosis of LVH.

The full baseline characteristics along with in group comparisons for continuous and categorical variables respectively are presented in tables 7.1 and 7.2.

Table 7.1. Baseline characteristics and in group comparisons (categorical)

	Sokolow - Lyon		**Romhilt-Estes**		**RT3DE**	
	x^2	**p**	**x^2**	**p**	**x^2**	**p**
SEX	2.921	0.087	6.000	**0.014**	4.671	**0.031**
DIABETES	1.136	0.287	2.172	0.141	1.918	0.166
ETHNIC GROUP	3.124	0.210	0.855	0.652	8.341	**0.015**

Table 7.2. Baseline characteristics and in group comparisons (continuous)

Parameter	Total	Sokolow -Lyon			Romhilt -Estes			RT3DE		
		LVH	No LVH	p	LVH	No LVH	p	LVH	No LVH	p
Age	62±13	63±13	58±12	0.198	60±13	64±12	0.344	63±12	59±13	0.259
SBP	143±19	147±21	141±18	0.390	147±25	138±17	0.131	149±19	133±16	**0.007**
HD vintage	5.1±2.9	5.5±3.3	4.3±2.5	0.547	5.7±3.0	4.0±2.3	0.069	4.4±2.4	6.2±3.4	**0.045**
Hb	106±11	108±10	106±12	0.649	106±12	107±10	0.819	104±11	110±11	0.124
Albumin	38±4	37±6	38±3	0.772	37±3	38±3	0.611	37±5	38±3	0.484
UF volume	2±0.8	2.3±1.0	1.8±0.8	0.108	2.0±1.0	1.8±0.6	0.333	2.0±0.8	2.0±0.9	0.948
BMI	25±4	24±3	26±4	0.106	25±4.8	26±3	0.344	26±4	24±3	0.120

RT3DE: Real time 3-dimensional echocardiogram, LVH: Left ventricular hypertrophy, SBP: Systolic blood pressure, HD: Haemodialysis, Hb: haemoglobin, UF: Ultrafiltration, BMI: Body Mass Index

In our cohort of prevalent haemodialysis patients the sensitivity of both ECG methods for diagnosis of LVH was very low. This was the case for the whole sample and also for individual groups. Romhilt – Estes was marginally better than Sokolow – Lyon and that was especially the case for male patients. When using the Sokolow-Lyon method, the sensitivity was just 27% overall and <50% in all sub-groups. The highest positive predictive value was 67%, in older patients. In obese patients the positive predictive value was just 10%. The Romhilt-Estes method performed slightly better. The sensitivity was 52% overall, and was ≥40% in all sub-groups. The highest positive predictive value was in female patients (100%), and was 75% in older patients. Full results are presented in table 7.3.

Table 7. 3. Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of different ECG methods of calculating left ventricular hypertrophy against a gold standard of 3D echocardiography.

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Sokolow –Lyon				
All patients	28	63	28	32
Men only	36	54	45	44
Women only	20	**100**	20	**100**
Age >70 years	18	75	67	25
Age < 70 years	33	58	55	37
BMI> 30 g/m^2	10	10	10	18
BMI<30 g/m^2	37	57	54	40
Romhilt-Estes				
All patients	52	33	28	26
Men only	64	23	47	38
Women only	40	**100**	**100**	25
Age >70 years	55	50	75	29
Age <70 years	50	33	53	31
BMI>30 g/m^2	40	0	67	0
BMI<30 g/m^2	58	43	58	43

BMI: body mass index

However, none of these results are sufficient for ECG to be considered a viable screening tool for LVH. This is further demonstrated when they are compared to the results for M mode 2D echocardiography.

Here, in an assessment of the sensitivity, specificity, PPV and NPV of 2D echocardiography versus the gold standard RT3DE, the overall sensitivity was 90%, overall specificity 100%, positive predictive value also 100%, and negative predictive value 84%.

Sensitivity was ≥80% in all sub-groups. Specificity was 100% in all sub-groups, as was positive predictive value. Negative predictive value ranged from 0% in female patients to 100% in men. The full results for 2D echocardiography are found in table 7. 4.

Table 7.4. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of 2D measurement of left ventricular hypertrophy against a gold standard of 3D echocardiography.

2D echo LV mass adjusted to Ht $^{2.7}$				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All patients	**90**	**100**	100	84
Men only	**100**	**100**	**100**	**100**
Women only	80	**100**	**100**	50
Age >70 years	**91**	**100**	**100**	80
Age <70 years	89	**100**	**100**	86
BMI >30 g/m^2	**90**	**100**	**100**	67
BMI< 30 g/m^2	89	**100**	**100**	88

BMI: body mass index

Discussion

In our cohort of prevalent haemodialysis patients we demonstrated that two of the most commonly used ECG methods of diagnosing LVH perform very poorly when compared to the 3DRTE which we used as a surrogate golden standard. This is even more prominent when compared with the sensitivity and specificity for LVH diagnosis using conventional M mode echocardiograms. This finding is in keeping with previous studies which have demonstrated discordance between ECG and echocardiographic methods of LVH estimation[26]. However this previous study was simply a comparison between ECG and 2D echocardiography and did not include any gold standard measurement.

The ECG methods of diagnosis of LVH we elected to use in this study are the Sokolow – Lyon and Romhilt – Estes. They are both easy to calculate from standard 12 lead ECGs, are easily reproducible and do not require any training or software. These characteristics mean that they could be easily used as a diagnostic test in clinical practice. The disadvantage of using these two ECG methods of LVH calculation is that they both rely on voltage criteria for assessment of LVH. As ECG voltage is influenced by body habitus and changes in volume status their results may be unreliable in changes of volume status and differences in body habitus. This may provide a degree of explanation for the poor performance of these measures in our study. Other factors are very likely to contribute. One simple factor may simply be body habitus. In our study, the effectiveness of ECG was better in patient with a body mass index <30 g/m^2 compared to obese patients.

It was noteworthy that within the subgroup analyses, there were very few useful findings except for a striking specificity and PPV for female patients using the Romhilt-Estes method. Here, both values were 100%. Specificity was also 100% for Sokolow Lyon method in female patients. This indicates that the presence of LVH on an ECG in a female patient should not be ignored and should lead to a diagnostic 2D echocardiography for quantification.

The reason for the apparent sex difference is not clear. It may relate to sex differences in body habitus for the same reasons as set out above, or may reflect a statistical anomaly in a small sample size study.

