The Effect of Circulatory Dysfunction upon the Liver in Haemodialysis Patients

Claire J. A. Grant, BMedSci, BMBS(Hons), MRCP(UK)

Student ID: 4206909

Thesis Submitted to the University of Nottingham

for the degree of Doctor of Philosophy

December 2018

Table of Contents

Abstract 9

Publications and Abstracts arising from this work 11

Acknowledgements 13

Declaration 16

Index of Tables and Figures..... 17

Tables 17

Figures 19

List of commonly used abbreviations 27

Chapter 1: Introduction and Literature Review..... 29

1 Chronic Kidney Disease 30

1.1 The Renal System in Health..... 30

1.2 Stages of Chronic Kidney Disease (CKD) 32

1.3 Complications of end-stage disease 32

2 HD in Chronic Kidney Disease 35

2.1 Introduction 35

2.2 Prevalence 36

2.3 Mortality 37

2.4 The challenges of HD..... 37

3 Circulatory Stress in HD 39

3.1 The effects of ESRD on the cardiovascular system 39

3.2 The haemodynamic effects of dialysis and IDH..... 40

3.3 Dialysis Associated Organ Injury 45
3.3.1 The heart 45
3.3.2 The brain 46
3.3.3 The kidneys 46
3.3.4 The gut and liver 48
4 The hepato-splanchnic system in circulatory stress 49
4.1 The Intestine and the Splanchnic Circulation 49
4.1.1 The intestinal circulation 50
4.1.2 Gut blood flow in other forms of circulatory stress 52
4.1.3 Gut blood flow regulation during fasting and feeding . 54
4.2 The Liver and the Hepatic Circulation 56
4.2.1 Introduction 56
4.2.2 Liver Cell Function 57
4.2.3 The Hepatic Circulation 59
4.2.4 The Hepatic Circulation in Circulatory Stress 64
4.3 The Intestine, Liver and Circulatory Stress in HD 67
5 The uremic toxin burden in dialysis patients 73
5.1 The impact of the intestine and liver upon toxin accumulation in HD patients 75
5.2 The impact of uremic toxins upon the gut and liver in HD patients 77

5.3 The potential link between circulatory stress and uremic toxins 78
6 Dynamic Imaging of the Hepato-splanchnic System 79
7 Hypothesis and Aims 81
7.1 Formulation of the hypothesis 81
7.2 The hypothesis 81
7.3 Aims 82
Chapter 2: Liver Stiffness in HD Patients – the effects of dialysis 83
1 Introduction 85
2 Methods 88
2.1 Study Design 88
2.1.1 Screening and Consent: 88
2.1.2 Eligibility 89
2.2 Study Procedures 89
2.2.1 Collection of personal characteristic data 90
2.2.2 Physical Examination 90
2.2.3 Blood tests 90
2.2.4 Measurement of Liver Stiffness 91
2.2.5 Bioimpedance Analysis 94
3.1.1 Participant Withdrawal 97
3.2 Outcome Measures 98
3.3 Data, Safety and Specimen Monitoring 98

3.3.1 Data monitoring 98
3.3.2 Patient Blood Samples 99
3.3.3 Safety monitoring 100
3.4 Analysis Plan and Sample Size Calculation 101
3.4.1 Analysis Plan 101
3.4.2 Sample Size Calculation 102
3.5 Ethics 103
4 Results 104
4.1 Study Participants 104
4.2 Liver stiffness 106
4.3 Haemodynamic Instability 114
4.4 Gamma Glutamyltransferase 117
5. Discussion 123
5.1 LS and hydration status 123
5.2. Change in LS after dialysis 124
5.3 Association of GGT with LS 126
5.4 Limitations 127
6 Conclusion 129
Chapter 3: Liver Congestion and Fluid Removal in HD Patients 130
1 Introduction 132
1.1 Hypervolemic stress in ESRD 132
1.2 Existing tools for measuring volume excess 134

1.2.1 Physical examination 134
1.2.2 Bioimpedance analysis 134
1.2.3 Measurement of IVC size 135
1.3 Study Rationale 135
1.4 Study Aims 136
2 Methods 137
2.1 Study Design 137
2.2 Ethical Review and Consent 137
2.3 Eligibility 138
2.4 Study Procedures 138
2.5 MRI Data Acquisition 139
2.6 Bioimpedance Measurement 142
2.7 Blood Sampling 142
2.8 Statistical Analysis 142
2.9 Data Recording and Storage 143
3 Results 145
3.1 Study Population and Dialysis Treatment 145
3.2 Pre-dialysis hydration and organ congestion 148
3.3 Dialysis-related changes in organ congestion and hydration markers 157
4 Discussion 167
4.1 Liver water content and hydration markers 167

4.2 Change in liver water with HD 169
4.3 Limitations 170
5 Conclusion 172
Chapter 4: Evaluation of the effects of HD upon hepatic perfusion and function 173
1 Introduction 175
2 Methods 177
2.1 Summary 177
2.2 Study Population 178
2.3 Dialysis Treatments 178
2.4 CT Assessment of Liver Perfusion 179
2.4.1 Basic Principles of CT perfusion imaging 179
2.4.2 CT perfusion imaging in the liver 181
2.4.3 Image acquisition for this study 184
2.5 CT Assessment of Intestinal perfusion 187
2.6 Measurement of ICG Clearance 188
2.7 Echocardiography 191
2.8 Blood sampling 194
2.9 Uraemic Toxin Measurement 194
2.10 Assessment of Symptom Burden 198
2.11 Statistical Analysis 201
3 Results 202

3.1 Study Population and Dialysis Treatment.....202
3.2 Hepatic Perfusion and Function during Dialysis.....205
3.3 Mesenteric perfusion 220
3.4 Relationship of hepato-splanchnic changes to dialysis-related organ injury.....222
3.5 Changes in uraemic toxin concentration 226
3.6 Symptoms and subjective experience 229
3.7 The effects of eating.....229
4 Discussion 231
Limitations 236
5 Conclusion.....239
Chapter 5: Overall Discussion.....240
1 Summary of Results 241
2 The liver and circulatory stress: the effects of fluid overload.242
3 The liver and circulatory stress: the acute effects of HD.....244
4 Clinical Significance 248
5 Future Work 250
6 Conclusion.....252
Appendices 253
References 255

Abstract

Intermittent haemodialysis (HD) leads to recurrent circulatory stress which causes subclinical ischaemic injury and cumulative dysfunction in a variety of vulnerable organs. The effects of HD upon the liver have never been explored in detail. However, the liver is a haemodynamically active organ and plays a key role in the chronic inflammatory response modulation, as well as host defence against infection.

This project was designed to study the effects of both volume overload and circulatory stress, both characteristic of HD, upon liver haemodynamics in HD patients using serial imaging. The aims of the project were to evaluate the hepatic haemodynamic response to dialysis and explore the hepatic consequences of HD-induced circulatory stress. Three studies were conducted. The first examining the effect of dialysis upon liver stiffness, the second examining hepatic water content before and after dialysis, in comparison to overall fluid status and peripheral fluid shifts, and the third examining hepatic perfusion serially during dialysis.

Hepatic congestion does not appear to be prevalent in HD patients even in the presence of significant fluid overload. However, HD

acutely affects hepatic perfusion and appears to lead to a degree of functional compromise. Perturbation of regional haemodynamics is associated with intra-dialytic haemodynamic stability and uraemic toxin clearance. There was considerable heterogeneity in the observed effects of dialysis, which appeared to relate to vulnerability to systemic circulatory stress.

The liver is affected by the circulatory stress of dialysis. Physiological mechanisms to preserve central circulating volume appear intact in HD patients. However, microcirculatory dysfunction and temporary functional compromise within the liver may impact upon the evolution of cardiac injury and toxin exposure in HD patients. Further work is needed to assess the full impact of hepatic disturbance in HD patients and its overall clinical significance.

Publications and Abstracts arising from this work

Publications

- The hepato-splanchnic effects of chronic hemodialysis. C Grant, SH Huang and CW McIntyre. (in press, Seminars in Dialysis 2019)

- Effect of Ultrafiltration During Hemodialysis on Hepatic and Total-body Water: An Observational Study. C Grant, T Wade, C McKenzie, G Filler, CW McIntyre and SH Huang. BMC Nephrology 2018 (accepted 23/11/2018, in press).

- Does hemodialysis affect the delivery of uremic toxins into the systemic circulation? The effects of circulatory stress upon hepato-splanchnic perfusion. C Grant, R Marants, T-Y Lee, N Tonial, T Velenosi, B Urquhart and C W McIntyre (submitted for publication CJASN).

Abstracts

- The hepatic response to hemodialysis: consequences for uremic toxin exposure and multi-organ injury. C Grant, R Marants, N Tonial, T Velenosi, B Urquhart, HS Leong, TY Lee and CW McIntyre. ASN Meeting 2016

- Paradoxical fluid diversion into the hepatic circulation in hemodialysis. C Grant, T Wade, C McKenzie, G Filler, A Sawant, CW McIntyre and SH Huang. ASN Meeting 2016

Acknowledgements

I would like to thank the many people who have helped me with this thesis. Some have played a large part, others small, but without each of them this piece of work would not be what it is. I am privileged to have been able to work with so many dedicated and bright people.

Thank you to everyone who has collaborated with me on these projects. Thank you to Professor Ting-Yim Lee and Dr Charles McKenzie for their direction and oversight. Thank you to Jarrin Penny, Tanya Tamasi, Jennifer Hadway, Tony Wales, Sal Treesh, Trevor Szekeres and David Reese for their assistance with study procedures. Thank you to Nicholas Tonial, Dr Thomas Velonosi and Dr Brad Urquhart for their laboratory work. Thank you to Ranaan Marants and Trevor Wade for their help with imaging analysis.

Thanks must also be given to the nursing staff on each of the dialysis units who often allowed me to disrupt their schedules for the sake of a study and always demonstrated interest in what we were doing.

To the patients who willingly gave their time to help me, I owe many thanks. Their motivation in sacrificing their time was always to try to

help improve care and symptom control for other dialysis patients. I am very grateful for their patience, humour and encouragement.

I must thank Dr Susan Huang for her support, direction and friendship during my time in Canada and beyond. Thank you for all the coffee, laughs, help with data collection and equipment transport. There are not many doctoral students who have been driven round with their equipment in a Porsche!

Thank you to Professor Maarten Taal for being my official supervisor and an encouraging mentor. I know stepping in halfway through wasn't easy, but many thanks for all the support.

To my supervisor, Professor Chris McIntyre, I owe much. Thank you for first approaching me to consider research and for the many opportunities which followed as a result. Thank you for including a non-nephrologist in your research group, encouraging me not to pursue "vanilla" and for all the support in moving to Canada. It has been a privilege to work as part of your team.

Finally, I will try to attempt thanks to my mother who has been unfailing in her encouragement and support, not just in this period of

study but throughout my entire life. Her dedication to learning has stimulated my scientific curiosity and her compassion taught me how to care for others. In thanking her, I must acknowledge my father who left us too soon but had already inspired me to pursue a caring career in medicine. I dedicate this thesis to them.

Declaration

I declare that this thesis is the result of work done whilst registered as a student of The University of Nottingham for a PhD. The studies were conducted in London, Ontario, Canada during a research fellowship with Western University. This work has been carried out by myself except where explicitly stated in the text and at the beginning of each results chapter.

Index of Tables and Figures

Tables

Table 1: GFR categories in CKD [1] 32

Table 2: Uremic Toxins implicated in cardiovascular disease in CKD (from Evenepoel et al. [87]) 74

Table 3: Sample size calculation for estimated effect size 103

Table 4: Study population characteristics 105

Table 5: Subjects' Dialysis Characteristics 106

Table 6: Liver stiffness and markers of hydration 109

Table 7: Characteristics according to liver stiffness response to HD113

Table 8: Relationship of GGT with markers of hydration 120

Table 9: Patient Characteristics (n=14) 147

Table 10: Pre-dialysis characteristics including measures of hydration 150

Table 11: Relationship between pre-dialysis liver water content and measures of hydration.....155

Table 12: Session Characteristics.....157

Table 13: Characteristics of those who demonstrate increased liver water content with ultrafiltration.....163

Table 14: UF volume, peripheral and hepatic water content.....165

Table 15: Demographic Information and Medical History of Study Subjects (n=15).....203

Table 16: Summary of delivered dialysis therapy 204
Table 17: Relationship between volume status and pre-HD hepatic blood volume 205
Table 18: Total liver blood flow before, during and after HD. LBF= liver blood flow. Shaded subjects ate after 1 hour but before 3 hours of dialysis. 207
Table 19: Statistical comparison of the distributions of overall, arterial and portal-derived perfusion compared to pre HD (all available ROIs included) 210
Table 20: Mesenteric perfusion before, during and after dialysis. The top table shows the absolute bowel perfusion, whereas the bottom shows relative change. Subject 3 (shaded) ate between 1 and 3 hours of HD. 221
Table 21: Measured uraemic toxins concentrations before and after dialysis.....228

Figures

Figure 1: Schematic representation of the nephron 31

Figure 2: Major consequences of ESRD 33

Figure 3: Schematic of the HD process (from NIDDK [2]) 35

Figure 4: Fluid Kinetics in HD 41

Figure 5: The pathophysiology of IDH (from Reeves et al. [20]) 43

Figure 6: The structure of a liver lobule (simplified schematic) 59

Figure 7: Liver microarchitecture, the oxygen gradient and metabolic zonation (from Kietzmann [61]) 61

Figure 8: From Millonig et al. 2010: The relationship between central venous pressure and liver stiffness [64] 64

Figure 9: Mechanisms limiting the entry and dissemination of LPS into the circulation (from Guerville et al. 2016 [71]) 67

Figure 10: A Fibroscan® machine and probe 92

Figure 11: Example of Fibroscan® data output 93

Figure 12: Graph to illustrate the agreement between gold-standard isotope dilution calculations of extracellular water (ECW ref) and bioimpedance derived measurement (ECW BCS) from Moissl et al. [106] 95

Figure 13:BCM®, Fresenius Medical Care, Bad Homburg, Germany 96

Figure 14: Distribution of liver stiffness pre-dialysis in HD patients, showing all but one patient with LS<10kPa. 107
Figure 15: The relationship between E/I ratio and liver stiffness, demonstrating a significantly lower E/I ratio in those with higher LS. The top panel includes the extreme LS outlier, bottom panel excludes the outlier. 110
Figure 16: Change in liver stiffness after HD, demonstrating the bidirectional response. The top panel is a boxplot of the group change; for the bottom panel, each line is individual change in LS with HD.....112
Figure 17: Boxplots to demonstrate the change in liver stiffness in subjects with IDH and those without. Although there is no statistical significance, a higher proportion of those with IDH exhibited a rise in LS post HD. 116
Figure 18: Pre-dialysis GGT is higher in those with higher liver stiffness. This figure excludes the extreme outlier (n=44) 118
Figure 19: Post-HD GGT correlates with liver stiffness. This figure excludes the extreme outlier (n=44) 119
Figure 20: Pre HD fluid overload is associated with a lower GGT....121
Figure 21: Boxplot to demonstrate the lack of relationship between change in liver stiffness and pre-dialysis GGT. Here both dots and asterisks represent outliers. 122

Figure 22: Example of MR images acquired in the abdomen pre HD 141
Figure 23: The positive association between weight and bioimpedance-derived measure of excess fluid (n=14) kg = kilograms; L=litres 149
Figure 24: The relationship between pre-dialysis thigh water content and total body water (n=11), demonstrating a non-significant trend towards higher peripheral water content with higher TBW. L= litres; g/mL = grams per millilitre) 151
Figure 25: The relationship between pre-dialysis thigh water content and diastolic BP, demonstrating significantly higher DBP with higher peripheral water content (mmHg = millimetres mercury; g/mL = grams per millilitre) 152
Figure 26: The relationship between pre-dialysis thigh water content and intracellular water content, demonstrating a significant association between higher thigh water content and intracellular water. L= litres; g/mL = grams per millilitre) 153
Figure 27: Plot showing the variability in pre-dialysis hepatic water content. Each bar represents a separate subject. g/mL = grams per millilitre.....154
Figure 28: The relationship between pre-dialysis liver water content and IVC diameter, demonstrating that those with higher IVD diameter

had a significantly lower hepatic water content. The dotted line represents an IVC diameter above 2cm, typically used as a marker for significant volume overload. 156
Figure 29: Boxplots to demonstrate lack of significant change in peripheral muscle water content with dialysis (n=11, p=0.55; g/mL = grams per millilitre) 158
Figure 30: The lack of relationship between UF volume and change in liver water content (n=14). The dotted line indicates where there is no change in liver water content. 160
Figure 31: The lack of relationship between pre HD IVC diameter and change in liver water content after HD. n=14, cm = centimetres, g/mL = grams per millilitre. 161
Figure 32: Relationship between pre-dialysis liver water concentration and change post-dialysis 164
Figure 33: The relationship between nadir BP and IVC diameter, demonstrating that those with higher IVC diameter pre HD were significantly less likely to suffer IDH (n=14). 166
Figure 34: From Stewart et al.[99], Time density curves generated from the aorta, portal vein and liver tissue 181
Figure 35: Pictorial representation of deconvolution-based CT perfusion. (a) Dynamic contrast-enhanced CT scan with aorta (arterial input), portal vein (venous input) and liver parenchyma

outlined in red, blue and orange, respectively. (b) Deconvolution equation.....183
Figure 36: Simultaneous CT perfusion imaging and HD (image shared with the subject's permission) 184
Figure 37: CT perfusion imaging of the abdomen using dynamic contrast imaging 185
Figure 38: Example of bowel ROI and generated perfusion map 187
Figure 39: From Vos et al. [150]. Example of a typical ICG indicator-dilution curve. ICG = indocyanine green; CICG = ICG blood concentration. A: primary peak, B: secondary peak (re-circulation phase), C: (hepatic) elimination phase. The shaded grey area represents the area under the primary curve, allowing cardiac output calculations.....189
Figure 40: DDG-2001 Pulse Dye Densitometer (Nihon Kohden, Tokyo Japan) 191
Figure 41: Types of left ventricular myocardial wall strains, from Blessberger and Binder, 2010 [154].....192
Figure 42: Example of speckle-tracking of the left ventricle to allow calculation of segmental longitudinal strain (from Marwick et al. [155]).....193
Figure 43: Boxplot to demonstrate the change in hepatic blood volume throughout dialysis, demonstrating a significant fall after 3

hours and post HD. Here dots and asterisks represent outliers (n=15) 208
Figure 44: Boxplot to demonstrate the change in mean transit of hepatic blood during dialysis, again demonstrating a fall throughout dialysis, although only to a statistically significant level post HD. Dots represent outliers.(n=15) 209
Figure 45: Each histogram outlines the distribution of hepatic perfusion for each imaging timepoint (all available ROIs) Top left=pre HD, top right=1 hour, bottom left=3 hours, bottom right=post HD) 211
Figure 46: Each histogram demonstrates the distribution of PV-derived perfusion for each imaging timepoint (all available ROIs). Top left=pre HD, top right=1 hour, bottom left=3 hours, bottom right=post HD There was no significant difference between the timepoints 212
Figure 47: Each histogram shows the distribution of HA-derived perfusion for each timepoint (all available ROIs) (top left=pre-HD, top right=1 hour, bottom left=3 hours, bottom right=post HD) 213
Figure 48: Pictorial example of heterogeneity of hepatic perfusion during dialysis. Each panel demonstrates an individual subject's liver blood flow histogram with the change in colour representing different timepoints. 214

Figure 49: ICG clearance falls throughout dialysis and is significantly lower than baseline at three hours and post HD (n=15). The error bars in this figure represent 95% confidence intervals. Top panel indicates absolute change and bottom panel percentage change. p values 0.02 at 3 hours and p=0.03 post HD (paired t test) (n=15) 216
Figure 50: Comparative origins of hepatic afferent blood flow throughout dialysis. Error bars represent 95% confidence intervals. Although there is a trend towards fall in portal flow and rise in HA flow, no statistically significant changes were observed.(n=15).....218
Figure 51: Schematic representation of the relative contributions to hepatic blood flow throughout dialysis. Blue bars represent PV contribution and red bars HA contribution to overall liver blood flow. 219
Figure 52: Boxplots to demonstrate the significant difference in pre-dialysis hepatic perfusion and portal blood flow in those who subsequently developed myocardial stunning during HD. The top panel illustrates the difference in hepatic perfusion whilst the bottom illustrates portal venous perfusion.....223
Figure 53: Serial blood flow comparisons in those with stunning and those without. The top panel illustrates the lower total liver blood flow before and after dialysis for those who had myocardial stunning. The bottom panel shows significantly lower portal venous perfusion for

those who without stunning. For each panel, the dotted line represents a group with myocardial stunning whereas a solid line represents those without myocardial stunning. Error bars represent 95% confidence intervals.....224
Figure 54: Boxplot demonstrating the relatively preserved ICG clearance in those without stunning compared to those who did stun. 225
Figure 55: Greater perturbation of hepatic perfusion was associated with lower reduction of indoxyl sulphate (Δ=fed patients). Left panel represents percentage change mean transit time, right panel represents percentage change in hepatic blood volume after 3 hours of HD.....227

List of commonly used abbreviations

AVF	Arterio-venous fistula
BP	Blood pressure
BMI	Body mass index
CAD	Coronary artery disease
CCF	Congestive cardiac failure
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
CT	Computerised tomography
CVC	Central venous catheter
DBP	Diastolic bloods pressure
DM	Diabetes mellitus
ESRD/ESRF	End-stage renal disease/failure
FBC	Full blood count
g	Grams
GI	Gastrointestinal
HA	Hepatic artery
HABR	Hepatic arterial buffer response
HD	Haemodialysis
HTN	Hypertension
ICG	Indocyanine green
IDH	Intra-dialytic hypotension
IVC	Inferior vena cava

JVP	Jugular venous pressure
kg	Kilogram(s)
kPa	Kilopascal(s)
L	Litre(s)
LS	Liver stiffness
LVF	Left ventricular failure
LVH	Left ventricular hypertrophy
m	Metres
m^2	Metres squared
min	Minute
ml	Millilitre(s)
mmHg	Millimetres of mercury
MRI	Magnetic resonance imaging
ms	Milliseconds
PD	Peritoneal dialysis
PV	Portal vein
ROI	Region of interest
RRT	Renal replacement therapy
SBP	Systolic bloods pressure
SMA	Superior mesenteric artery
UF	Ultrafiltration

Chapter 1: Introduction and Literature Review

1 Chronic Kidney Disease

1.1 The Renal System in Health

In health, the kidneys exert a variety of functions, with the most significant being the excretion of water, electrolytes and toxins to maintain homeostasis. These duplicate organs are located in the retroperitoneal space and are each connected to the bladder within the pelvis by a ureter. Their arterial blood is supplied by the right and left renal arteries which directly branch from the aorta. Venous drainage occurs via the renal veins into the inferior vena cava.

The main functioning unit of the kidney is the nephron (see Figure 1). Through the action of these units, excess water and solutes as well as toxins are removed from the circulation to create urine. The nephron also functions to maintain the blood pH and blood pressure.

Figure 1: Schematic representation of the nephron



1.2 Stages of Chronic Kidney Disease (CKD)

Chronic kidney disease is defined as abnormality of kidney structure and/or function for more than three months [1]. Progressive renal damage leads to a reduction in renal function, which is conventionally expressed as a reduction in the rate of glomerular filtration and staged as such (see Table 1). CKD, regardless of aetiology, usually does not manifest symptomatically until there is a severe reduction in function.

Table 1: GFR categories in CKD [1]

Stage	Glomerular Filtration Rate (ml/min/1.73m^2)	Description
G1	>90	Normal or high
G2	60-89	Mildly decreased
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure

1.3 Complications of end-stage disease

CKD Stage 5 is also referred to as end-stage renal failure or disease (ESRD). Patients with this stage of disease suffer from multiple symptoms resulting from the loss of various functions of the kidney. Urine output is often significantly reduced and thus fluid accumulates as volume input greatly exceeds output. Untreated, patients suffer

from the consequences of hypervolemia, including peripheral and pulmonary oedema. The accumulation of urea and other toxic solutes leads to fatigue, pruritus and ultimately, if untreated, fatal encephalopathy. Multifactorial anaemia as a result of iron and erythropoietin deficiency additionally contributes to severe fatigue. Disruption of calcium and phosphate metabolism leads to renal osteodystrophy, which may manifest as bone pain, arthralgia or fracture, as well as widespread vascular calcification.

Figure 2: Major consequences of ESRD

Problem	Consequence
Fluid accumulation	Peripheral oedema Pulmonary oedema (dyspnoea) Hypertension Left ventricular hypertrophy
Uremia/Toxin accumulation	Encephalopathy Fatigue Pruritus Pericarditis Nausea/vomiting/loss of appetite Peripheral neuropathy
Disrupted calcium/phosphate homeostasis	Renal osteodystrophy Vascular calcification
Erythropoietin deficiency	Anaemia
Hyperkalaemia	Dysrhythmias Muscle weakness

Untreated, ESRD is invariably fatal, with the exact mode of death dependent upon co-morbidity and rapidity of disease progression. The development of various modalities of renal replacement therapy (RRT) has led to vastly improved outcomes for this disease.

There are three modalities of RRT which are delivered to those with ESRD in the UK and Canada: renal transplantation, peritoneal dialysis and intermittent HD. Renal transplantation offers the best prognosis in ESRD but is limited to those few who are fit to undergo significant surgery and to those for whom a suitable organ is available. Peritoneal dialysis (PD) is the use of the peritoneum as an exchange membrane for fluid and solutes. Chronic, intermittent haemodialysis (HD) is the more commonly delivered modality of dialysis and is outlined in the following section.

2 HD in Chronic Kidney Disease

2.1 Introduction

HD is a form of renal replacement therapy which can be used acutely or chronically to replace several critical roles of the kidney including waste and excess fluid removal. It involves the passage of blood from a patient into a dialysis machine and back to the patient. Within the machine, blood is pumped in the opposite direction to a solution known as dialysate (which contains purified water and regulated amounts of physiological electrolytes) separated by a semi-permeable membrane, across which small molecules can pass including waste products and water.

Figure 3: Schematic of the HD process (from NIDDK [2])



Blood can be removed from patients through a centrally placed access catheter or, for those receiving dialysis long-term, an artificially created arteriovenous fistula, usually in the upper limb. Patients requiring chronic renal replacement therapy typically receive intermittent dialysis with four-hour sessions delivered three times per week.

2.2 Prevalence

In 2016, there were approximately 25,000 patients in the UK receiving maintenance HD (385 people per million), with the number of total patients receiving dialysis continuing to rise by around 3% each year [3]. The average age of those on dialysis is also rising with a growing number of patients over the age of 75. Although the absolute number per million of the population is low, to achieve adequate care of these patients requires the dedication of a considerable proportion of healthcare resources.

In Canada, there are a similar number of patients receiving treatment for end-stage renal failure, despite the population being approximately half that of the UK. Comparable patterns of incidence are being observed with an increasingly ageing and co-morbid population of patients [4]. In Ontario, healthcare is also free at the

point-of-use. The most recent available data from the Ontario Renal Network [5] showed that there were 2,358 incident HD patients in 2016/7. Total prevalence amounted to 8,966 patients (635 people per million).

2.3 Mortality

There is significantly increased morbidity and mortality associated with receiving HD. In particular, risk of cardiovascular death is massively elevated, being up to 27 times higher than that of the general population [6]. Such rates do not appear to be associated with typical cardiovascular risk factors observed in the general population such as hypertension, hypercholesterolemia and obesity [7]. Increasingly, it is being recognised that the direct consequences of the procedure itself may contribute to this elevated risk and accordingly research has been focussed upon understanding the underlying pathogenesis of cardiovascular disease and organ dysfunction in HD.

2.4 The challenges of HD

Two key challenges for those caring for ESRD patients are to alleviate uraemic symptoms and prevent premature death. The processes which underpin reduced life quality and expectancy in those with end-stage renal failure are disrupted fluid and sodium homeostasis and

the systemic accumulation of uremic toxins. Intermittent HD was conceived as a treatment to address both of these issues, avoiding mortality associated with unremitting severe uremia and fluid overload. However, it appears that the imposed circulatory stress of HD from the necessity of fluid removal is linked to the development of cardiovascular disease and early mortality [8]. Furthermore, HD in its current form fails to sufficiently remove many protein-bound uremic toxins causing significant end-organ damage and a failure to deliver adequate symptom control [9]. Thus, the process of dialysis itself contributes to both the pathophysiology of morbidity and mortality in HD patients.

3 Circulatory Stress in HD

3.1 The effects of ESRD on the cardiovascular system

The effects of chronic kidney disease upon the cardiovascular system prior to reaching end-stage disease render the patient with ESRD more vulnerable to the circulatory changes imposed by HD. Progressive vascular calcification (VC) occurs with advancing CKD and is associated with disruption to mineral homeostasis, inflammation and malnutrition [10,11]. Such calcification leads to arterial stiffness and reduced arterial compliance and is associated with higher rates of cardiovascular mortality [12,13]. At a microvascular level, endothelial dysfunction occurs as a result of disruption to vasoregulatory factors such as nitric oxide and endothelin [11]. Autonomic dysfunction is common with the progression of CKD and related to both endothelial dysfunction and vascular calcification [14]. The baroreceptors located in the central circulation fail to invoke a sufficient autonomic vasomotor response to changing central haemodynamics. This loss of vasoregulatory ability renders the circulation less able to adapt to any acute fall in perfusing pressure, increasing the risk of demand ischaemia in vulnerable organs during periods of circulatory stress.

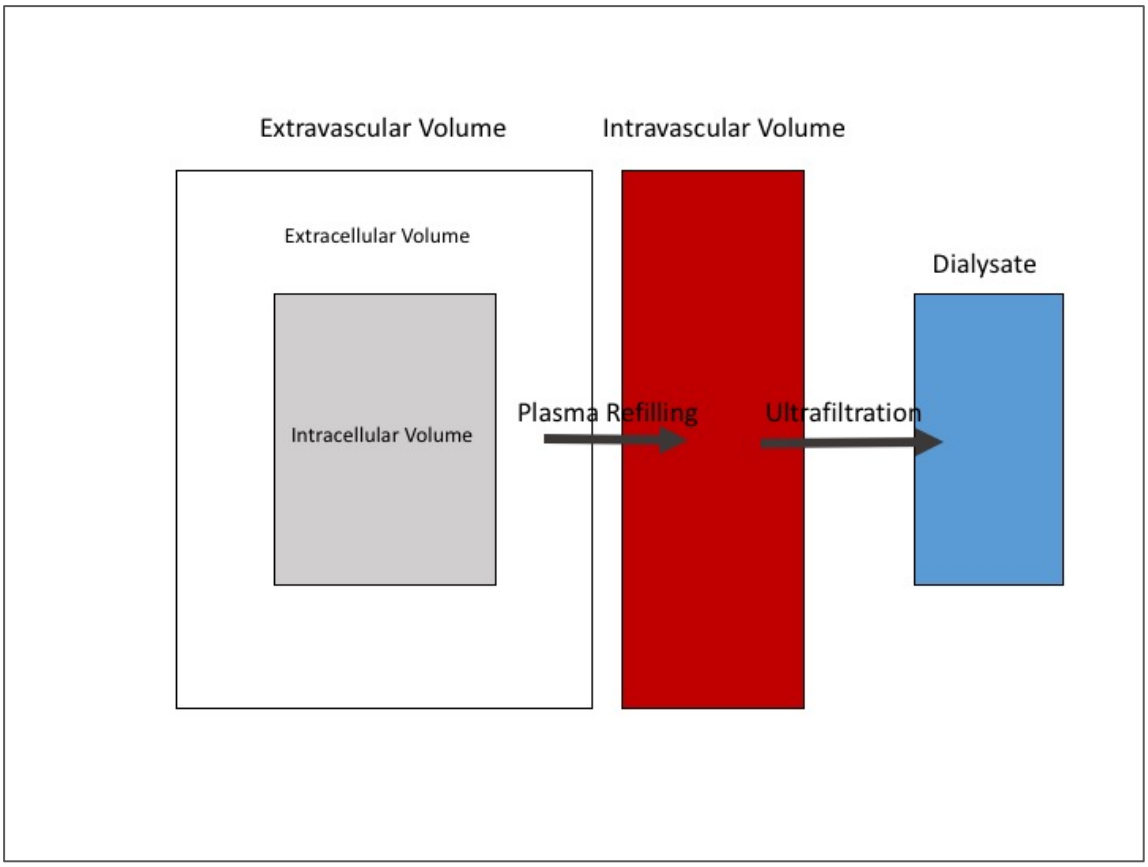
Furthermore, with progressive renal impairment, fluid overload becomes common as urine output falls. This is an important factor

implicated in the development of hypertension and left ventricular hypertrophy (LVH) in advanced CKD [15,16]. LVH is strongly coupled with cardiovascular mortality and the development of chronic heart failure in CKD [17], thereby adding to the susceptibility of the cardiovascular system to haemodynamic stress.

3.2 The haemodynamic effects of dialysis and IDH

The process of HD is capable of exerting significant stress upon the cardiovascular system. The removal of blood into the dialysis circuit at the beginning of dialysis leads to an immediate drop in circulating volume of approximately 100-200mL. Removal of fluid through the process of ultrafiltration (UF) during the dialysis session leads to a further reduction in circulating volume which is only attenuated by plasma refilling (Figure 4).