Converse to this, the performance of standard M mode 2D echocardiography was far superior. Indeed, compared to the gold standard of RT3DE, the sensitivity in the whole population was 90% and specificity 100%. This appears to demonstrate its suitability for LV mass monitoring in the future should this become a standard therapeutic target.

However, to this end and in summary, our study shows that ECG methods for assessment of LVH that rely on voltage criteria have very low sensitivity and unreliable specificity compared to RT3DE and also conventional M –Mode echocardiography and as a result they could not be reliably used as a quick and inexpensive method of LVH estimation in clinical practice in the case of haemodialysis patients. It cannot therefore be recommended that ECG be used as a screening tool for LVH in dialysis patients.

The key limitation of this study is its small sample size. This was due to the limitations in obtaining high quality echocardiographic windows during RT3DE that are necessary to accurately measure left ventricular mass using this modality. RT3DE requires a higher frame rate for image acquisition than 2D echocardiography which is likely to explain some of the reason for the lower numbers of patients with RT3DE results. Alongside this, the RT3DE software uses concurrent ECG to capture the timing of image acquisition by measuring LV dimension relative to the ECG R wave. Artefact from suboptimal ECG tracing may also affect the ability to acquire RT3DE even with appropriate windows.

The option was available to perform ECG versus 2D echocardiography in a larger population of the SKS (a total of 192 cases with sufficiently high quality 2D echocardiographic windows were available, 99 of which had LVH). However, alongside the analysis of ECG versus 2D imaging, we undertook an assessment of the suitability of 2D echocardiography to be a surrogate of the RT3DE gold standard. It was felt most appropriate to undertake all analyses

within the same population for consistency and to draw like for like comparisons.

Nonetheless, it is unlikely that a larger sample would change the trend seen herein to the point that ECG could be seen as viable. What may differ in a larger study is a more accurate comparison of results between sub-groups.

In conclusion, ECG is not suitable to be used as a bedside screening tool for the presence of LVH in a dialysis population.

Chapter 8

The association of plasma KIM 1 with echocardiographic and electrocardiographic parameters in haemodialysis

Rationale

Previous studies have demonstrated an association between KIM-1 and cardiovascular mortality in ESRD populations. This includes work done collaboratively between the Salford Renal Vascular Research Group and peers from the Karolinska Institute. However, these studies have not explored whether this association is retained independently when KIM-1 is included in a multivariate model that also contains other, more established cardiovascular biomarkers. Parallel to the study described here, the work contributing to this thesis has been exploring the utility of biomarkers captured using ECG rather than laboratory samples.

It was felt appropriate to evaluate how combining approaches to biomarker modelling would change the association of each type of biomarker with outcome.

In this thesis, previous chapters have tested whether ECG has the potential to be a useful bedside test for cardiovascular risk assessment. This chapter was therefore an exploratory study to assess whether KIM-1 retains its predictive utility in a model that also contains ECG data *i.e.* whether there is any additional usefulness in adding KIM-1 to a cardiovascular assessment that will already contain pre-existing standard tests such as ECG. Also, by doing this the chapter would also assess whether ECG retains its significance when included in a model with emerging laboratory biomarkers of cardiovascular risk.

Further to this, because the Salford Kidney Study, from which this chapter derives, also contains echocardiographic assessment of patients, the study contained herein also explored KIM-1 in respect of echocardiographic parameters as well as ECG.

Further to the above, the mechanism behind the association of KIM-1 with cardiovascular end points is unclear. Given that the increased cardiovascular risk in ESRD has a very significant component of sudden death and arrhythmia, this chapter explored whether KIM-1exhibits any association or correlation with ECG recorded parameters of abnormal cardiac conduction and arrhythmia. This was with a view to explore the mechanism of its association with poor outcome.

Abstract

Background. Cardiovascular mortality is disproportionately elevated in end stage renal disease (ESRD) but biomarkers with proven use in the general population have limited use in ESRD. KIM-1 is a novel laboratory biomarker of cardiovascular risk, although the mechanism behind its association with poor outcome is unclear. This study explored the association between KIM-1 and electrocardiographic markers in an attempt to establish mechanistic patterns and assess whether the addition of KIM-1 in ECG / echocardiographic models improves their risk prediction potential.

Methods. A prospective single centre cohort study of prevalent haemodialysis patients. Electrocardiography and echocardiography were performed on a mid-week non-dialysis day. KIM-1 assays were measured by electrochemiluminescence on citrated plasma. Correlations were performed between plasma KIM-1 levels and other biomarkers derived from ECG, echocardiography and laboratory data.

In previously published data using MIMICK and SKS data cohort, plasma KIM-1 maintained statistical significance for prediction of cardiovascular mortality in a multivariable Cox regression model adjusted for age, sex, and dialysis vintage. We replicated the methodology in previously published data using MIMICK and SKS data, but with additional ECG and other parameters to determine whether KIM-1 retained significance when included in models alongside important, more established cardiovascular risk measurements.

Results. 186 patients were included. 136 (73.1%) male. Median age 65 years (IQR 20), median time on dialysis 1.8 years (IQR 3.6). Median KIM-1 543.5 ± 320pg/ml. The biomarkers we examined were: BNP, heart rate, PR interval, QTc, QRS axis, T axis, ejection fraction, LVMI, GLS and end diastolic volume. There was no correlation between KIM-1 and any of these.

In a multivariate Cox regression analysis, KIM-1 maintained statistically significant association with cardiovascular outcomes, independent of ECG and echocardiographic biomarkers (HR: 1.456, CI: 1.021-2.077, p: 0.038).

Conclusion. Based on our data, we could not identify a linear correlation between standardised values of plasma KIM-1 and the above electrocardiographic and echocardiographic biomarkers. Additionally, KIM-1 maintained statistical significance in a multivariate model that included ECG and echocardiographic biomarkers.

Introduction

Cardiovascular mortality is disproportionately elevated in end stage renal disease (ESRD) compared to the general population. Cardiovascular risk factors and associated biomarkers that are key to improving outcomes in the general population, such as in dyslipidaemia, have proven to be of limited use in the ESRD population. The search for population specific biomarkers has therefore become a key focus of bench to bedside research in Nephrology.