Figure 4: Fluid Kinetics in HD

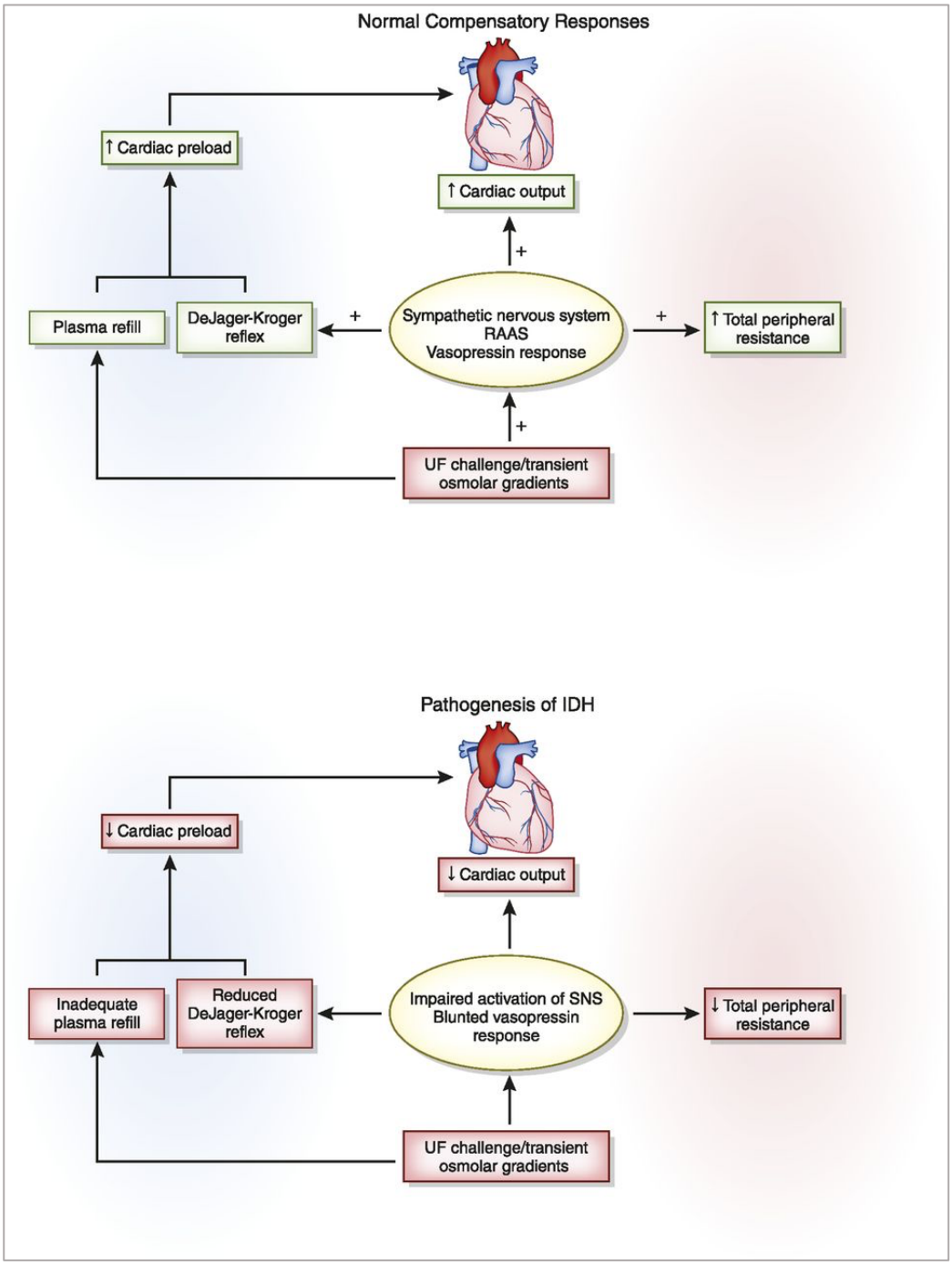


Intradialytic hypotension (IDH) has no consistent definition in nephrology literature and is described both in terms of a fall from pre-dialysis baseline and absolute nadir, with or without symptoms. IDH is common, complicating around 20-30% of all dialysis treatments [18], and is strongly associated with increased morbidity and mortality, with the definition of a nadir of less than 90mmHg (with or without symptoms) being the strongest predictor of death in a recent systematic review [19]. Predictors of IDH have been shown to include

higher UF rate, advanced age, lower pre-dialysis blood pressure and the presence of diabetes [18].

The pathophysiology of IDH is complex and dependent upon a number of patient and dialysis-specific factors. Maintenance of organ perfusion in the event of falling intravascular volume requires maintenance of cardiac output (through elevation of heart rate and contractility), the augmentation of cardiac preload through increased venous return, arteriolar vasoconstriction and maintenance of plasma refilling. Failure of any or all of these compensatory mechanisms can lead to a reduction in effective perfusing pressure (Figure 5).

Figure 5: The pathophysiology of IDH (from Reeves et al. [20])



Whilst IDH is strongly associated with poor outcomes, it does not completely discriminate between a stressed and an unstressed circulation; that is, it is not a requirement for end-organ damage during dialysis [21,22]. A recent study demonstrated cardiac injury and a fall in myocardial perfusion with only a drop of 20mmHg of systolic pressure from baseline [23].

3.3 Dialysis Associated Organ Injury

3.3.1 The heart

The temporal relationship between major cardiovascular events, including sudden death, and HD treatment led to the hypothesis that cardiac injury occurs during dialysis. In the mid 2000s, HD was shown to lead to acute, reversible regional myocardial wall motion abnormalities, a phenomenon usually associated with temporary cardiac ischaemia, known as myocardial stunning [24]. In 2008, it was shown that a reduction in both global and segmental myocardial blood flow during dialysis was strongly associated with the development of these reversible RWMAs in patients without coronary artery disease [21]. Subsequent studies have demonstrated the link between the development of progressive cardiac dysfunction and cardiovascular mortality with the severity of acute myocardial stunning during dialysis [25,26]. This type of cardiac injury is not dependent upon the presence of traditional cardiovascular risk factors and has been shown to even occur in children receiving HD treatment [27]. It is however, strongly associated with haemodynamic instability during dialysis [25], with amelioration of circulatory stress shown to reduce the severity of injury and improve outcomes [28,29].

3.3.2 The brain

Leukoaraotic lesions consistent with recurrent ischaemic insult have been demonstrated in HD patients. Such white matter injury has been shown to progress with time spent on dialysis, relate to haemodynamic instability during HD and relate to functional cognitive impairment. Measures to improve haemodynamic stability have been demonstrated to slow the progression of brain injury [22]. More recently, a significant fall in cerebral blood flow has been demonstrated during dialysis in elderly HD patients [30].

3.3.3 The kidneys

Most ESRD patients who begin HD treatment are not completely anuric [31,32]. The presence and preservation of some level of residual renal function (RRF) following dialysis initiation is related to improved control of serum phosphate, hypervolemia and hypertension, as well as improved nutrition, less severe anaemia and lower middle molecule plasma concentrations [33,34]. In addition, RRF is strongly associated with improved HD patient survival [35] and the importance of long-term RRF maintenance has been emphasized [36,37].

Despite its importance in conferring health and survival benefits, RRF characteristically declines in patients with ESRD after the initiation of

HD. This decline has consistently been linked to poorer outcomes and increased mortality [35,38]. Preservation of RRF in ESRD, even to a degree below that which was previously considered meaningful, has been shown to improve survival [39]. However, there remains a lack of robust measures to achieve this.

Observational studies have reported several factors associated with RRF decline, including age, aetiology of renal disease, use of bioincompatible dialysis membranes and elevated blood pressure [40,41]. Following this, larger epidemiological studies have confirmed many of these findings, and shown additionally that hemodynamic instability or lower blood pressure during HD is an independent factor associated with RRF loss [42,43]. The hypothesis-generation from many of these studies has unfortunately failed to result in either robust experimental trials to examine the pathophysiology of RRF decline in HD-treated patients, or interventional trials to evaluate potential strategies for RRF preservation. Nevertheless, it is widely hypothesised that haemodynamic instability during dialysis contributes significantly to the decline of RRF [8,36,44].

3.3.4 The gut and liver

The impact of recurrent circulatory stress upon the hepato-splanchnic system has rarely been studied in HD patients. The following section outlines why this system is uniquely vulnerable and yet potentially of critical importance to overall haemodynamic stability in HD.

4 The hepato-splanchnic system in circulatory stress

The intestine and liver have an intricately linked circulation, forming a unique connection between these two major organs. The hepato-splanchnic system receives approximately 25% of the cardiac output in the resting state, more than any other organ system in the human body. This circulation has a unique regulation and plays an important role in the physiological response to circulatory stress.

4.1 The Intestine and the Splanchnic Circulation

The lower gastrointestinal tract is formed by the small and large intestine. This stretch of the alimentary canal is where the majority of digestion and absorption of nutrients occur.

The small intestine is formed of the duodenum, jejunum and ileum. Secretion of bile and pancreatic enzymes to aid the process of digestion and absorption occurs within the duodenum. Absorption of macronutrients (protein, lipid and carbohydrate) occurs largely in the jejunum and ileum. The small intestine also functions to absorb water and micronutrients. The large intestine is formed of the caecum, ascending, transverse, descending and sigmoid colon and the rectum. Colonic function was previously thought to be limited to water absorption and the storage of faeces ready for excretion, however its

role is now considered to be more complex, including hosting several trillion commensal bacteria, known as the microbiome. These bacteria play an active role in the metabolism of non-digested products from the small intestine, in the maintenance of the gut epithelium and in immune development.

The preservation of gut function during disease is critical for two main reasons. Firstly, the functionality of the gut barrier is required to prevent the free translocation of pathogenic bacteria and toxins from within the GI tract into the systemic circulation. Secondly, absorption of nutrients is critical when calorific needs may be significantly higher.

4.1.1 The intestinal circulation

The arterial blood supply of the intestinal or splanchnic circulation is formed from three tributaries of the aorta – the coeliac axis, the superior and inferior mesenteric arteries. An arteriolar and subsequent capillary network arising from these vessels forms the largest network of vessels within the body. The portal vein, which is formed by the union of the splenic and mesenteric veins, forms the route of blood efflux for the splanchnic circulation (see Section 4.2.3).

The intestinal circulation is regulated by both intrinsic and extrinsic factors. Autoregulatory factors include local metabolic control, locally-produced vasoactive substances and local reflexes. These are best demonstrated acting in combination during the physiological response to feeding, where post-prandial hyperaemia occurs as a result of increased gut metabolism, a reflex response to the presence of gut contents and the secretion of locally-produced hormones, such as gastrin and secretin.

Despite these intrinsic autoregulatory mechanisms, gut blood flow is heavily impacted by systemic hemodynamics, the action of the sympathetic nervous system and circulating vasoactive substances. Moreover, the intestinal circulation plays a key role in the physiological systemic adaptive response to circulatory stress. A reduction in cardiac output triggers vasoconstriction of the post-capillary mesenteric venules leading to the emptying of the splanchnic reservoir of blood into the systemic circulation – a so-called 'auto-transfusion" - which confers an additional 200-300mls to the circulating volume, maintaining cardiac preload. A further drop in circulating volume or pressure leads to splanchnic arteriolar constriction, sustaining total peripheral resistance and therefore perfusion to critical circulations such as the coronary or cerebral

vascular beds. The oxygen extraction capacity of the intestine appears to increase to maintain metabolic function, at least in the short-term [45]. However, maintaining a lower relative blood flow renders the gut highly vulnerable to ischemic injury during profound or prolonged shock. A sustained drop in splanchnic perfusion leads to tissue hypoxia and ultimately intestinal dysfunction with increased gut permeability, allowing products of intestinal putrefaction and bacterial toxins to traverse into the systemic circulation, provoking a widespread inflammatory response, often worsening systemic haemodynamic dysfunction [46,47].

4.1.2 Gut blood flow in other forms of circulatory stress

Direct quantitative measurement of gut blood flow in humans is extremely difficult for those studying physiology or disease owing to the early requirement of surgery for anatomical access. There is, therefore, much methodological variation in studies of different disease states and their effect on gut blood flow. However, from experimental animal models it appears that there are two distinct phenotypes of splanchnic circulatory response to circulatory stress depending on the aetiology – one found in low flow states such as cardiogenic shock or hypovolemia, the other in inflammatory conditions, specifically sepsis.

In cardiogenic shock, cardiac surgery or hypovolaemia due to haemorrhage there is reduced gut blood flow without increased tissue metabolic demand. Although this reduction in blood flow appears to be disproportionate to that seen in other central organs (due to the physiological protection in place), the intestine appears to cope with such low-flow states as oxygen extraction capacity is well-maintained. However, sustained hypoperfusion results in tissue hypoxia and eventually mucosal injury [46].

Additionally, in such states, the restoration of intestinal blood flow after tissue hypoxia appears to paradoxically lead to a temporary worsening of tissue injury – a phenomenon known as ischaemia-reperfusion injury. The mechanism behind this observed effect is not fully understood but it has been shown that reperfusion of hypoxic gut tissue provokes an additional cell injury through the activation of complement, the release of inflammatory mediators and free radicals as a result of oxidative stress. During this phase of injury, gut permeability may be significantly compromised, leading to an additional phase of gut-derived systemic inflammatory injury.

The effect of systemic inflammation or sepsis upon the gut is considerably different to the "low-flow" states. With sepsis, the metabolic demand within the splanchnic tissue is increased. There is increased gut blood flow, even in the face of systemic hypotension, and oxygen extraction capacity is high [48]. However, the eventual development of mismatch between oxygen supply and demand in shock, myocardial depression, ventilatory dysfunction, the presence of endothelial injury and microvascular dysfunction all contribute to tissue hypoxia. Gut permeability has consistently been shown to increase in septic states, leading to the further translocation of bacterial toxins, exacerbating, or even driving, the systemic inflammatory response and resulting multiple organ failure [49–51].

4.1.3 Gut blood flow regulation during fasting and feeding

Eating/feeding may improve the ability of the splanchnic circulation to autoregulate in the presence of systemic haemodynamic compromise, as luminal contents act as a powerful stimulus to increase intestinal blood flow. However, the experimental evidence for such an effect in humans is largely limited to the critical care setting where feeding tends to be continuous and non-physiological (often post-pyloric). In this setting, there has been debate as to the benefit of feeding in haemodynamic compromise, with some maintaining that it increases

the risk of bowel ischaemia due to increased metabolic demand [52]. Some studies show that the hyperaemic post-prandial response to feeding remains intact even in those receiving inotropic support and does not compromise haemodynamic stability for any sustained length of time [53,54]. However, others have reported a link between enteral feeding and small-bowel ischaemia [55,56]. The exact nature of the relationship between enteral feeding and preservation of gut barrier function in multiple organ dysfunction is yet to be well characterized.

4.2 The Liver and the Hepatic Circulation

4.2.1 Introduction

The liver is the largest solid organ in the human body. It is situated within the abdominal cavity and receives 20-25% of the cardiac output. It has a wide variety of functions, all of which are critical to the maintenance of health:

- Protein Metabolism
- Carbohydrate metabolism
- Lipid metabolism
- Hormone metabolism
- Drug metabolism
- Excretion of bile
- Storage
    - Minerals – copper, iron
    - Vitamins – A, B, C
- Immune function
    - Antigen presentation
    - Macrophagocytic destruction of foreign material

The liver is functionally divided into eight segments, each with separate vascular inflow, outflow and biliary drainage. There is further

division at a microscopic level into functioning units known as liver lobules (Figure 6).

4.2.2 Liver Cell Function

The dominant cell type in the liver is known as the hepatocyte. These cells are responsible for executing the majority of synthetic and metabolic functions of the liver including protein synthesis and storage, carbohydrate and drug metabolism. They make up around 80% of liver mass.

Cholangiocytes are epithelial cells which line the biliary tree both within and outside the liver. They aid the excretion of bile through secretion of fluid and electrolytes to promote bile flow.

Several other cell-types found within the liver are found in close proximity to the sinusoids. Although they account for only a small proportion of total cell mass, these cells are responsible for many of the adaptive functions of the liver including the immune response.

Hepatic stellate cells (HSCs) can be found within the perisinusoidal space (also known as the Space of Dissé). Their role in healthy liver tissue is not entirely clear and it is thought that they are relatively quiescent although recent evidence suggests that they are partly

responsible for antigen presentation to macrophages [57]. Activation of these cells plays a crucial role in the development of liver fibrosis in chronic liver disease.

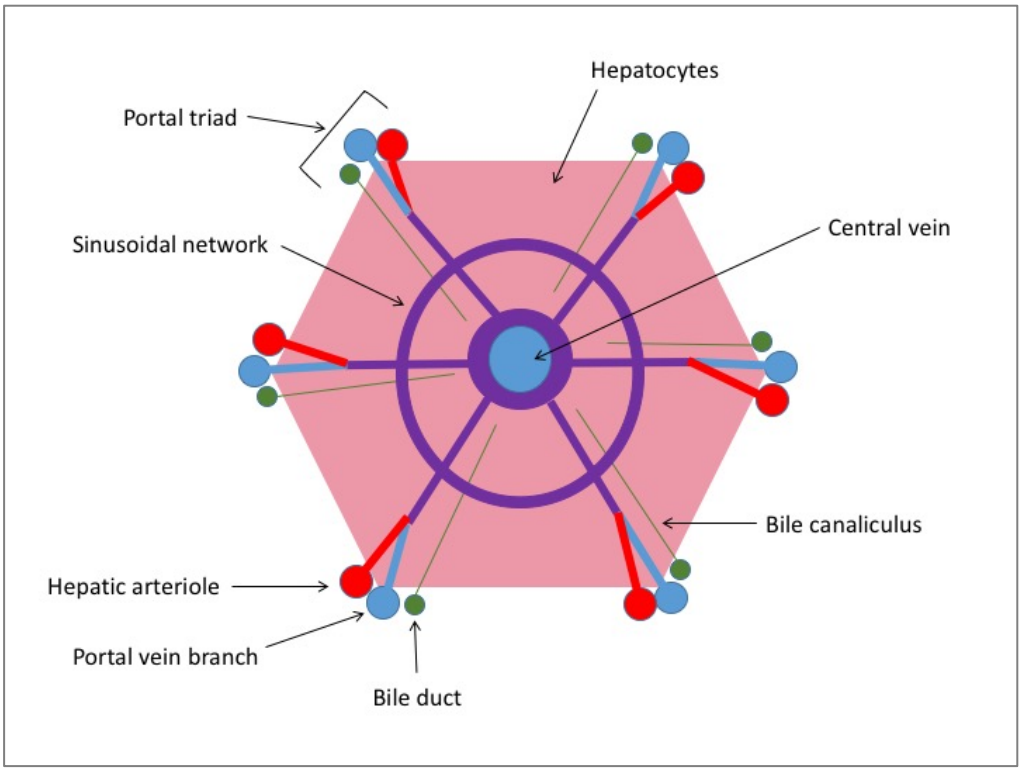
Sinusoidal endothelial cells (SECs) form a fenestrated lining to the sinusoids to allow efficient nutrient and oxygen exchange. However, in addition to endothelial function, these unique cells also appear to play an important part in immune function in health, inducing T cell tolerance, and the pathogenesis of inflammation in disease, stimulating cytokine release [57–59].

Kupffer cells are the macrophage population in the liver which line the endothelium of sinusoids. Their response to exposure to various gut-derived bacteria and microbial debris is a critical part of the innate immune response by releasing various reactive cytokines and activating complement pathways. They possess the ability to alter the phenotype of their cell population as well as the other cell populations in proximity to the sinusoids including SECs, HSCs and hepatocytes and are therefore thought to be early mediators of liver response to damaging stimuli [60].

4.2.3 The Hepatic Circulation

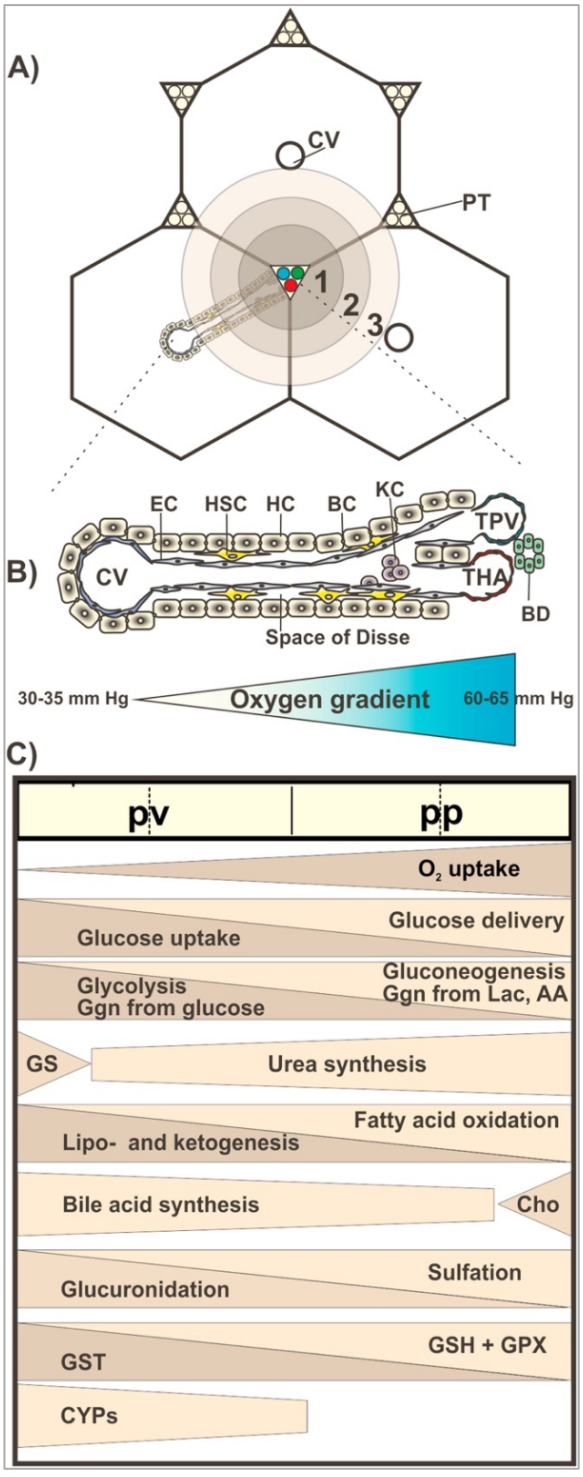
The liver has a unique dual blood supply and the most complex circulation of any organ within the body. 70% of its blood inflow comes from the portal vein, which is formed by the union of the splenic and mesenteric veins (of splanchnic origin). The remainder is supplied by the hepatic artery, a direct branch of the coeliac artery (of systemic circulatory origin). Blood flows through these vessels into the sinusoids where nutrient and oxygen exchange occurs. The blood then flows to a central vein, through to a hepatic vein and eventually the inferior vena cava.

Figure 6: The structure of a liver lobule (simplified schematic)



Due to the configuration of sinusoidal flow, hepatocytes are not capable of intrinsically regulating hepatic arterial flow according to their metabolic requirements. Hepatic metabolic activity is different according to the location of hepatocytes (due to differing oxygen supply), known as zonation. Oxidative metabolism and gluconeogenesis tend to occur in the periportal zone whereas glycolysis largely occurs in the perivenous zone. A change in liver blood supply (portal venous vs hepatic arterial fraction) can affect the metabolic activity of the liver both acutely and chronically. Regulation of blood flow through the liver is therefore critical in determining liver function.

Figure 7: Liver microarchitecture, the oxygen gradient and metabolic zonation (from Kietzmann [61])(reproduced under CC BY NC ND license)



A) Classic hexagonal shaped liver lobule with a central vein (CV) in the middle and portal triad (PT) corners with branch from the portal vein also called terminal portal vein (TPV, blue dot), and a branch from the hepatic artery also called terminal hepatic arteriole (THA, red dot) as well as a bile duct (BD, green dot). The acinus extends from a PT into the direction of two adjacent central veins. Three zones can be distinguished. 1, the periportal zone; 2, the intermediary zone; 3, the perivenous, pericentral, or centrilobular zone.

(B) Liver sinusoid and oxygen gradient. Hepatocytes (HC), are connected with each other, bile canaliculi (BC) transport the bile formed in HC into the bile duct (BD). Sinusoids are wrapped with fenestrated endothelial cells (EC). HC and EC are separated by the Space of Disse which is the residence niche for hepatic stellate cells (HC). Resident macrophages, the Kupffer cells (KC) are also to be found in the sinusoid.

(C) Distribution of major metabolic pathways. pp, periportal; pv, perivenous; AA, amino acids; Cho, cholesterol synthesis; CYP, cytochrome P450 enzymes; Ggn, glycogen; Lac, lactate; GPX, glutathioneperoxidase; GS, glutamin synthesis; GST, glutathione transferase.

Portal inflow is determined by the outflow from the splanchnic bed and is therefore under splanchnic regulation, unaffected by any intrinsic hepatic regulation. However, the hepatic artery is able to autoregulate its flow, through the vasoactive effects of adenosine, to accommodate for any changes in portal flow – a phenomenon known as the hepatic arterial buffer response (HABR) [62]. Hepatic arterial flow is also influenced by the sympathetic nervous system, with adrenergic stimulation leading to vasoconstriction. However, this activation has a short duration in the face of ongoing systemic circulatory stress as the drop in portal pressure activates the HABR.

Under normal physiological conditions, sinusoidal resistance is low, with little pressure gradient between sinusoidal blood and that in the portal vein, despite the influx of blood from the hepatic artery. However, vasoactive substances produced locally or systemically can affect sinusoidal resistance with profound effects upon intra-hepatic blood flow [63].

The hepatic venous system is formed of a network of interlobular weaving valveless veins which ultimately drain into the inferior vena cava (IVC). This system has a significant capacitance, acting as a physiological blood reservoir. Hepatic venous flow is affected by

sinusoidal flow, the sympathetic nervous system, circulating vasoactive substances and direct central haemodynamics. The venous system has an adaptive role to play in the physiological response to both low flow states such as haemorrhage and to elevated central venous pressure or hypervolemia. In the former, sympathetic activation leads to venoconstriction with significant expulsion of blood into the central circulation, thereby decreasing liver volume. Conversely, elevated central venous pressure or right atrial pressure directly affects the resistance within the hepatic venous system, with an almost immediate rise in liver volume. The non-elastic liver capsule surrounding the liver means that the response to rising central venous pressure cannot indefinitely continue proportionally, with an eventual rise in liver stiffness demonstrable (see Figure 8).

Figure 8: From Millonig et al. 2010: The relationship between central venous pressure and liver stiffness [64]

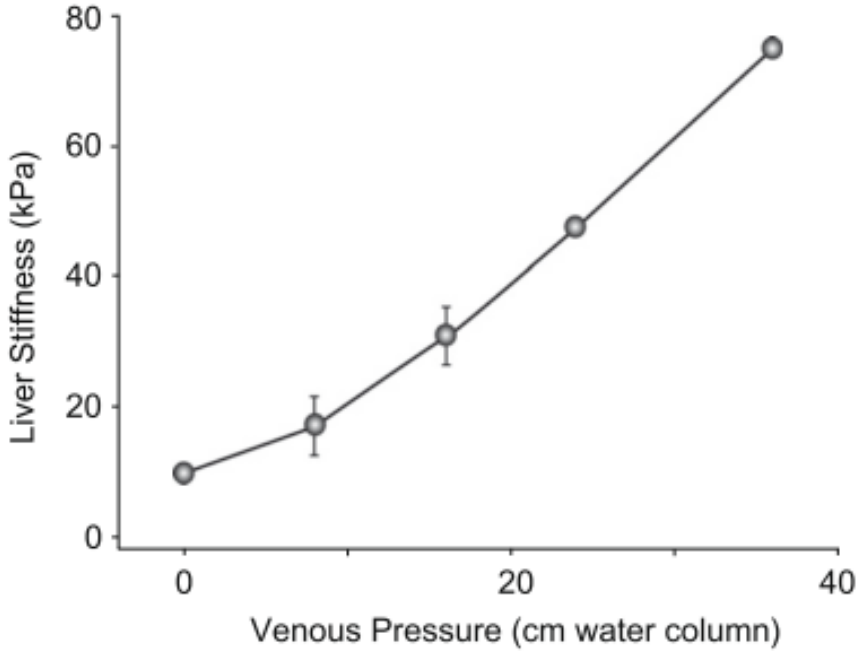


Fig. 3. Liver stiffness in the isolated pig liver directly and linearly depends on intravenous hydrostatic pressure. After clamping the caval vein distally and proximally of the liver, hepatic artery and portal vein, intravenous hydrostatic pressure was stepwise increased up to 50 cm water column by infusion of isotonic saline solution. Liver stiffness increased linearly ($r = 1$, $p < 0.01$) until 36 cm water column intravenous pressure where it reached the maximum measurable liver stiffness of 75 kPa. Data are the average from three animals. The error bars represent SEM. Bars are sometimes too small to be visible.

4.2.4 The Hepatic Circulation in Circulatory Stress

Like the intestinal circulation, the hepatic circulation also plays a role in the maintenance of haemodynamic stability in the face of circulatory stress, acting as a physiological reservoir, expelling up to

60% of its held blood volume in response to adrenergic stimulation, through post-sinusoidal vasoconstriction [65].

The liver, however, has unique protection against ischaemic injury. Owing to its dual blood supply, injury from typical arterial thromboembolic disease is extremely rare. The ability of hepatocytes to extract oxygen efficiently is highly sophisticated, allowing preservation of metabolic activity in the face of significant fall in delivered oxygen. Furthermore, metabolic activity is down-regulated in the face of a fall in perfusion and glycogenolysis activated, to provide metabolic substrate to other organs [65].

However, profound or prolonged systemic shock, where both the portal and arterial inflow are reduced, may lead to ischemic or "hypoxic" liver injury, manifesting overtly as acute liver failure in the severest form in critically ill patients. Typically, centrilobular necrosis is seen histologically, with ischemic burden rising with the degree/duration of hypoperfusion [66]. One key factor increasing the risk of ischaemic necrosis is pre-existing hepatic congestion [67].

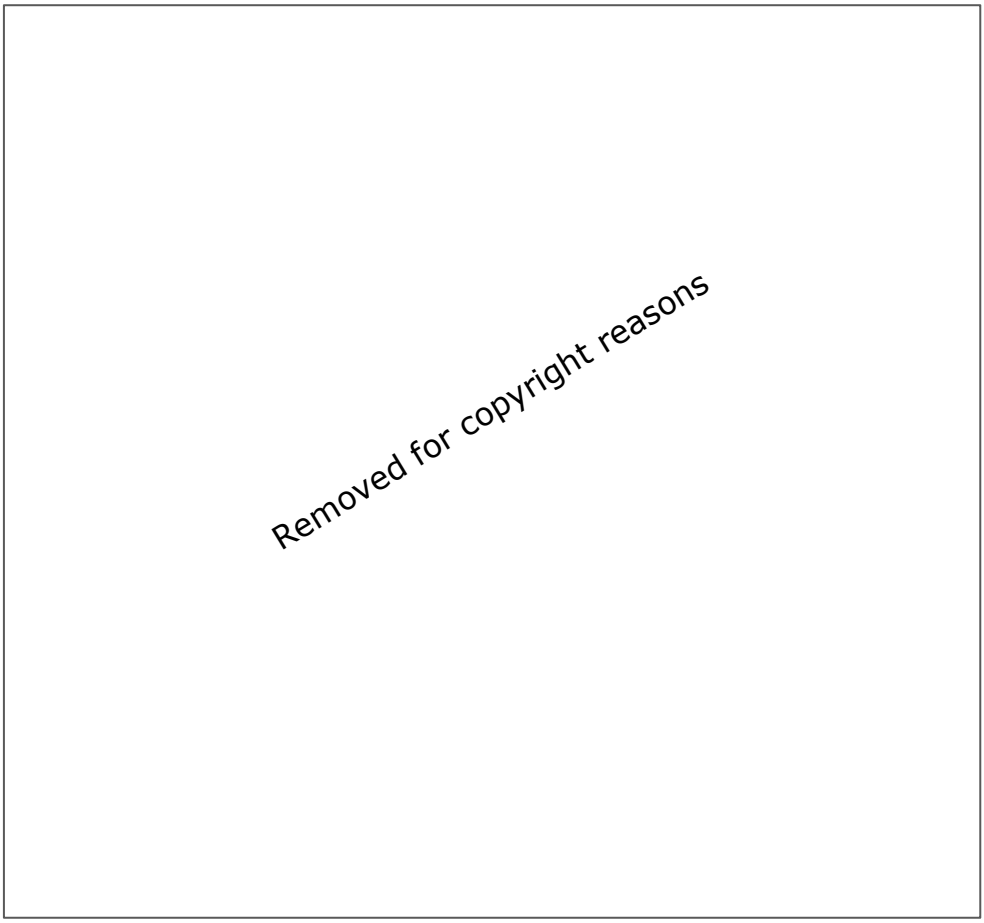
In a manner comparable with the splanchnic circulation, a different pattern of dysfunction is seen in the hepatic circulation with sepsis.

Metabolic demand increases and oxygen extraction becomes defective, leading to relative tissue hypoxia despite preserved or even increased apparent hepatic blood flow [68]. The liver is also the site of massive inflammatory and vasoactive cytokine release in sepsis, leading to local exposure to substances which potentiate endothelial dysfunction and worsen tissue hypoxia [69]. Animal studies have shown that, during sepsis, the HABR is dysfunctional, with vasodilatation in the hepatic artery leading to increased overall liver blood flow, possibly due to a direct effect of endotoxin [62].

The liver also has an important host defence role, acting as a functional barrier to the translocation of bacterial fragments including endotoxin from the gut into the systemic circulation. Even in health, minute amounts of bacterial fragments traverse the fully functional intestinal barrier. These do not reach the systemic circulation as they are destroyed through the action of Kupffer and other immune cells lining the sinusoids. However, in the presence of a failing intestinal barrier, such as in sepsis or in low-flow states, this process becomes saturated and the liver is unable to effectively deal with the quantity of toxins received in the portal inflow [70]. Furthermore, endotoxin itself both directly and indirectly affects liver function. Few studies have examined the liver's immune function in the context of low-flow

states or sepsis to date, although recently researchers have highlighted this important step in the pathophysiology of multi-organ dysfunction in shock [69,71].

Figure 9: Mechanisms limiting the entry and dissemination of LPS into the circulation (from Guerville et al. 2016 [71])



4.3 The Intestine, Liver and Circulatory Stress in HD

HD is a unique challenge to the hepatic and splanchnic circulations. Firstly, it is a repeated, predictable circulatory stressor. Secondly,

ESRD is associated with chronic activation of the systemic inflammatory response, with elevated levels of vasoactive cytokines [72]. Thirdly, patients are frequently volume overloaded and subject to hypervolaemic stress, a factor associated with intestinal oedema and gut dysfunction [73] as well as rendering the hepatic circulation more vulnerable to ischaemia. Lastly, there is pre-existing autonomic dysfunction which renders each circulation more vulnerable to a fall in circulating blood volume [14].

The potential consequences of intestinal and liver injury in HD patients are greater than in the normal population. The almost universal presence of intestinal dysbiosis in ERSD not only renders the non-cellular portion of the intestinal barrier weaker, but also leads to an increased intra-colonic burden of bacterial toxin available to translocate into the circulation in the presence of mucosal injury [74]. As previously outlined, bacterial translocation is potentially a key pathophysiological step in many of the processes contributing to increased morbidity and mortality in this group. It leads to a cascade of pro-inflammatory effects including accelerated atherogenesis, myocardial depression, free-radical damage and malnutrition. Many of these factors also contribute to hemodynamic instability during

dialysis and thus gut injury may perpetuate a vicious cycle of organ injury [8].