One candidate biomarker is the kidney injury molecule 1 (kim-1 in rodents, KIM-1 in humans). KIM-1 is a type 1 transmembrane protein which contains an immunoglobulin and a mucin like domain. KIM-1 is also known as Hepatitis A virus cellular receptor 1 (HAVcr-1) as well as T-cell immunoglobulin and mucin domain 1 (TIM-1). It is an apoptotic-cell phagocytosis and scavenger receptor, but its gene expression was found to be significantly up regulated after ischaemic renal injury in rats[116] and KIM-1 protein production was increased in the post ischaemic proximal tubule in other animal studies[116,117]. Several studies in humans have indicated that the release of KIM-1 is increased in the proximal tubule following ischaemic injury[116,118,119]. This, in combination with the fact that KIM-1 is almost absent in the normal kidney, has led to an interest in the potential role of KIM-1 as a novel biomarker for tubular ischaemia.

There has been some interest in the role of KIM-1 beyond acute kidney injury and in particular the association between KIM-1 and cardiovascular outcomes. A recent study that examined 92 cardiovascular proteins measured in plasma by a proteomics assay demonstrated an association between KIM-1 and cardiovascular mortality in two independent cohorts of haemodialysis patients totalling 369 patients (MIMICK and SKS)[120]. Of all candidate cardiovascular biomarkers, KIM-1 was the only one to be independently associated with cardiovascular mortality after correction for dialysis vintage, cardiovascular risk factors and inflammation (hazard ratio per standard deviation increase 1.84, CI 1.26-2.69, p=0.002).

Importantly, the elevated cardiovascular risk in ESRD is mediated predominantly by non-atherosclerotic disease. This includes a very high risk of

arrhythmia and sudden cardiac death, possibly accounting for more than half of all cardiovascular deaths in this population. Approximately 1 in 4 dialysis patients will die suddenly, compared with 11% in the general population. The nature of the arrhythmogenic precipitants remains contentious. In the general population, 80% of sudden deaths are due to ventricular tachyarrhythmia, most often caused by myocardial infarction. However, recent studies in ESRD populations that have used implanted loop recorders have consistently demonstrated that bradyarrhythmia are both highly prevalent and highly pathological in ESRD. Other studies show association with precursors to arrhythmia such as prolongation of the QT interval, PR interval, and QRS duration. These are outlined in significant detail in the narrative review found in chapter 1.

Previous studies demonstrating the association of KIM-1 with cardiovascular mortality in ESRD populations have not explored whether this association is retained independently when KIM-1 is included in a multivariate model that also contains other, more established cardiovascular biomarkers, specifically ECG biomarkers of arrhythmia or aberrant conduction. To this end, it is not clear whether measuring KIM-1 would provide any additional value to a cardiovascular assessment that will already contain pre-existing standard tests such as ECG. Furthermore, there is as yet no clear mechanistic understanding of why KIM-1 demonstrates such a strong association with cardiovascular outcome in this population. Exploring the correlation between KIM-1 values and other measureable cardiovascular abnormalities, such as those found on standard 12 lead ECG, may provide some tentative hypothesis generating information in this regard.

I. Aims

The aims of this study were therefore threefold. Firstly, to explore the correlation between KIM-1 and established and novel ECG biomarkers in a tentative attempt to identify mechanistic processes to explain its association with cardiovascular outcome in haemodialysis patients. Secondly, to determine whether KIM 1 maintained predictive value for cardiovascular death in a multivariate model that also contains other ECG and laboratory biomarkers.

Third, to explore whether the association of KIM-1 with cardiovascular outcome can be more specifically described in respect to types of sub-types of cardiovascular outcome, for example atherosclerosis.

Further to this, because the Salford Kidney Study, from which this study derives, also contains echocardiographic assessment of patients, the study contained herein also explored KIM-1 in respect of echocardiographic parameters as well as ECG.

Methods

I. Study protocol

In this study we used the haemodialysis arm of SKS which consists of incident and prevalent haemodialysis patients who received thrice weekly treatment either in Salford Royal Hospital or in one of its four satellite units between March 2012 and 2014. .Local ethical approval was granted (UK REC 05/Q1404/187) and the study complied with the Declaration of Helsinki. Exclusion criteria were age under 18 years, home haemodialysis, and non-standard dialysis hours (4 hours three times per week).

Patients who participated in the study underwent detailed cardiovascular assessment including SKS study specific extended protocol echocardiography and electrocardiography on a non-dialysis day during a short, mid-week interdialytic break. All 12 lead ECGs were obtained on single visits during a mid-week non-dialysis day. They were performed with the patients in a recumbent position and with standard lead placement using a Philips Pagewriter TC 20 device for ECG acquisition at a sampling rate of 500 Hz.

Transthoracic echocardiography was performed immediately after ECG. All patients were assessed in the left lateral decubitus position, using Philips echocardiography equipment with 3.5 MHz transducers. Measurements were obtained according to published guidelines by the European Society of Echocardiography.

Patients also undergo blood sampling. Standard clinical tests were performed immediately and additional samples centrifuged and plasma and serum stored at -80°C. Samples used for KIM-1 analyses which were measured on citrated plasma by electrochemiluminescence, using the MESO QuickPlex SQ 120 automate from Mesoscale Discovery Systems (Rockville, Maryland, USA). Demographic characteristics and co-morbidities were collected using the hospital's electronic patient records or self-reported by the patients. Follow-up in was from the date of a study protocol echocardiogram until death, transplantation, re-location, or November 16, 2015.

II. Statistical analysis

For baseline data, continuous variables were expressed as mean ± standard deviation for normally distributed data and median plus interquartile range (IQR) for non-normally distributed data. Normality was assessed graphically with Q-Q plots and was subsequently tested with the Shapiro – Wilk test. Statistical significance was set at $p< 0.05$.

Correlations were performed between plasma KIM-1 levels and other biomarkers derived from ECG, echocardiography and laboratory data. Pearson correlation coefficient was used for normally distributed data and Spearman's rank correlation coefficient for non- normally distributed data.

In the previously published study using MIMICK and SKS data cohort[120], plasma KIM-1 maintained statistical significance for prediction of cardiovascular mortality in a multivariable Cox regression model adjusted for age, sex, and dialysis vintage. Cardiovascular mortality was defined as death due to any disease coded as ICD-10 I00 to I99. Follow up was censored at transplantation or most recent dialysis visit.

We replicated this process using the SKS data with additional ECG and other parameters included. The chosen parameters were PR interval, QRS duration, QTc interval, heart rate, atrial fibrillation, LV ejection fraction, $LVMIht^{2.7}$, LV end diastolic volume, global longitudinal strain, and serum NTproBNP. The approach was to perform univariate Cox regression models for each of these variables, and for any where the result of this provided us with $p<0.1$, to then

include that variable in a multivariate model alongside KIM-1, age, sex, and dialysis vintage as per the previous published study and from this determine whether KIM-1 retained significance when included in models alongside important, more established cardiovascular risk measurements.

Finally, we performed additional analyses in our sample to identify whether an association between plasma values of KIM-1 and only atherosclerotic cardiovascular outcomes existed, and again whether this was independent of ECG parameters and echocardiography. We defined an endpoint of ischaemic death which comprised deaths due to myocardial infarction and ischaemic stroke. The rationale behind this was that previous studies have identified potential associations, albeit inconsistent, between *TIMD4-HAVCR1* (otherwise known as KIM-1) and lipid phenotypes and risk of atherosclerotic disease in the form of coronary heart disease and ischaemic stroke[121].

Results

I. Population

186 patients were included in the final analysis. Of these, 136 (73.1%) were male. Diabetes was present in 55 (29.6%), CAD in 48(25.8%), AF in 14 (7.5%), PVD in 18 (9.7%) and CVD in 18 (9.7%). Median age was 65 years (IQR 20) and median time on dialysis 1.8 years (IQR 3.6). The median HR was 73bpm (IQR19), median QRS axis 14 (IQR 49), median T axis 51 (IQR 55). The mean QTc was 415±39ms. The median left ventricular biplane Ejection Fraction (EF) was 62.6% (IQR 13), median $LVMIht^{2.7}$ was 4.7(IQR 3.6) and the median Global Longitudinal Strain (GLS) was -13.6 (IQR 4.9). The mean left ventricular End Diastolic Volume (EDVTeicholz) was 108.9 ml ±35.1. The median value of KIM-1 in our sample was 543.5 ± 320pg/ml. For the purposes of statistical analysis, KIM-1 values were transformed to a mean of 0 and standard deviation of 1. The descriptive statistics for all parameters measured in this study are presented in table 8.1.

Table 8.1 Descriptive statistics

Normally distributed	N	Mean ± SD
QTc	186	415±40ms
EDV	184	108.9±35.1ml
Non-normally distributed	N	Median (IQR)
Age	186	65 (20) years
Dialysis vintage	186	1.8 (3.6) years
Heart Rate	186	73 (19) bpm
QRS axis	175	14 (49) °
T axis	175	51 (55) °
EF	172	62.6 (13) %
LVMI ht $^{2.7}$	185	4.7 (3.6)g/m $^{2.7}$
GLS	181	-13.6 (4.9) %
KIM-1	186	543.5 (320) pg/ml
NTproBNP	181	140 (399) pg/ml
Frequencies	N	%
Male	136	73.1
Diabetes	55	29.6
Smoking	120	64.5
CAD	48	25.8
PVD	18	9.7
CVD	18	9.7
Heart Failure	27	14.5

EDV: End Diastolic Volume, EF: Ejection Fraction, LVMIHt2.7: Left ventricular mass indexed to $Ht^{2.7}$, GLS: global longitudinal strain, KIM-1: Kidney Injury molecule 1, BNP: brain natriuretic peptide, CAD: Coronary artery disease, PVD: peripheral Vascular Disease, CVD: Cardiovascular disease

II. Correlations between plasma KIM-1 and other biomarkers

Using Pearson correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non- normally distributed data we identified no statistically significant correlations between the standardised

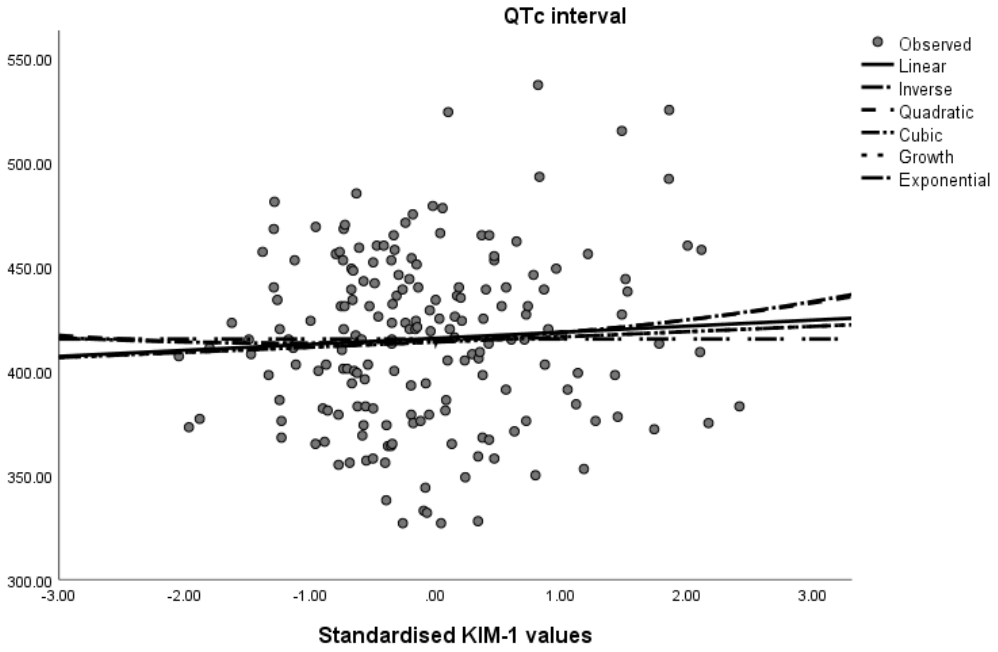
values of plasma KIM-1 and selected electrocardiographic, echocardiographic and laboratory biomarkers.

In detail, the biomarkers we examined were: BNP (rho -0.107, p= 0.187, CI: -0.255, 0.63); HR (rho -.0127, p=0.119, CI: -0.278, 0.33); PR interval (rho 0.144, p=0.075, CI-0.024,0.314); QTc (r 0.062, p=0.448, CI -0.090, 0.212); QRS axis (rho -0.035, p=0.664, CI: -0.193, 0.117); T axis (rho 0.125, p= 0.124, CI: -0.024, 0.274); Biplane LVEF (rho 0.053, p=0.518, CI: -0.001, 0.080), $LVMIHt^{2.7}$ (rho 0.058, p= 0.476, CI: -0.102, 0.203); GLS (rho 0.058, p= 0.478, CI: -0.094, 0.210); EDVTeich (r -0.002, p=0.985, CI: -0.003, 0.082).