Liver function is relatively unstudied in HD patients, presumably as the frequency of clinically measurable ischaemic injury or dysfunction is rare. However, the preservation of liver function is highly significant in HD patients. The liver has an enhanced role in drug metabolism and excretion in ESRD; many uremic toxins are activated through hepatic metabolism; controlled storage and breakdown of nutrients is highly relevant in the catabolic state of most patients. Perhaps most importantly, it acts as the final functional immune defence between the intestinal luminal microenvironment and the systemic circulation.

Whether the protective haemodynamic mechanisms in the hepato-splanchnic system continue to function during the repetitive stress of chronic HD has yet to be conclusively established. There is a small body of evidence to suggest that splanchnic perfusion falls disproportionately in the chronic HD patient. Yu et al. showed that splanchnic erythrocyte content decreased after 2 hours of dialysis with UF, in the absence of cardiovascular instability [75]. Schneditz et al. demonstrated lower hepato-splanchnic blood flow in stable HD patients using non-invasive measurement of indocyanine clearance

[76]. Finally, Ribitsch et al., using a similar technique, showed that splanchnic perfusion fell significantly throughout dialysis with a rise in hepato-splanchnic resistance, implying vasoconstriction [77]. One limitation of this group of studies is that there is no clear assessment of the splanchnic response in those with autonomic dysfunction. There are no studies to date which examine the acute effect of HD upon liver blood volume or hepatic perfusion.

There is a small body of existing evidence to suggest that GI mucosal injury occurs during dialysis. One study showed that mucosal ischemia, inferred through a fall in measured pH, occurred acutely within the upper GI tract during dialysis without evidence of systemic hemodynamic instability [78]. McIntyre et al. demonstrated that levels of endotoxin, a bacterial toxin found within the gut which translocates into the systemic circulation with gut injury, are massively elevated in those receiving chronic dialysis, far exceeding those with end-stage renal failure receiving conservative care, implying a specific association with the dialysis process rather than uraemia per se [79]. In this study, systemic levels of endotoxin increased approximately threefold within a few months of commencing dialysis, implying gut injury and increased permeability. Higher levels were shown to be associated with hemodynamic

instability, cardiovascular injury during dialysis and increased mortality. A subsequent study showed that more frequent, prolonged dialysis schedules with greater hemodynamic stability were associated with lower gut-derived endotoxin levels [80].

The effects of HD specifically upon the hepatic circulation or hepatic function have rarely been examined. Ribitsch et al. in their study examining hepato-splanchnic circulatory changes during dialysis used indocyanine green as a surrogate measure of hepatic blood flow, due to its high extraction rate from hepatic sinusoids. They demonstrated that there was a significant fall in ICG clearance from the circulation with dialysis, suggesting a fall in hepatosplanchnic blood flow. However, discriminating between a fall in intestinal blood flow, portal flow and total liver blood flow was not possible through this methodology, nor was assessing the impact of dialysis upon hepatic function.

Nevertheless, data from other conditions where a temporary fall in circulating pressure occurs, such as liver surgery, suggest that ischaemia-reperfusion does occur within the liver and is not necessarily clinically detectable through conventional measures of liver injury [81]. Furthermore, hepatic dysfunction is potentiated by

heart failure [66], additional hypoxic injury [82], venous congestion, increased myocardial demand [83], endotoxemia [84] and microcirculatory dysfunction [85] - all of which are present during HD [8] and suggest that the liver in HD is uniquely vulnerable.

5 The uremic toxin burden in dialysis patients

Another important consequence of chronic kidney disease is the systemic accumulation of toxic solutes. These toxins are a group of heterogeneous substances which have a plethora of effects and are closely linked to the development of premature cardiovascular morbidity and mortality [86]. It is now increasingly recognised that it is not decreased renal clearance alone which contributes to the increased systemic exposure to these toxins. Many of the solutes are produced exogenously and absorbed, having been either ingested or generated from bacteria within the GI tract [87], a process which is augmented with declining renal function.

Table 2: Uremic Toxins implicated in cardiovascular disease in CKD (from Evenepoel et al. [87])

Toxin	Group	Endogenous metabolism	Gut microbial metabolism	Exogenous intake
ADMA	Dimethylarginines	x		
p-cresyl sulphate	Phenols		x	
Phenylacetic acid	Phenols		x	
Indoxyl sulphate	Indoles		x	
Indole 3-acetic acid	Indoles		x	
AGEs	n/a	x		x
Homocysteine	n/a	x	x	
Oxalate	n/a	x		x

The process of HD aims to remove these solutes from the systemic circulation, thereby decreasing their harmful effects. However, many are highly protein-bound and therefore their removal is limited. Furthermore, maximising solute clearance is often limited by the cardiovascular tolerability of additional time spent on dialysis. Therefore, addressing the influx of toxins into the circulation is of critical importance to reduce exposure and its subsequent harm – providing additional impetus for the study of the hepato-splanchnic system in dialysis.

5.1 The impact of the intestine and liver upon toxin accumulation in HD patients

Impaired renal function is associated with the increased formation of harmful solutes with the intestine, particularly within the colon. The pathophysiology behind this process is highly complex and appears to be multifactorial, with significant inter-individual variation [88]. Moreover, the rate of production of these substances does not appear to be directly proportional to reduction in glomerular filtration [87]. Disruption of the normal gut flora within the gut lumen underpins this process as disproportionate proliferation of bacteria which produce nitrogenous waste occurs. Gut dysbiosis is multifactorial in CKD and the factors which contribute have been comprehensively reviewed [87,89]. It is worth noting that the recognized factors which lead to dysbiosis are more numerous and often more severe in hemodialysis. Functional disturbance of the intestine also contributes to uremic toxin production and is well-recognized in hemodialysis patients, with colonic transit time slow and constipation common, although the exact pathophysiology underlying this is not fully understood [90,91].

In addition to contributing to increased toxin production, gut dysbiosis also results in the increased permeability of the intestinal barrier. This affects both the non-cellular and cellular layer [92]. The mechanisms

by which gut-derived uremic toxins cross the intestinal barrier have not yet been established, nor is it therefore known whether its impaired function impacts upon toxin delivery into the systemic circulation. In dialysis patients, the additional vulnerability of the gut barrier to ischemic injury may be of significant import to the overall process of toxin exposure.

Having traversed into the portal circulation, these substances are taken up by hepatocytes and metabolized prior to the release into the systemic milieu. The role of the liver in the production of toxic solutes has been the subject of little study and the degree to which systemic exposure from gut-derived solutes depends upon hepatic function has yet to be conclusively established. One study of cirrhotics with end-stage renal failure suggests that the accumulation of the potent toxins indoxyl-sulphate and p-cresyl sulphate was affected by both stage of kidney and stage of liver disease [93]. However, cirrhosis itself affects gut permeability and causes gut dysbiosis therefore may be a significant confounder when considering toxin production and absorption. Nevertheless, animal studies conducted as part of this body of work appear to confirm that an acute reduction in hepatic function leads to a reduction in the glucuronidation or sulfation of absorbed solutes [93].

5.2 The impact of uremic toxins upon the gut and liver in HD patients

As with circulatory stress, the accumulation of uremic toxins appears to be both influenced by and influences alimentary system function, creating a vicious cycle of injury and accumulation. Understanding in this area is limited by a paucity of research and the necessity of examining the impact of individual rather than collective toxins. One better recognized effect upon the gut is the influence of various toxins upon appetite and food intake [91], which in turn affects bacterial composition and activity within the colon, thereby affecting the generation of colonic toxins.

Hepatic metabolic function has been more widely shown to be affected by individual uremic toxins. For example, indoxyl sulphate has been repeatedly shown to affect hepatic synthetic production and elements of drug metabolism [94–96]. p-Cresol has also been shown to affect drug metabolism within the liver, as well as influencing hepatic uptake of other toxic substances such as aluminium [97,98].

5.3 The potential link between circulatory stress and uremic toxins

Recognizing the complex pathophysiology of uremic toxin generation and accrual and its relationship to the alimentary system has several implications for hemodialysis patients.

Firstly, in this patient group, there is an intricate link between fluid overload and removal and the accumulation of gut-derived uremic toxins, as recurrent circulatory stress potentially impacts both the intra-luminal environment, gut permeability and liver blood flow/function.

Secondly, this relationship means that exposure to and subsequent accumulation of protein-bound toxins that are difficult to remove with dialysis, might be reduced through amelioration of dialysis-associated circulatory stress.

6 Dynamic Imaging of the Hepato-splanchnic System

Our understanding of the physiology of the hepatic and splanchnic circulations has been largely derived from animal studies, owing to the need for invasive access in humans. However, as Jakob has noted [46], animal studies are usually conducted with non-physiological interventions such as splenectomy and ligation of the coeliac trunk or portal venous branches, affecting the translatability of findings. Furthermore, successful manipulation of the circulation in animal models of low-flow states does not translate consistently to corresponding human pathological studies.

Advances in imaging technology have created the ability to assess not only organ structure but also function and perfusion. Functional assessment in the gastrointestinal system has allowed non-invasive measurement as diverse as organ water content, fat content, gut motility, intestinal permeability and metabolic activity.

Both magnetic resonance imaging (MRI) and computed tomography (CT) are used during physiological stress to assess perfusion in organs such as the brain, heart and kidneys. The hepato-splanchnic circulation has traditionally been more challenging to image and assess, owing to the motile nature of the intestine and also due to the

dual hepatic blood supply. Hence, perfusion-based imaging has yet to be robustly applied to this circulatory system. However, recent advances in dynamic, contract-enhanced CT imaging have demonstrated accuracy and precision in measurement of the dual venous and arterially-derived hepatic circulation [99].

7 Hypothesis and Aims

7.1 Formulation of the hypothesis

HD exerts haemodynamic stress upon a circulation that is vulnerable to demand ischaemia. This results in measurable reduction in organ blood flow and cumulative ischaemic injury, as demonstrated in the heart and brain [8].

The acute and cumulative effects of HD upon the liver are unknown. It may be uniquely vulnerable to ischaemia in the ESRD patient due to a combination of factors including fluid overload, endotoxemia, oxidative stress and autonomic dysfunction. Furthermore, the liver may have a hitherto unappreciated but significant role as part of the functional barrier to both bacterial and uremic toxin translocation from the gut milieu, as well as in the initial haemodynamic response to the stress of dialysis, being a major organ of capacitance.

7.2 The hypothesis

The liver is affected by the combined circulatory stress of HD and ESRD. Hepatic capacitance plays a role in the maintenance of haemodynamic stability during HD, leading to a fall in liver stiffness.

Hepatic perfusion falls during HD and leads to temporary functional compromise.

7.3 Aims

The specific aims of this work are to:

- Describe the relationship between hepatic congestion and haemodynamic stability during dialysis
- Describe the impact of HD upon hepatic congestion
- Describe the effect of HD upon liver blood flow
- Describe the effect of HD upon hepatic function

Chapter 2: Liver Stiffness in HD Patients – the effects of dialysis

Declaration of Contributors

Dr Susan Huang assisted in obtaining bioimpedance measurements.

1 Introduction

The liver plays an active role in the systemic response to circulatory stress. It contributes to the augmentation of cardiac preload in response to a fall in circulating volume through active venoconstriction. During periods of elevated venous pressure or hypervolaemia, a fall in hepatic venous resistance leads to accommodation of additional volume. Therefore, the liver's ability to adapt to support the systemic circulation in periods of stress is important in the HD patient, potentially affecting the evolution of symptoms associated with fluid overload and the development of IDH. However, this aspect of circulatory function in HD is relatively unstudied.

Liver stiffness (LS) describes a quantitative measure of the resistance of the liver to deformation. It is affected by several factors including fibrosis, inflammation and, importantly, blood flow. Fluid overload has been shown to affect LS. Millonig et al. showed in 2010 that acute elevation of central venous pressure (CVP) in pigs significantly yet reversibly elevated LS, as well as a significant elevation in human subjects with decompensated heart failure [64]. Hopper et al. went on to show that both left and right ventricular failure were associated

with elevated LS compared to controls, with a greater increase in right-heart failure (median 9.7kPa vs. 4.7kPa) [100]. However, unlike Millonig et al., they found that acute diuresis failed to affect LS. Taniguchi et al. showed that LS of above 10.6kPa was a sensitive marker for raised right atrial pressure (RAP) and compared favourably to echocardiography in detection of right-sided heart failure [101].

In HD, one small study demonstrated that change in liver stiffness after HD was proportional to net fluid withdrawal [102], whereas another showed a tendency for LS to rise with HD [103].

This study was therefore designed to assess whether liver stiffness can be used to detect fluid overload in HD patients and to describe the effect of HD upon liver stiffness, specifically in relation to haemodynamic stability during HD.

Hypothesis:

Liver stiffness is proportional to fluid status in HD patients and decreases after HD. Absence of a fall in liver stiffness is associated with IDH.

Study Objectives

- Describe the relationship between liver stiffness and other measures of hydration in HD patients
- Describe the relationship between liver stiffness and haemodynamic stability during dialysis
- Describe the impact of HD upon liver stiffness
- Assess the feasibility of using the Fibroscan device as a tool to assess hydration in HD patients

2 Methods

2.1 Study Design

This was a cross-sectional non-invasive study involving a single centre recruiting participants for a single study visit. The study measurements were taken before and after a participant's dialysis session, in their usual place of care. Subjects for the proposed study were drawn from the population of HD patients enrolled in the London Health Sciences Centre Renal Program in London, Ontario.

2.1.1 Screening and Consent:

Potential study candidates were identified by a dialysis nurse familiar with all patients in the HD Program. At a routine dialysis visit, the nurse asked the patient whether they were happy to be introduced to the research team. The research team member then explained the study and gave patients an opportunity to ask questions. All patients were given written information to take home and review. All patients willing to participate in the study were required to sign and date the informed consent form (ICF) before any study-related assessments were performed.

2.1.2 Eligibility

To be eligible to participate, patients had to meet all of the inclusion criteria, and none of the exclusion criteria.

Inclusion criteria:

- Patients who have been on HD for at least 3 months

Exclusion criteria:

- Patients who have not given consent
- Patients who have a known history of liver cirrhosis, hepatitis C or hepatitis B
- Patients who have bilateral leg-amputations
- Patients who have BMI > 30 kg/m^2

2.2 Study Procedures

Once participants gave their informed consent, the study date was arranged. There were no specific instructions for preparation for the study day and participants continued to take their medication as normal. Subjects were asked to fast throughout dialysis.

All dialysis treatments were delivered using Fresenius 5008 machines (Fresenius Medical Care, Bad Homburg, Germany) according to the participants' usual prescriptions determined by their nephrologist.

The study day consisted of the following interventions before and after dialysis:

2.2.1 Collection of personal characteristic data

- Demographics
- Primary aetiology of disease
- Dialysis history and prescription
- Co-morbidities
- Cardiac status – risk factors, vascular history, ECG and echocardiogram findings, coronary angiography
- Medication
- Alcohol and smoking history

2.2.2 Physical Examination

Participants underwent a cardio-respiratory examination including the measurement of BP. This was performed by a physician in appropriate clinical conditions.

2.2.3 Blood tests

Blood samples were taken for standard biochemical and hematological tests pre and post dialysis at the visit. These included urea, creatinine, sodium, potassium, calcium, magnesium, phosphate, albumin, bicarbonate, full blood count and liver function tests.