Based on our data, we could not identify a linear correlation between standardised values of plasma KIM-1 and the above electrocardiographic and echocardiographic biomarkers.

Following that, we tested for non-linear relationships using curve estimation regression models. The relationships we tested were inverse, quadratic, cubic, growth and exponential. All of these models were a poor fit for our data. Figure 8.1 provides a graphic representation of these results.

Figure 8.1. Correlation between KIM-1 and selected electrocardiographic and echocardiographic parameters



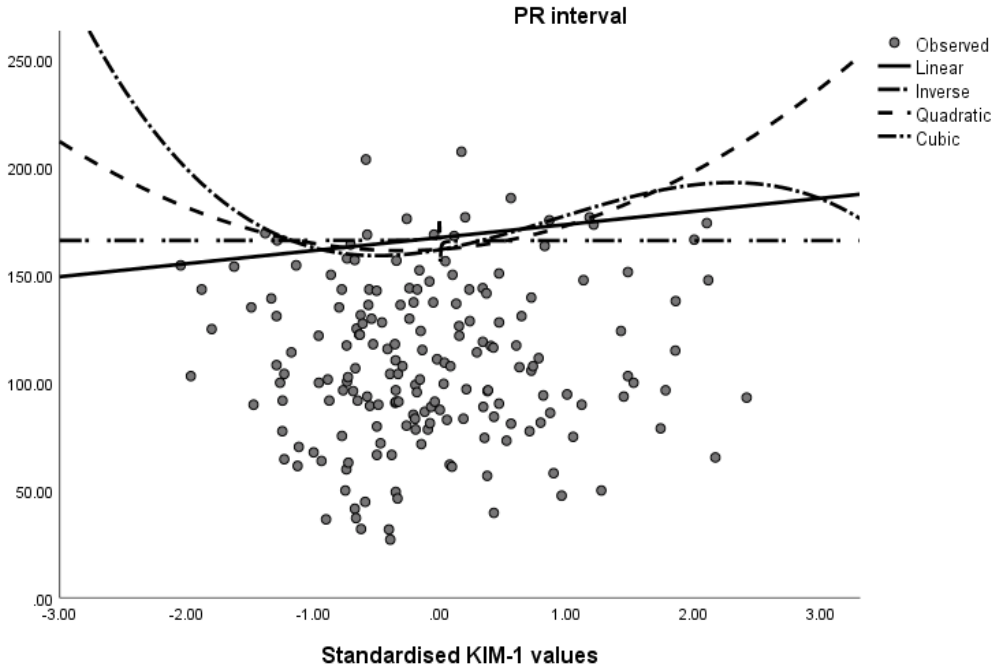
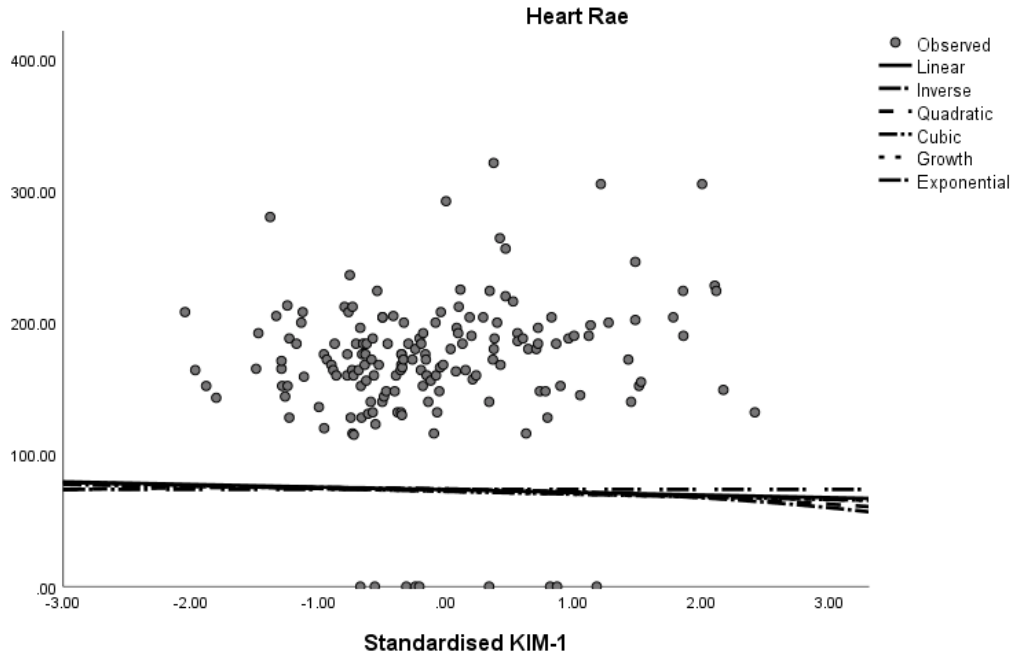


Figure 8.1 (continued). Correlation between KIM-1 and selected electrocardiographic and echocardiographic parameters



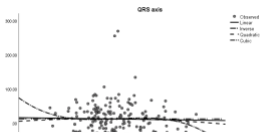
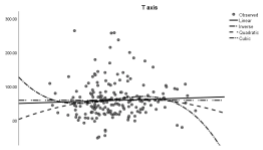


Figure 8.1 (continued). Correlation between KIM-1 and selected electrocardiographic and echocardiographic parameters



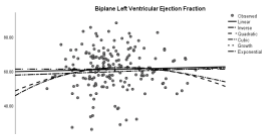
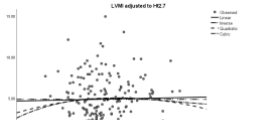


Figure 8.1 (continued). Correlation between KIM-1 and selected electrocardiographic and echocardiographic parameters


LVM adjusted to HGT
Observed
Linear
Inverse
Quadratic
Cubic

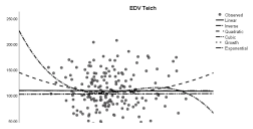
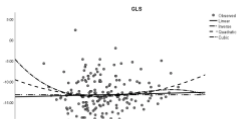

EDV Teich
Observed
Linear
Inverse
Quadratic
Cubic
Growth
Exponential

Figure 8.1 (continued). Correlation between KIM-1 and selected electrocardiographic and echocardiographic parameters



LVMI: Left Ventricular Mass Index, EDV Teich: End Diastolic Volume using the Teicholz method, GLS; Global Longitudinal Strain

III. KIM-1 in multivariate models including ECG biomarkers

In a model (model A) that included age, sex, dialysis vintage and standardised values of KIM-1, KIM-1 maintained independent association with cardiovascular death as previously reported (also in table 8.2) The ECG and echocardiographic biomarkers that showed independent association with cardiovascular death in univariate Cox regression survival analysis were QTc (HR: 1.008, CI: 1.001-1.016, p: 0.028), biplane EF (HR 0.93, CI: 0.930-0.997, p<0.001) and EDV (HR 1.008, CI: 1.000-1.016, p:0.045). Univariate analysis for all ECG and echocardiographic parameters examined is presented in table 8.3.

We then performed multivariate Cox regression analysis adding to the original model the electrocardiographic and echocardiographic parameters that showed

statistically significant associations with cardiovascular mortality in univariate analysis in order to assess whether KIM-1 retains statistical significance in the presence of more conventional cardiovascular biomarkers.

The results are presented in table 8.3. In the multivariate model KIM-1 maintained a statistically significant association with cardiovascular outcomes, independent of ECG and echocardiographic biomarkers.

Table 8.2 Multivariate Cox regression analysis for cardiovascular death adjusted for age, sex and time on dialysis – Model A

Variable	Hazard Ratio	95% CI	P
Age	1.014	0.989 – 1.0390	0.282
Sex	1.442	0.756 – 2.753	0.267
Dialysis Vintage	0.990	0.901- 1.088	0.830
Standardised KIM-1	**1.454**	**1.031 – 2.050**	**0.033**

Table 8.3. A comparison of the univariate association of ECG biomarkers and other measured parameters with cardiovascular mortality

Parameter	HR	95% CI	p
PR (ms)	1.000	0.994-1.006	0.877
QTc (ms)	**1.008**	**1.001-1.016**	**0.028**
ECG strain	0.922	0.548-1.550	0.759
Heart Rate	0.980	0.958-1.002	0.070
AF	0.720	0.466-1.113	0.139
EF biplane	**0.953**	**0.930-0.997**	**<0.001**
EDV	**1.008**	**1.000-1.016**	**0.045**
LVMIHt$^{2.7}$	1.022	0.922-1.133	0.681
GLS	1.026	0.953-1.105	0.489
NTproBNP	1.001	1.000-1.001	<0.001

Key: EF: Ejection Fraction, EDV: End Diastolic Volume, LVMIHt$^{2.7}$: Left Ventricular Mass Index adjusted for Height, GLS: Global Longitudinal Strain, NTproBNP: N- Terminal pro-hormone of Brain Natriuretic Peptide

Table 8.4. Multivariate Cox regression analysis for cardiovascular deaths adjusted for parameters in model A and QTc interval, Ejection fraction and End Diastolic Volume – Model B

Variable	Hazard Ratio	95%CI	P
Age	1.018	0.991-1.046	0.203
Sex	1.882	0.934- 3.789	0.077
Dialysis Vintage	0.997	0.904-1.100	0.959
Standardised KIM-1	**1.456**	**1.021-2.077**	**0.038**
QTc interval	1.005	0.997-1.012	0.228
Biplane EF	**0.954**	**0.930-0.978**	**<0.001**
EDV	**1.010**	**1.001-1.019**	**0.036**

EF: Ejection Fraction, EDV: End Diastolic Volume

IV. Association between standardised KIM-1 values and atherosclerotic endpoints

There were 11 ischaemic deaths in our sample of 186 patients in a mean period of follow up of 907 ±389 days. In a univariate Cox regression survival model, standardised plasma KIM-1 values did not significantly associate with the risk of ischaemic death (HR 0.686, p=0.334, CI: 0.319- 1.474). For the purposes of our analysis, ischaemic death (fatal atherosclerotic events) comprised fatal myocardial infarction and fatal ischaemic stroke. We then performed univariate analyses for the ECG and echocardiographic biomarkers analysed in the section above, in respect of atherosclerotic events rather than cardiovascular events as a whole. Here, the only statistically significant association between these parameters and fatal atherosclerotic events was in the case of NTproBNP (p = 0.005). Because of this limited finding, no multivariate analysis was performed. Full details of the univariate analyses are found in table 8.5

Table 8.5. A comparison of the univariate association of ECG biomarkers and other measured parameters with fatal atherosclerotic events

Parameter	Fatal atherosclerotic event		
	HR	95% CI	p
PR (ms)	1.004	0.992- 1.017	0.502
QTc (ms)	0.993	0.979 – 1.008	0.383
ECG strain	0.626	0.290-1.353	0.234
Heart Rate	1.026	0.984 – 1.068	0.228
AF	4.765	0.029-795.255	0.550
EF biplane	1.002	0.943-1.063	0.959
EDV	0.997	0.981-1.013	0.720
LVMIHt2.7	1.017	0.829-1.247	0.873
GLS	1.108	0.958-1.283	0.168
NTproBNP	1.001	1.000-1.002	0.005

Key: EF: Ejection Fraction, EDV: End Diastolic Volume, $LVMIHt^{2.7}$: Left Ventricular Mass Index adjusted for Height, GLS: Global Longitudinal Strain, NTproBNP: N- Terminal pro-hormone of Brain Natriuretic Peptide

Discussion

In this analysis, there was no correlation between KIM-1 levels and any measured ECG parameter. This means that it is highly unlikely that there is any arrhythmic mechanism underlying the reason for the association of KIM-1 with cardiovascular outcome in this and another haemodialysis population. Further to this, there was also no correlation between KIM-1 and any echocardiographic parameter, or any association between KIM-1 and a fatal atherosclerotic event, meaning that the reason for KIM-1 being associated with cardiovascular risk in haemodialysis patients remains unclear.

Plasma KIM-1 showed promising results with regard to cardiovascular death risk assessment in a previous study in haemodialysis patients[117]. In our cohort, it was associated with cardiac death in a model that did not include other

cardiovascular risk biomarkers, but this association failed to maintain statistical significance when ECG, echocardiographic and laboratory markers of cardiac disease were added. This was despite the fact that plasma KIM-1 did not correlate with any electrocardiographic parameters, echocardiographic markers or plasma BNP. This means that the lack of independent statistical association is not due to a competing effect between KIM-1 and these other measured variables. This could indicate that KIM-1 has the potential for truly independent prognostic capability. This is, however, countered by the loss of statistical significance in the prognostic models that include other cardiovascular parameters such as ECG markers.