2.2.4 Measurement of Liver Stiffness

A non-invasive medical device, FibroScan®, has been devised for the measurement of liver stiffness. It uses ultrasonic technology, with both high and low frequency elastic waves (50 Hz to 5 MHz) produced by a specialized ultrasound probe which is applied to the body wall. It is coupled with 1D ultrasound imaging that measures the propagation speed of these waves. Liver stiffness is calculated from the velocity of the waves in a ROI of liver tissue approximately 2-4cm below the body wall using the following equation [104]

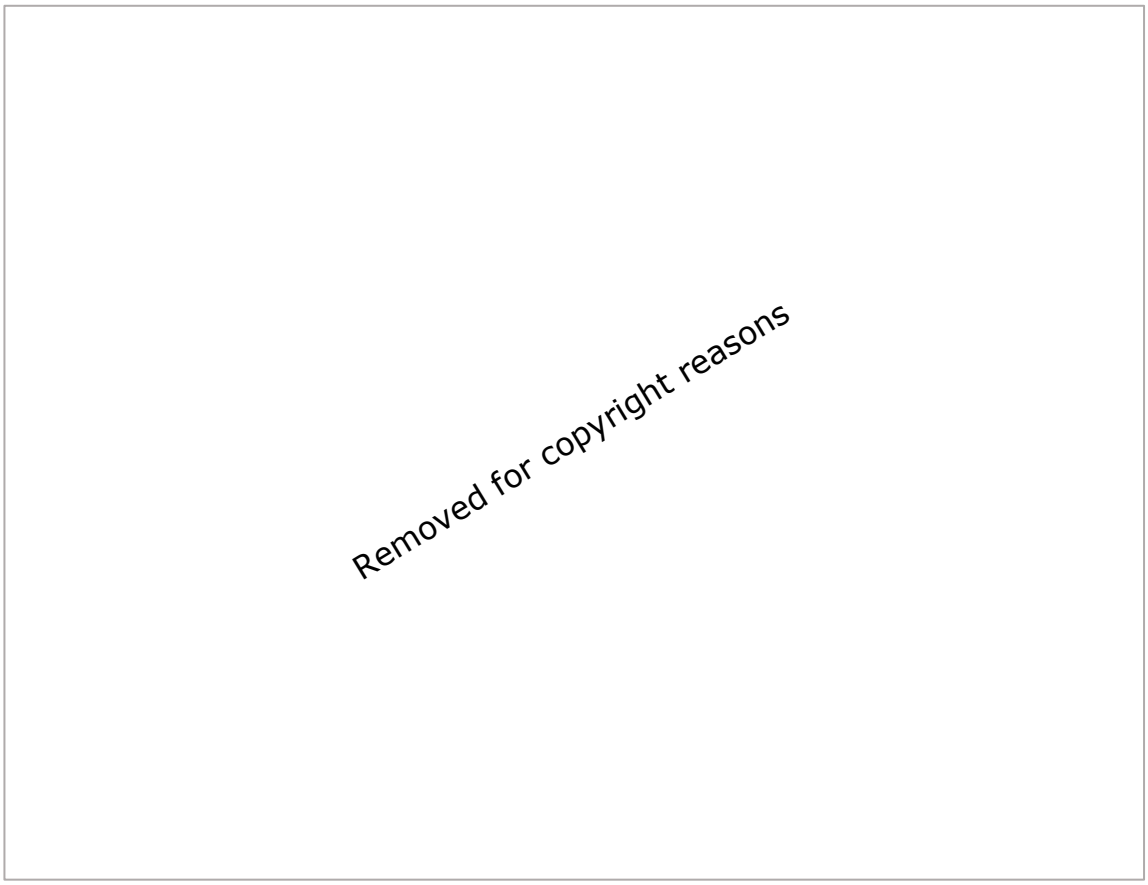
$$E = 3\rho Vs^2$$

where E is the stiffness, ρ is the density and Vs is the shear velocity. This provides a rapid quantitative assessment of the hepatic stiffness which takes less than 5 minutes to collect. It has been shown to have excellent intra and inter-observer reliability [104] and is now widely used in clinical practice.

Transient elastography measurement using Fibroscan® is considered to be a reliable method for the diagnosis of significant fibrosis (AUC = 0.84), severe fibrosis (0.89), and cirrhosis (0.94) [105]. However, stiffness measurements also reflect liver congestion, and are directly affected by central venous pressure and hypervolemia [64,100]. The

Fibroscan® therefore represents a potentially useful non-invasive tool in the assessment of liver congestion, as well as structural damage.

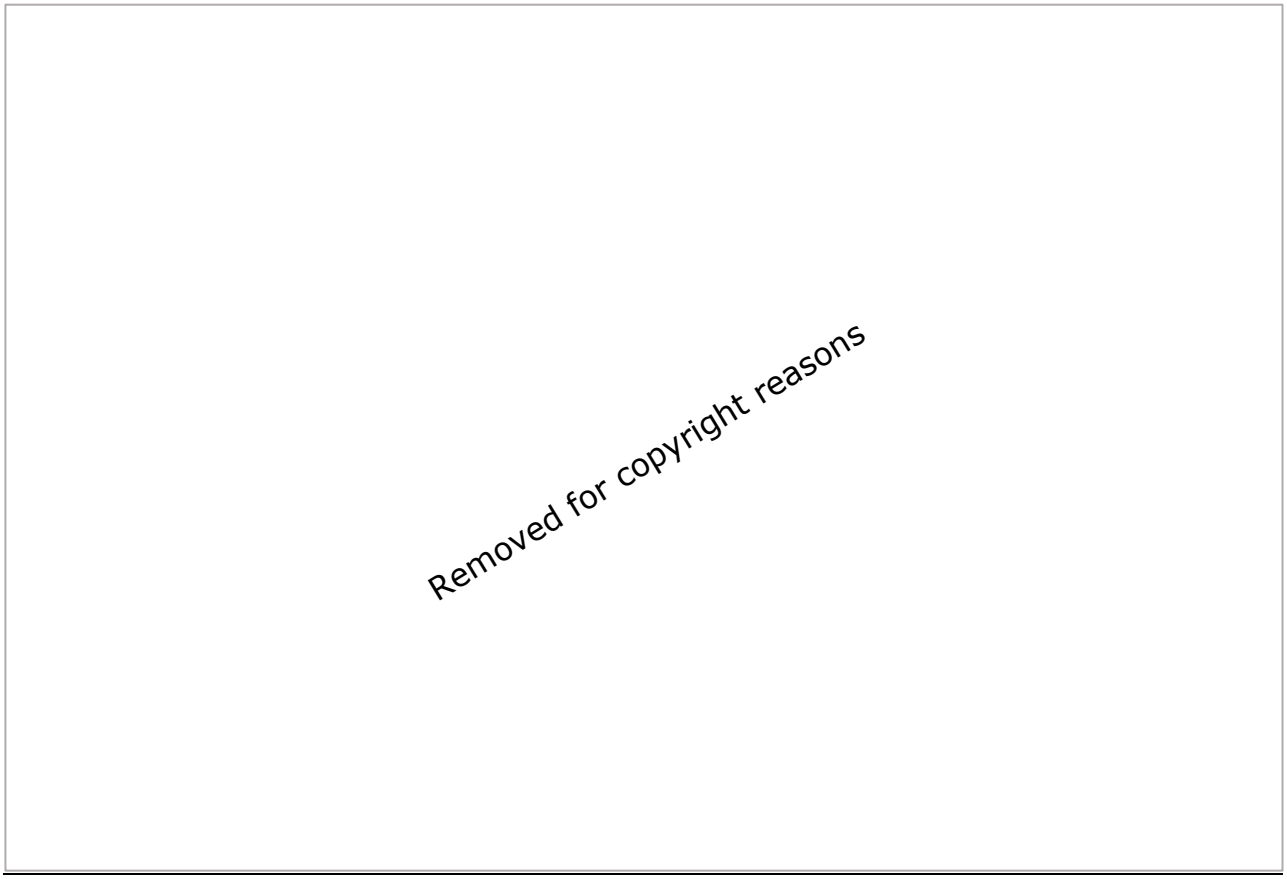
Figure 10: A Fibroscan® machine and probe



For this study, Fibroscan® measurement was performed both pre and post dialysis. Measures of liver stiffness were obtained by a single, trained operator (CG) using transient elastography (Fibroscan® GE Healthcare). Measurements were obtained by the conventional clinical method, with the probe applied in the mid-axillary line to an appropriate right intercostal space, with the subject supine. Adequate

positioning of the probe over the liver was confirmed using both the M mode and A mode ultrasound images (see Figure 11). Ten valid measurements were obtained and the median value and IQR were recorded. Unreliable results were deemed as those with an IQR of less than 33% of the median or in those in whom fewer than 10 valid results could be obtained.

Figure 11: Example of Fibroscan® data output

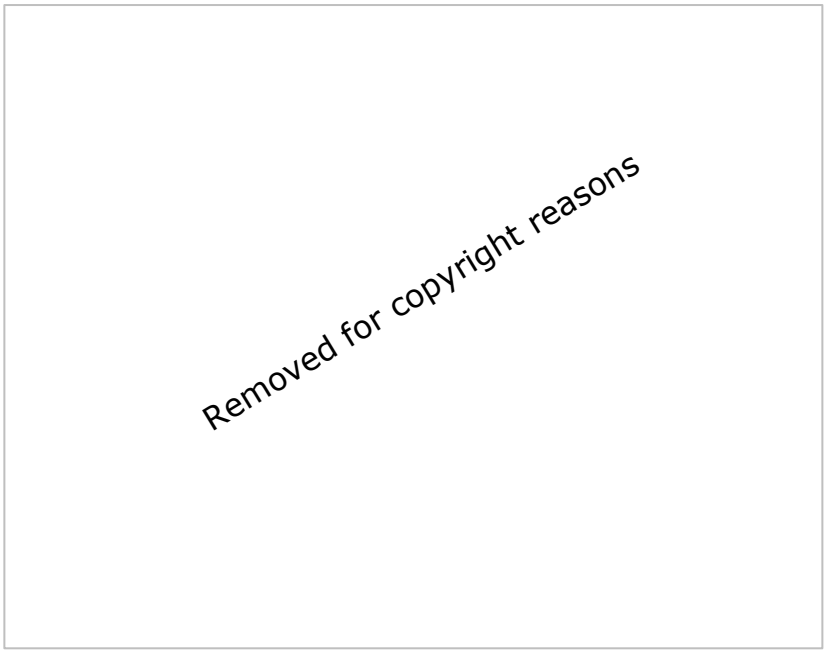


2.2.5 Bioimpedance Analysis

Bioimpedance analysis (BIA) is a non-invasive technique to assess body composition. In hemodialysis, it has been studied as a tool to assess fluid status and estimate dry weight. It has been shown in non-dialysis patients to correlate with the gold-standard assessment of total body water (TBW) – isotope dilution [106].

The speed of passage of a small alternating electric current through the body is measured and the relative electrical resistance and reactance of different body tissues is assessed. The measurement of resistance provides data pertaining to the water content and the reactance to the cell mass of the tissue measured. These values may then be transformed into indirect measures of total body water, extracellular and intracellular volume, muscle and fat mass using anthropometric data, with the application of corrections for gender, BMI and tissue composition. Multifrequency analysis – the use of multiple electric frequencies to assess resistance and reactance – improves the accuracy of calculation of both intracellular and extracellular volume.

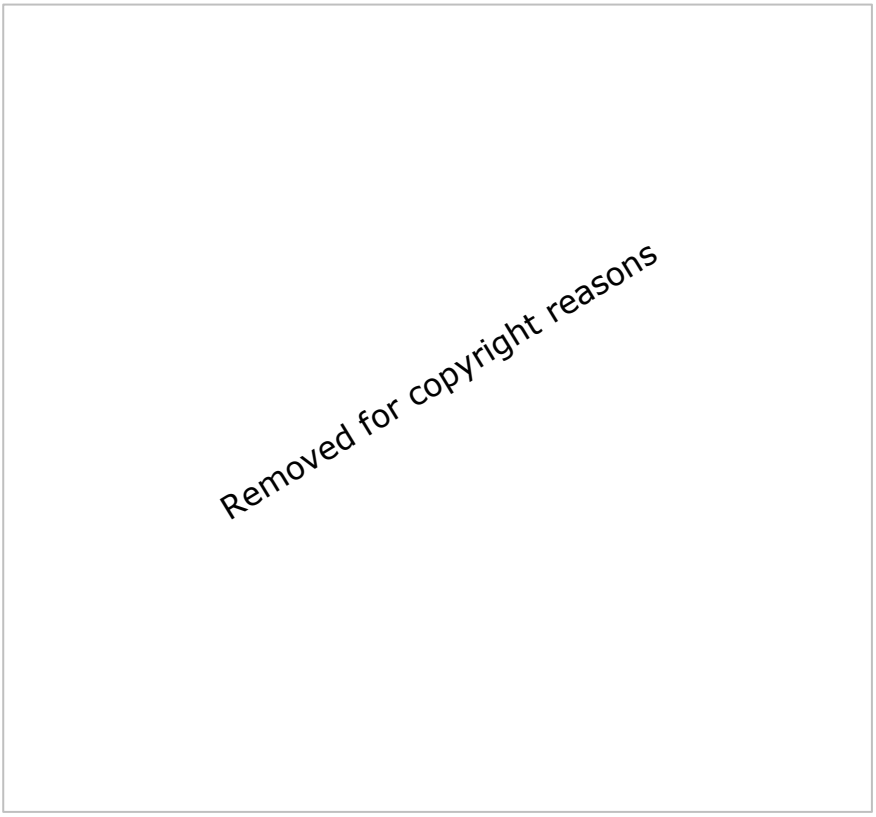
Figure 12: Graph to illustrate the agreement between gold-standard isotope dilution calculations of extracellular water (ECW ref) and bioimpedance derived measurement (ECW BCS) from Moissl et al. [106]



3

One device to assess bioimpedance is the Body Composition Monitor® (BCM, Fresenius Medical Care, Germany) which has been validated [107,108] and is now widely used in clinical practice.

Figure 13:BCM®, Fresenius Medical Care, Bad Homburg, Germany



For this study, body composition and assessment of total body water (TBW) were measured using a multifrequency BIA analyzer (Body Composition Monitor®, Fresenius Medical Care, Germany). Measurements were performed using the standard tetrapolar

technique, with electrodes placed at both wrists and both ankles. The patient was supine for at least 10 minutes before measurements were taken. The extracellular fluid space was expressed both as an absolute volume and as a proportion of the estimated TBW (E/I ratio).

3.1.1 Participant Withdrawal

Subjects were withdrawn from the study if they withdrew consent at any time or if it was deemed unsafe or impossible to proceed with the study procedures. If a patient failed to attend for their visit a member of the research team tried to contact them by telephone within 24 hours of their appointment before stating that they had been lost to follow up. The participants were aware that withdrawal would not affect their future care. Participants were made aware (via the information sheet and consent form) that, should they withdraw, the data collected to that date cannot be erased and may have still been used in the final analysis.

3.2 Outcome Measures

Primary Outcome:

- Liver stiffness in kPa pre and post HD

Secondary Outcomes:

- Change in weight in kg pre and post HD
- Change in total body water pre and post HD
- The presence of intra-dialytic hypotension during HD
- UF volume during HD
- Change in extracellular and intracellular water pre and post HD
- Liver function tests pre and post HD

3.3 Data, Safety and Specimen Monitoring

3.3.1 Data monitoring

Data collection forms were completed by the research team. Upon completion the forms were housed in duplicate within the Kidney Clinical Research Unit (KCRU) at Western University (UWO). Quality control included regular data verification and protocol compliance checks by the research team.

Study data was collected and recorded on the Data Collection Form by members of the research team. On all data collection forms and study documents, specific patient data was identified with unique study identifiers. A master list that linked patient identifying information to the unique identifier was stored separately from any other study documents, in a secure location, on a network drive on the London Health Sciences Centre (LHSC) network. Only study investigators and staff involved in the study had authorization to access study data. All study data will be securely stored in the KCRU storage facilities for 5 years. At the end of the storage period, all study related paper and electronic stored information will be destroyed by a bonded company according to local healthcare organisation privacy standards.

3.3.2 Patient Blood Samples

Samples were sent to the LHSC core labs. Test results were obtained by chart review by the research team. Samples analyzed at the LHSC core lab were stored until the results were confirmed, then destroyed as per routine lab biohazard procedures. No samples were stored as part of this study.

3.3.3 Safety monitoring

An adverse event was defined as any unfavourable and unintended sign, symptom, syndrome or illness that developed or worsened during the period of observation in the study. The overall risk of an adverse event occurring that was directly connected to this study was deemed to be extremely low, given the non-invasive nature of the study procedures.

A Serious Adverse Event (SAE) was defined as any adverse event occurring following study mandated procedures, directly related to the treatment or intervention that results in any of the following outcomes:

1. Death
2. A life-threatening adverse event
3. Inpatient hospitalization or prolongation of existing hospitalization
4. A disability / incapacity
5. A congenital anomaly in the offspring of a participant

All study-related serious adverse events were recorded and reported to the Research Ethics Board (REB). Unexpected serious adverse events were reported within the mandated timeframes to the REB. All

AEs were documented in detail on the appropriate data collection form.

The following are recognized complications related to chronic HD and were recorded on the CRF but were not reported separately on an AE/SAE form:

- Dizziness, cramps or headache during or after dialysis session
- Hospital admission for cardiovascular disease, arrhythmias, stroke, pneumonia, infections
- Death

There were no specific anticipated risks from participating in this study. Both Fibroscan® measurement and bioimpedance analysis are painless and have no reported side effects.

3.4 Analysis Plan and Sample Size Calculation

3.4.1 Analysis Plan

All analyses were conducted using SPSS version 22 software (IBM, New York). Continuous data were analyzed for normal distribution. Mean and standard deviation are reported for normally distributed data; otherwise, median and inter quartile range (IQR) are given. Pre and post-HD variables were compared using the paired t test (parametric data) or Wilcoxon signed rank test (non-parametric

data). The amount of total excess body fluid prior to the HD treatments, and the amount of fluid removed during HD, were compared to the changes in FibroScan® values pre and post-HD, using Pearson's or Spearman's correlation coefficient, depending on distribution. An alpha error of less than 0.05 was deemed statistically significant.

3.4.2 Sample Size Calculation

There have been limited studies assessing the effects of HD haemodynamic changes, including volume congestion and hypoperfusion from fluid removal, on liver stiffness measured by FibroScan®. Therefore, the number of patients required to adequately assess these differences was difficult to determine beforehand. In this pilot study, a total of 55 patients was selected, based on an estimate of correlation between liver stiffness and a bioimpedance-derived measure of overhydration (excess fluid). If it were assumed that the coefficient of determination (r^2) lay between 0.13-0.75, which corresponds to an effect size (f^2) between 0.15-3, with a power of 0.8 and alpha error of 0.05, the minimum sample size required was 55 (using G*Power® 3.1.5.1 Sample Size Calculator) [109].