Our understanding of the role of KIM-1 in CKD cardiovascular risk prediction remains very limited despite the recent advances in understanding the role of KIM-1 in the development of tubular damage in AKI[117,119] and the progression of AKI to CKD[122,123]. Our results are a reflection of the poor understanding of the mechanisms and roles of KIM-1 in haemodialysis.

It might therefore be plausible that the biomarkers we elected to correlate KIM-1 against were not the right ones. To balance against this view, persistently searching for correlations carries a risk of false positive results, even after Bonferroni correction.

On this theme of potential statistical error, that our results do not support the hypothesis that plasma KIM-1 associates with atherosclerotic events could be explained by the small number of such endpoints. Although the overall cardiovascular event rate (atherosclerotic and non-atherosclerotic) was very high, non – atherosclerotic endpoints predominate. However, results from animal studies have identified a potential link between lipids, atherosclerotic events and KIM-1 expression, and a human study in a Chinese population suggested that variants of *KIM-1* gene (referred to by the name *HAVCR1* - hepatitis A virus cellular receptor 1 in the paper) may be associated with increased risk of coronary heart disease and ischaemic stroke[121]. In another general population study, elevated KIM-1 demonstrated correlation with a number of traditional cardiovascular risk factors, such as blood pressure, cholesterol, age, and smoking[124]. In the same study, there was no relationship

between KIM-1 and either creatinine or cystatin C. The study did not explore the correlation between KIM-1 and ECG abnormalities. Therefore, this area is potentially worth re-evaluating when more atherosclerotic end points have occurred (i.e. after a longer follow up period of SKS) or by collecting data on non-fatal atherosclerotic events from this point.

Although not directly related to the aims of this chapter, there were noteworthy differences in the association between ECG parameters and all-cause cardiovascular deaths versus atherosclerotic deaths, albeit in a series of univariate analyses, they are pertinent to the overall themes of this thesis. In particular, of the electrocardiographic markers, QTc duration was significantly associated with cardiovascular deaths in general, but not atherosclerotic deaths (HR: 1.008, p: 0.028, CI: 1.001-1.016 and HR 0.993, p: 0.383, CI: 0.979-1.008, respectively. This could be explained by the hypothesis that the cardiovascular mortality in ESRD is mainly non-atherosclerotic, and in fact associated with conduction abnormalities. Similarly, for echocardiographic variables, we noted that Ejection Fraction and End Diastolic Volume showed a significant association with cardiovascular, but not atherosclerotic mortality. The likely reason for these differences could be that echocardiographic parameters reflect anatomical changes in the heart and could therefore be associated with heart failure and fibrotic damage of the myocardium leading to death, than atherosclerotic mortality.

Chapter 9

Evaluation of findings

Introduction

This final chapter of the thesis will provide a summary and critical evaluation of the findings of the studies in the previous chapters in the context of the aims of this thesis and also discuss plans for potential studies arising from this work.

The aims of the thesis were to assess the prognostic value of electrocardiographic markers of heterogeneity of repolarisation in haemodialysis patients, whether the presence of ECG strain has prognostic value independent of LVH and whether the combined use of echocardiography and echocardiography in assessing LVH in ESRD can provide improved risk stratification.

Chapter 1: *Cardiovascular disease in Chronic and End Stage Renal Disease; an overview with particular focus on the use of electrocardiography*

This chapter provided a brief overview of cardiac disease in chronic kidney disease (CKD) with particular focus on end stage renal disease (ESRD), reviewing the current hypotheses for the development of cardiac disease in renal patients. Following that, it examined the literature regarding conventional and novel ECG parameters and their association with cardiac risk in CKD and dialysis. A major limitation on their use in dialysis is their inconsistency and lack of reproducibility, especially in the case of the ones whose measurements are affected by fluid and electrolyte shifts (QT, PR interval, and some of the methods of LVH calculation). LVH calculation

methods, such as Novacode, that do not utilise voltage criteria are more reliable; however they require software interpretation of ECG which makes their use in everyday practice challenging.
Novel methods, as the QRS –T angle, have shown promising results in small trials. One of the limitations in their use is the lack of standardization of the measurements. In fact, the definitions of abnormal QRS – T angle vary significantly depending on the method of calculation used.

Chapter 4: *Repolarization heterogeneity and cardiac risk in prevalent heaemodialysis patients*

Chapter 4 evaluated a novel electrocardiographic marker, QRS –T angle, which in our study was expressed as TCRT and its association with cardiac outcomes and all-cause mortality. We demonstrated association with cardiac outcomes, but not with all-cause mortality. The advantage of using TCRT as opposed to other methods of QRS-T expression is that it can be derived from standard 10 second ECGs which are routinely used in everyday clinical practice. The main limitation in extrapolating these findings to the whole haemodialysis population is that our study sample consisted of prevalent haemodialysis patients; therefore survival bias cannot be excluded. Some consideration should also be given to the potential practical limitations of using TCRT in clinical practice. While it can be calculated using relatively easy methods, such as the Dower inverse matrix we used in our study, automatic calculation is not routinely included in the software that most standard ECG machines use. Therefore, use of TRCT in clinical practice would require some familiarity of the operators with the conversion methods.

Chapter 5. *Strain repolarisation on ECG, not left ventricular mass, associates with cardiovascular outcome in a prospective cohort of haemodialysis patients*

In chapter 5 we examined whether adding electrocardiographic criteria of LVH to an echocardiography based model would improve the prognostic

ability of that model. In our cohort of prevalent haemodialysis patients we demonstrated that it LVH strain rather than the presence of LVH itself correlates with cardiac outcomes after adjustment for other risk factors as diabetes, age, prior history of cardiovascular disease. This finding is in keeping with results in general population and also CKD patients. The possible significance of this finding in the haemodialysis population is that it points to potential mechanisms by which LVH leads to adverse outcomes in haemodialysis patients. In particular, it supports the hypothesis that aberrant conduction because of fibrotic myocardial remodelling plays a main role, as opposed to left ventricular mass. This is also supported by the finding that approximately 10% of the patients in these cohorts were noted to have heart block or other bradyarrhythmia.