Table 3: Sample size calculation for estimated effect size

Effect size f^2 (r^2)	0.15 (0.13)	1 (0.5)	3 (0.75)
Number of subjects required	55	11	7

3.5 Ethics

The study was approved by the Western University Health Sciences Research Ethics Board (HSREB ref: 106868) prior to initiation of any study related procedures. The trial was conducted in compliance with the protocol, the International Council on Harmonisation Good Clinical Practice (ICH/GCP) guidelines and all applicable regulatory requirements.

4 Results

4.1 Study Participants

Seventy-two patients consented to be part of the study from February 2015 to April 2016. Ten subjects were excluded due to the presence or development of exclusion criteria and three subjects withdrew consent prior to any study procedures. Five subjects did not complete the study (four due to inability to acquire reliable elastography readings), leaving fifty-four subjects. Demographics and the medical background of the included population are shown in Table 4.

Median IDWG was 2.1kg prior to dialysis. Mean UF was 25.9±13ml/kg with total ultrafiltration volumes ranging from 0–4000mL. Pre-dialysis bloodwork revealed that 32 out of 54 subjects were anaemic, with haemoglobin of less than 110g/L. Liver biochemistry tests (bilirubin, aspartate aminotransferase, gamma glutamyltransferase) were within normal range for 34 out of the 45 subjects for which results were available. Of the 11 subjects with abnormal liver tests, 5 had raised ALP alone, two had raised ALP and GGT alone, one had raised ALT alone, one had raised bilirubin alone and one subject had raised ALP, GGT, ALT, AST and normal bilirubin levels. This patient was known to

have chronic pancreatitis, with normal liver imaging and chronically, stable abnormal liver biochemistry.

Table 4: Study population characteristics

	Participants (n=54)
Age (yrs)	68.0±15
BMI (kg/m^2)	26.9±2.3
Gender (M/F)	37/18
Aetiology of ESRD (DM/HTN/Other)	22/13/19
Duration of ESRD (months)	16.5(45)
Duration of HD (months)	14.0 (38)
Previous Transplant (Y/N)	6/48
History of CHF (Y/N)	18/36
History of IHD (Y/N)	20/34
History of Diabetes (Y/N)	23/31
ACEi/ARB (Y/N)	15/39
Beta blocker (Y/N)	29/25
Diuretic use (Y/N)	14/40
Systolic BP (mmHg)	143±27
Diastolic BP (mmHg)	64.4±15
Hb (g/l)	104±17
Sodium (mmol/l)	137±11
Albumin (g/l)	37.8±6.5
Calcium (mmol/l)	2.21±0.4
Phosphate (mmol/l)	1.83±0.5
Bilirubin (µmol/L) (n=45)	5.00(2.4)
ALT (IU/L) (n=45)	14.0 (8)
ALP (IU/L) (n=45)	82.0 (51)
GGT (IU/L) (n=45)	22.5 (22)

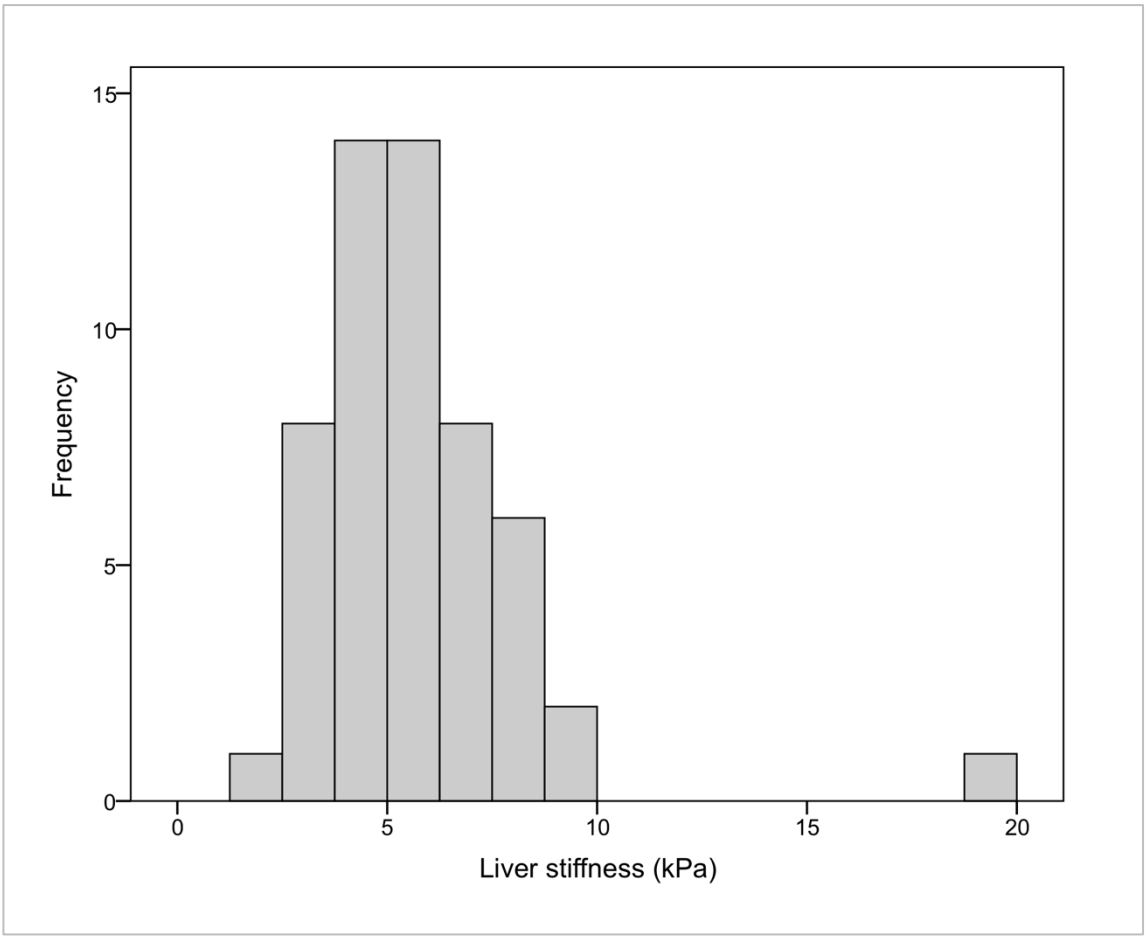
Table 5: Subjects' Dialysis Characteristics

IDWG (kg)	2.10 (2.3)
Total Ultrafiltration (mL)	1970±1000
UF by weight (mL/kg)	25.9±13
Access used (CVC/AVF)	40/14
HD duration (4/3.5/3hours)	24/20/10

4.2 Liver stiffness

Pre-dialysis liver stiffness (LS) was 5.30(3.8) kPa. Five participants had stiffness values above 7.5kPa, including one patient with significantly elevated value of 19.5kPa. This subject was a 43-year-old man with no specific identifiable risk factors for liver cirrhosis. He had end-stage renal disease due to glomerulonephritis and had been receiving dialysis for 15 years, having also had a failed renal transplant four years previously. The success rate for measurement of this subject's liver stiffness pre-dialysis was 91% with an IQR of 2.6kPa for the 10 successful readings. This subject was included in further analyses but also excluded from specified analysis to examine the effect of this extreme outlier.

Figure 14: Distribution of liver stiffness pre-dialysis in HD patients, showing all but one patient with LS<10kPa.



Pre-dialysis LS was not associated with age, gender, BMI, a history of transplant or dialysis vintage. LS was not significantly different in those with a history of ischaemic heart disease (IHD), cardiac failure or diabetes, nor was it significantly different in those taking diuretics.

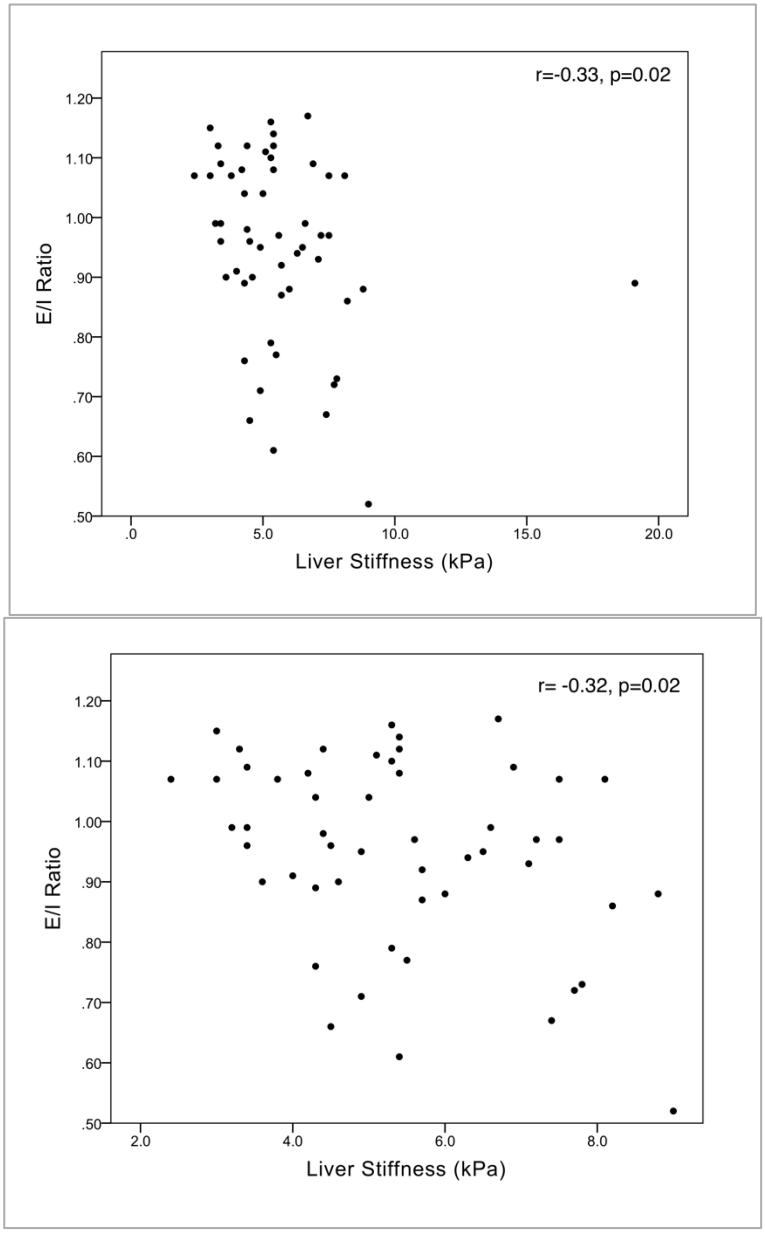
Pre-dialysis LS was higher in those with a higher GGT level (r=0.34, p=0.037), however it was not significantly associated with other measures of liver biochemistry.

Pre-dialysis LS was not associated with pre-dialysis weight, BP, bioimpedance-derived total body water or overhydration volume. However, a higher liver stiffness was weakly associated with a significantly lower E/I ratio (r=-0.35, p=0.03). The subject with significantly elevated LS was 1.5kg under their target weight, although bioimpedance indicated that they had 2.2L excess fluid and their BP was 165/97.

Table 6: Liver stiffness and markers of hydration

	With high LS patient		Without high LS patient	
Hydration Measure	R value	P value	R value	P value
Amount over target weight (kg)	0.03	0.84	0.09	0.53
Total body water (L)	0.16	0.25	0.12	0.38
Extracellular water (L)	0.05	0.72	-0.04	0.79
E/I ratio	**-0.33**	**0.02**	**-0.32**	**0.02**
Overhydration volume (L)	-0.09	052	-0.08	0.58
Systolic BP (mmHg)	0.02	0.87	-0.08	0.58
Diastolic BP (mmHg)	0.16	0.22	0.15	0.29

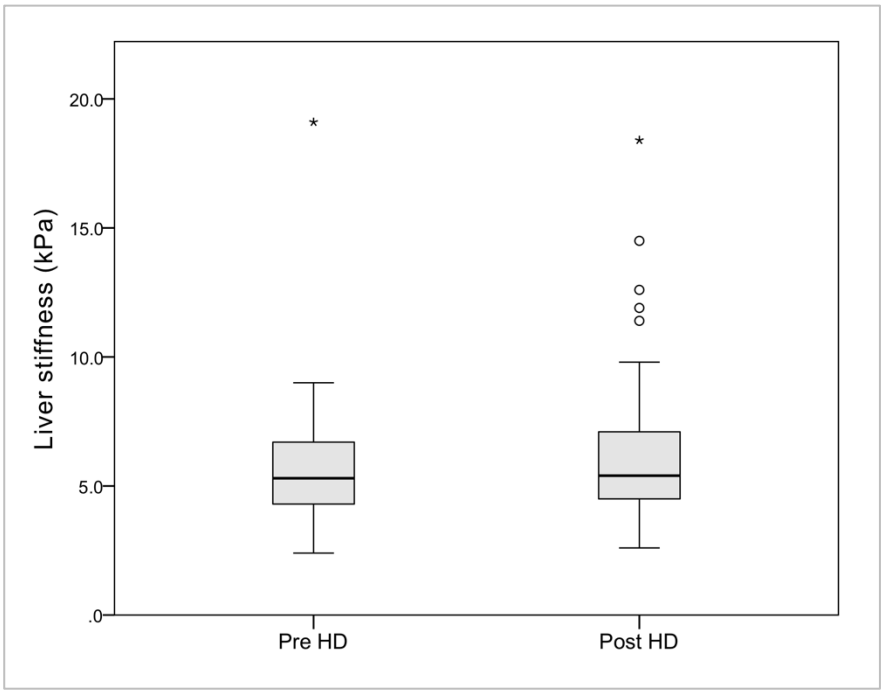
Figure 15: The relationship between E/I ratio and liver stiffness, demonstrating a significantly lower E/I ratio in those with higher LS. The top panel includes the extreme LS outlier, bottom panel excludes the outlier.

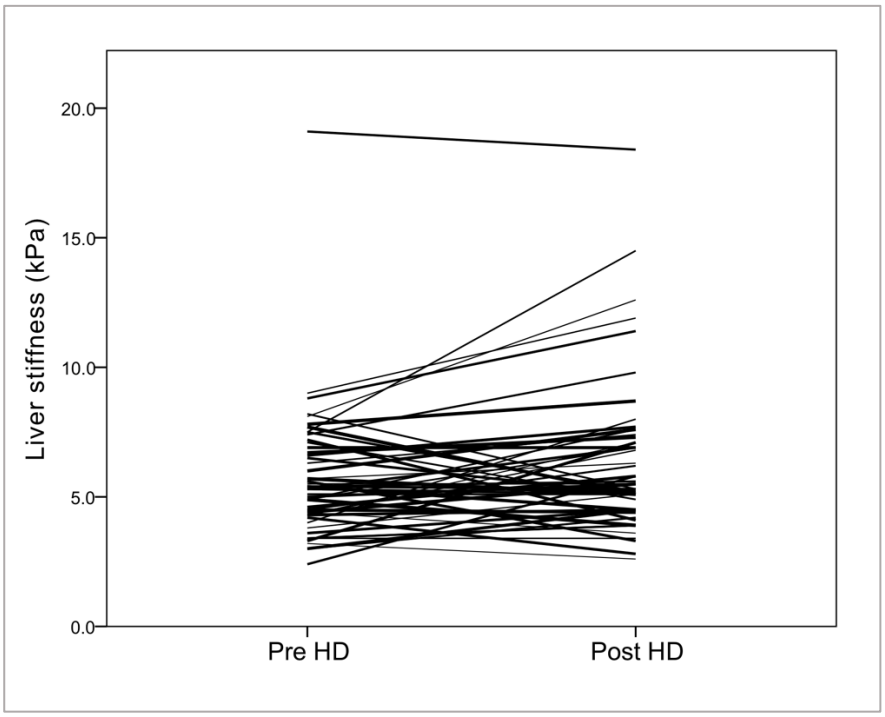


Liver stiffness did not significantly fall after dialysis as hypothesised. Conversely, there was a small but statistically significant change in LS post-dialysis with a mean increase of 0.65kPa (95% confidence interval 0.12-1.19kPa). There was wide variability observed with the change in LS ranging from a fall of 3.10kPa to an increase of 7.00kPa.

The change in LS remained statistically significant after exclusion of the extreme outlier. This subject's LS fell by 0.7kPa.

Figure 16: Change in liver stiffness after HD, demonstrating the lack of consistent response. The top panel is a boxplot of the group change; for the bottom panel, each line is individual change in LS with HD.





There was no clear relationship between the magnitude or direction of change in LS and ultrafiltration volume; nor was there a relationship with markers of hydration, including change in weight, total body water or ECW.

Table 7: Characteristics according to liver stiffness response to HD

	Up (n=31)	Down (n=23)	P value
Age (yrs)	69.2±14	67.5±15	0.69
Time on HD (months)	12(35)	18(61)	0.28
CHF (%)	26%	41%	0.25
Diabetes (%)	42%	41%	0.94
Pre-dialysis SBP (mmHg)	138±28	151±27	0.09
Pre-dialysis DBP (mmHg)	63.0±13	64.0±15	0.80
Pre-dialysis Over TW (kg)	2.09±1.8	1.75±1.5	0.48
Pre-dialysis TBW (L)	37.9±6.4	34.8±9.0	0.16
Pre-dialysis Overhydration (L)	1.16±2.1	0.89±1.2	0.60
Pre-dialysis ECW (L)	18.1±3.2	16.7±3.6	0.15
Pre-dialysis E/I ratio	0.94±0.2	0.96±0.2	0.67
SBP <100 during HD (%)	48%	32%	0.23
Ultrafiltration volume (mL/kg)	25.7±15	26.1±11	0.91
Reduction in TBW (L)	0.89±7.0	1.99±2.8	0.50
Post-dialysis Overhydration (L)	-0.68±1.9	-1.00±1.9	0.56
Pre-dialysis liver stiffness (kPa)	5.21±1.8	5.65±1.4	0.34

Neither the direction nor the magnitude of change were significantly associated with age, dialysis vintage, presence of cardiac disease, markers of hydration or ultrafiltration volume. There was a tendency for those with a rise in liver stiffness to have a lower pre-dialysis systolic BP, although this was not statistically significant (p=0.09).