Chapter 6. *The use of ECG parameters for diagnostic test evaluation of future cardiac events in haemodialysis patients*

In chapter 6 we assessed the ECG biomarkers used in previous chapters as diagnostic tests beyond any individual statistical significance. The overarching aim was to evaluate whether an ECG based model can be reliably used as a diagnostic test for pending adverse cardiovascular outcomes in haemodialysis patients. Our results showed very low sensitivity and AUC values on the ROC curves of all of them. This means that in our cohort of haemodialysis patients ECG biomarkers were overall very poor as a screening tool for cardiovascular outcomes and all-cause mortality. Limitations to the study include the sample size and the potential for there to be missing MACE event data because not all fatal MACE events will be captured following the cessation of cause of death data from the Office of National Statistics during the follow up period of this study.

Conversely, the specificity of the ECG parameters was high, especially in the case of incident and de novo changes. This could theoretically indicate that ECG parameters may have some use as a “rule out” test if used in conjunction with another diagnostic test with high sensitivity.

Chapter 7. *Diagnostic accuracy of electrocardiographic methods of estimation of Left Ventricular Hypertrophy*

The aim of chapter 7 was to assess the diagnostic accuracy of two of the most commonly used methods of electrocardiographic diagnosis of LVH, Sokolow-Lyon and Romhilt-Estes. In our cohort of prevalent haemodialysis patients the diagnostic accuracy of both of these methods was found to be very low compared with RT3DE and even conventional 2D echocardiogram. As a result, despite the fact that they are a quick and inexpensive method of LVH estimation in clinical practice, their use cannot be recommended for screening in the haemodialysis population.

Chapter 8. *The association of plasma KIM 1 with echocardiographic and electrocardiographic parameters in haemodialysis*

This chapter assesses whether KIM-1 retains its predictive utility in a model that also contains ECG data. Plasma KIM-1 showed promising results with regard to cardiovascular death risk assessment in a previous study in haemodialysis patients. In our cohort, it was associated with cardiac death in a model that did not include other cardiovascular risk biomarkers, but this association failed to maintain statistical significance when ECG, echocardiographic and laboratory markers of cardiac disease were added. In addition, in this analysis, there was no correlation between KIM-1 levels and any measured ECG parameter. This means that it is highly unlikely that there is any arrhythmic mechanism underlying the reason for the association of KIM-1 with cardiovascular outcome.

Summary

The overarching aim of this thesis was to assess the prognostic value of conventional and novel electrocardiographic biomarkers with regard to all-cause mortality and cardiac outcomes in the haemodialysis population. The hypothesis was that ECG markers of repolarisation and autonomic imbalance

would correlate with outcomes and would provide some insight into the pathogenesis of cardiac morbidity in the haemodialysis population. In addition to this, we hypothesised that in that case, the ECG would have the potential to be used as a valuable screening tool in haemodialysis.

Our results do not support the above hypotheses. Individual ECG markers demonstrate variable degrees of association with cardiac outcomes in particular group of patients, but this association is not reliably reproduced and often ceases to exist with the addition of other risk factors. Additionally, the sensitivity and positive predictive value of ECG biomarkers has been low making ECG unreliable as a screening test in haemodialysis especially when compared with the much improved sensitivity of echocardiographic biomarkers.

A distinction needs to be made between traditional and novel biomarkers; novel biomarkers, especially ones of repolarisation heterogeneity have shown promising results with regard to prediction of cardiac outcomes. However, their use in everyday practice is for the time being limited by the fact that their automatic calculation is not included in the software of a standard ECG machine, and their calculation depends on the familiarity of the operator with conversion matrices.

The high specificity for cardiac outcomes that some of the ECG parameters have demonstrated could mean that there still might be a role for ECG as a "rule out" test in haemodialysis, meaning that if it is completely normal the clinician may deduct that the risk of cardiac adverse outcomes is low.

Potential future work

Were we ever able to re-establish links to ONS cause of death data, we would be in a position to re-evaluate the MACE end point rate and potentially repeat the statistical analyses with MACE as an outcome with the inclusion of any new end points. This may provide the studies with more power to draw conclusions and also remove some bias and error which may be present due to missing data.

The potential for future follow up studies may vary between traditional and novel ECG biomarkers. Emerging measurements such a HRV, QT, TCRT dynamics, HRT, DC etc as discussed in chapters 1 and 4 are at a stage of relative infancy in our understanding of them. In their current form, proprietary software is needed and reference values are not universally agreed but were these barriers to be overcome, a repeat study following the approach taken in chapter 6 could be considered with the inclusion of these new biomarkers.

Chapter 5 details that ECG strain in association with LVH may be a more suitable therapeutic target than LVH itself. However, this is without a full understanding of whether regression of LVH through management of blood pressure, volume status and other contributory pathologies will also lead to improvements in the conduction pathway. Previous studies have shown the ability to improve LVH in dialysis patients and a future study could be undertaken to establish wither this also leads to improvements in cardiac conduction. Additionally, in our cohort of patients the prevalence of ECG strain was low. That could be reflective of the small number of patients in our study overall. A repeat validation study in a larger cohort of patients should be considered.

As detailed in the discussion in chapter 6, the ROC methodology used was not optimal for predicting future events. Approaches to build on the pilot data it provides could be through a more concentrated study undertaking very frequent ECG to evaluate the setting in which new ECG changes emerge. This would be very feasible given that patients attend hospital for dialysis thrice weekly. Alongside this, with greater granularity of baseline ECG, were patients to then present to hospital with a new symptom burden (e.g. syncope, presyncope, new breathlessness), any new changes that occur with the onset of these symptoms could be determined.

A follow on study to the work described in chapter 7 would be viable with greater recruitment. Given the challenges of obtaining high quality 3D echocardiographic windows, this would need to be a multicentre study. However, the findings within the female population do indicate some potential

use, or at least potential for a better understanding of how to interpret ECG LVH if a larger scale study were to be performed.

The results in chapter 8 show that in a model including mixed methods of capturing biomarkers (ECG, echocardiography, laboratory tests, there is potential for each of them to provide added benefit. Possibly because of the diversity of both the cardiovascular risk factors and the mechanisms for death in ESRD patients, future studies could follow a different approach, one that focuses not on the utility of a single tool. Instead, perhaps a full cardiovascular assessment using a multitude of assessment tools, including echocardiographic, biochemical biomarkers may prove better in cardiovascular risk prediction than ECG alone.

Chapter 10

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