Eight subjects (excluding the subject with LS in cirrhotic range) had liver stiffness values above 7.5kPa after dialysis. Only 50% of these subjects had pre-dialysis values over 7.5kPa. Liver stiffness did not correlate significantly with any of the clinical or bioimpedance markers of hydration post-dialysis, including E/I ratio.

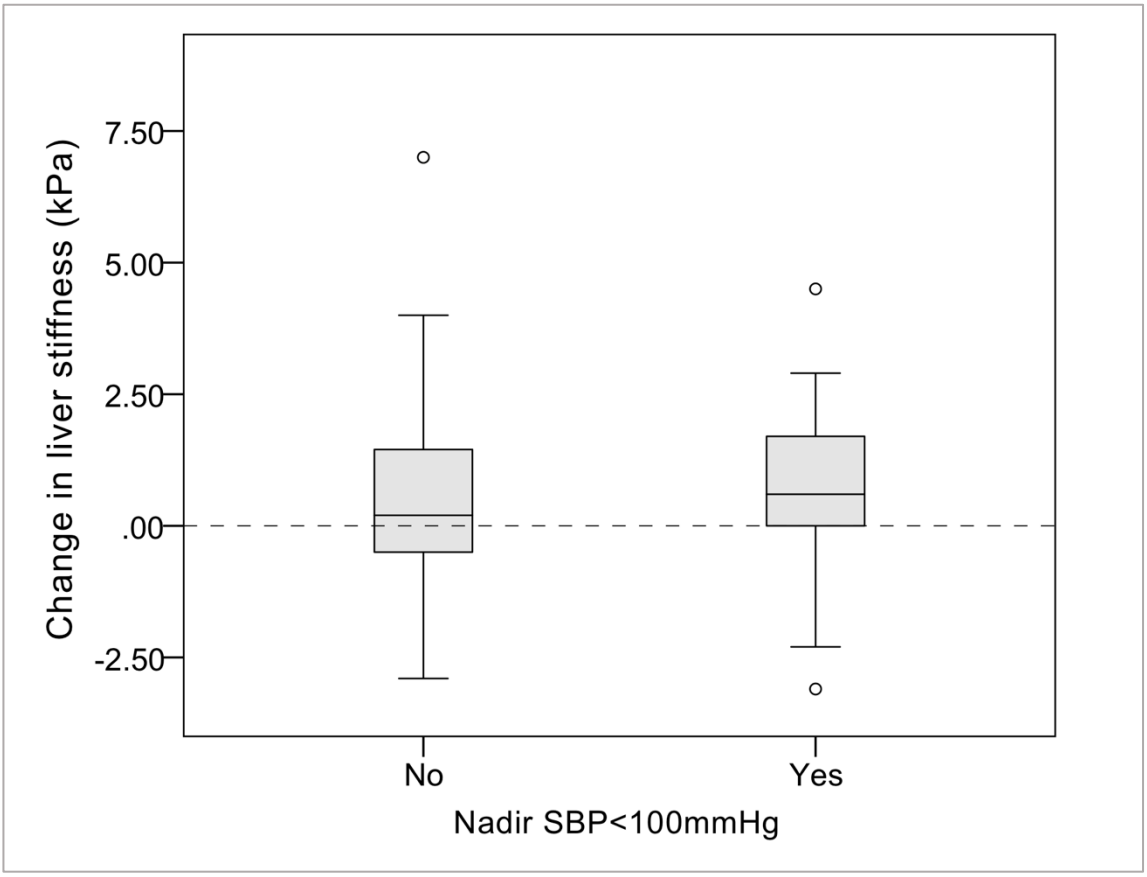
4.3 Haemodynamic Instability

Nineteen subjects experienced a fall in systolic BP below 100mmHg during dialysis. Fifteen subjects experienced a fall of at least 40mmHg from baseline. Nine patients experienced symptoms associated with hypotension during dialysis (six with cramps, three with severe headache). One subject had a syncopal event, associated with profound hypotension, towards the end of dialysis with a transient loss of consciousness.

Age, dialysis vintage and UF volume did not predict haemodynamic stability during dialysis in this cohort. Pre-dialysis bioimpedance-derived measures of hydration were also not associated with intra-dialytic hypotension. As expected, there was a significant association between pre-dialysis BP and intra-dialytic hypotension (systolic BP 126±26mmHg vs. 152±26mmHg, p=0.004).

Those who had intra-dialytic hypotension during HD (defined as nadir systolic BP<100mmHg) did not exhibit a significantly different pre-dialysis liver stiffness (p=0.82) or change in liver stiffness (p=0.70). However, liver stiffness increased in 68.1% of those with IDH, compared to 50% in those without. Change in liver stiffness in those with IDH was 0.60(2.3)kPa compared to 0.10(2.1)kPa in those without.

Figure 17: Boxplots to demonstrate the change in liver stiffness in subjects with IDH and those without. Although there is no statistical significance, a higher proportion of those with IDH exhibited a rise in LS post HD.



4.4 Gamma Glutamyltransferase

GGT was measured in 45 subjects pre-dialysis with levels ranging from 4 to 137IU/L, median 22IU/L. Post-dialysis levels were significantly higher ($p=0.016$, Wilcoxon signed rank test) than pre-dialysis values, with a median (range) of 25 (9-144IU/L). There was a significant association between change in GGT and change in haematocrit to suggest the effect of haemoconcentration ($r=0.75$, $p<0.001$). Serum levels both pre and post dialysis were significantly associated with higher liver stiffness ($r=0.39$, p-0.008 pre HD; $r=0.45$, $p=0.003$ post HD, excluding extreme outlier; $r=0.41$, $p=0.004$ pre HD, $r=0.33$, $p=0.029$, post HD including extreme outlier). Haematocrit was not significantly associated with liver stiffness.

Figure 18: Pre-dialysis GGT is higher in those with higher liver stiffness. This figure excludes the extreme outlier (n=44)

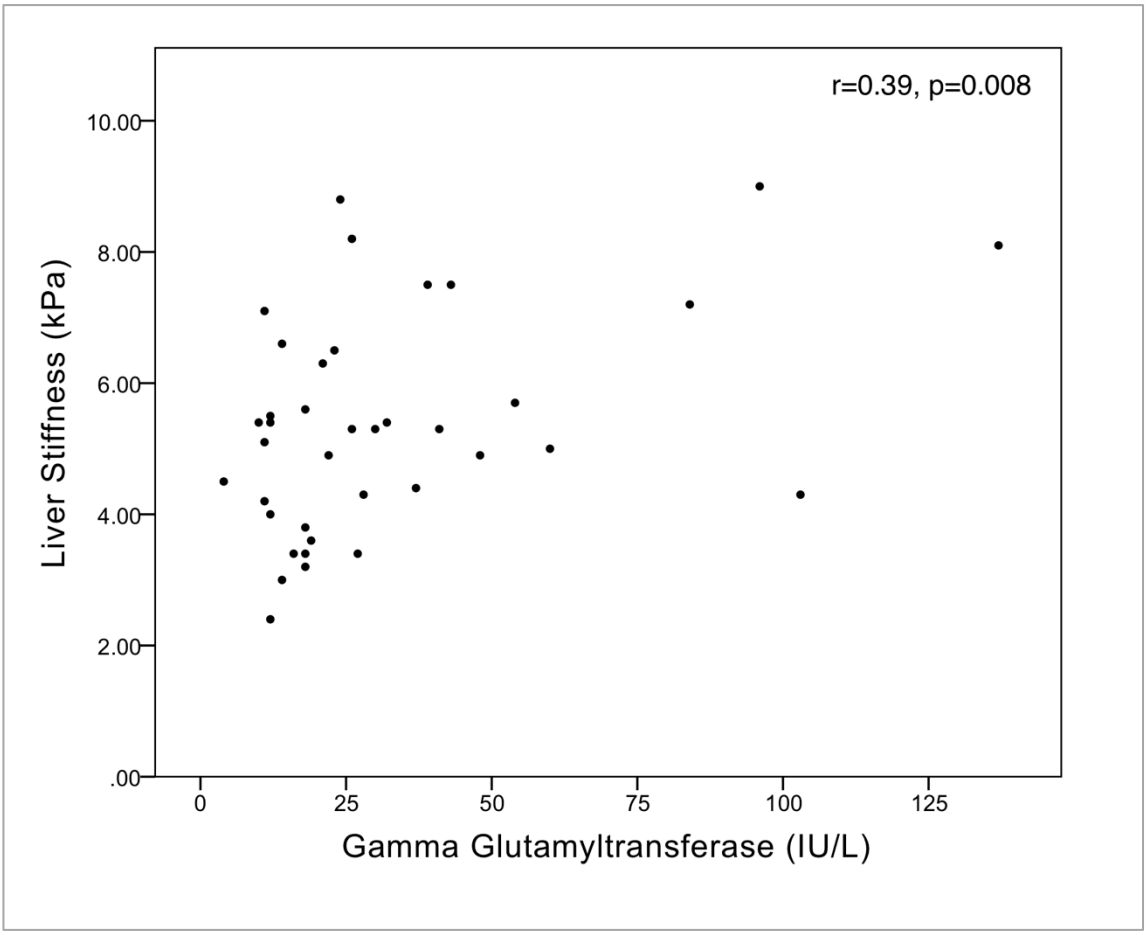
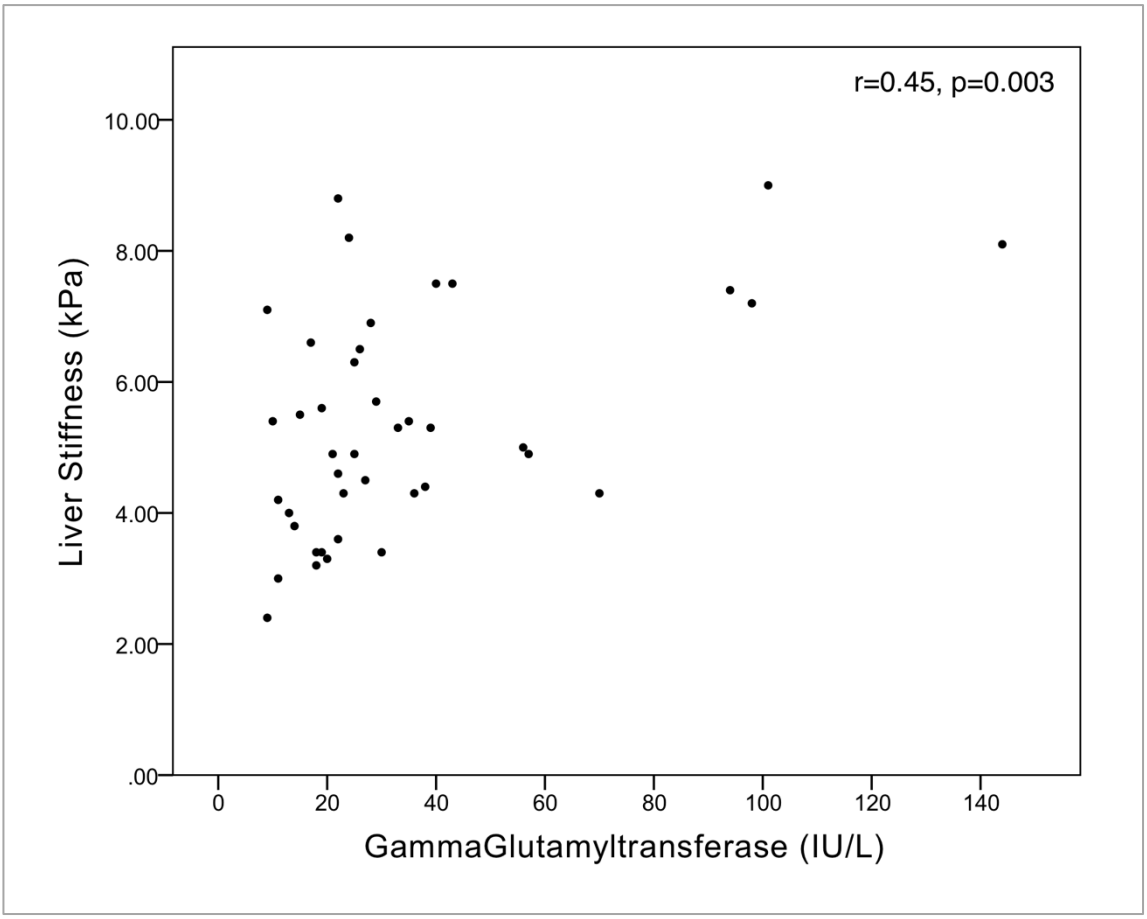


Figure 19: Post-HD GGT correlates with liver stiffness. This figure excludes the extreme outlier (n=44)

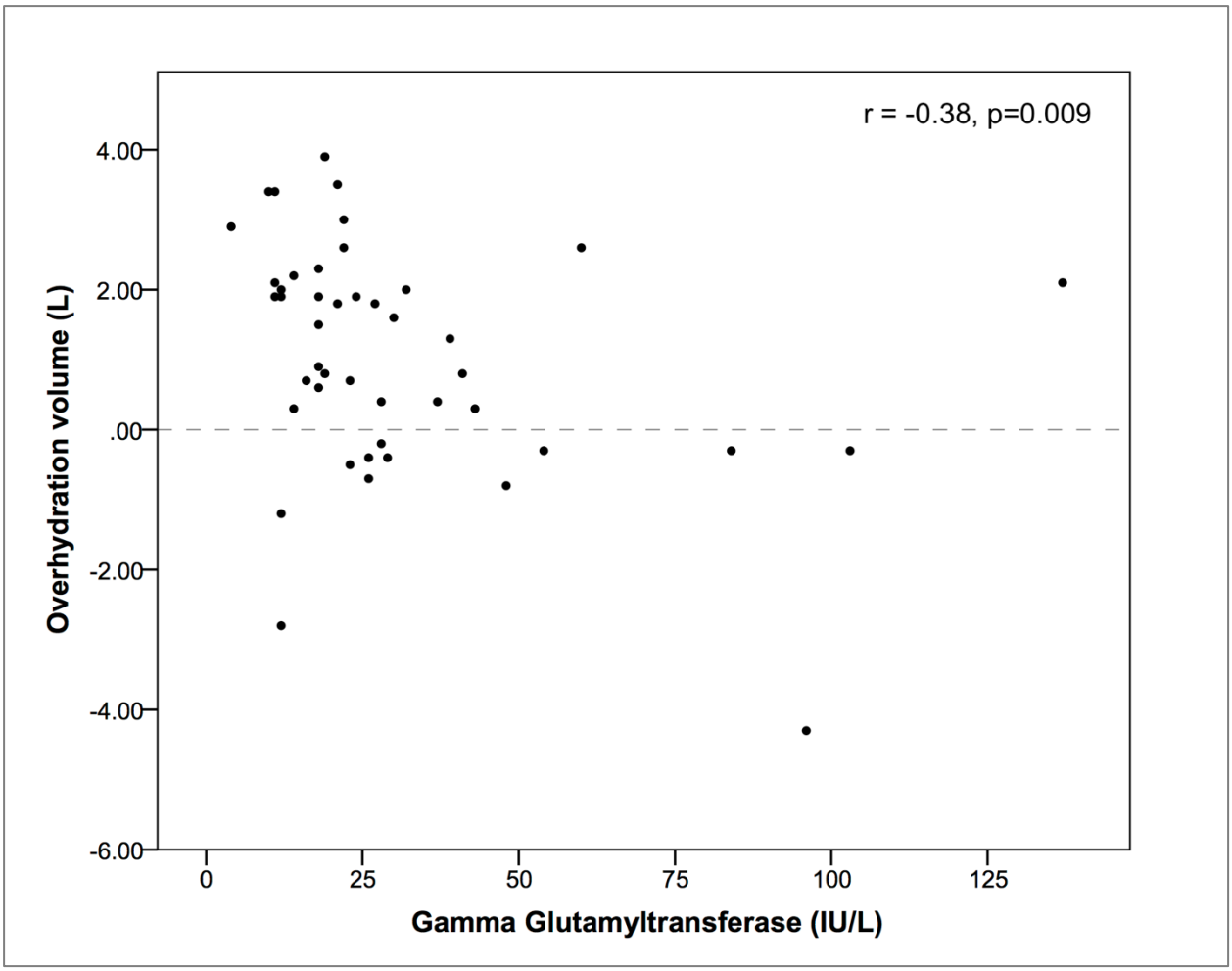


Fluid overload, as measured by bioimpedance was associated with a significantly lower GGT pre-dialysis (r=-0.38, p=0.009), but not post-dialysis.

Table 8: Relationship of GGT with markers of hydration

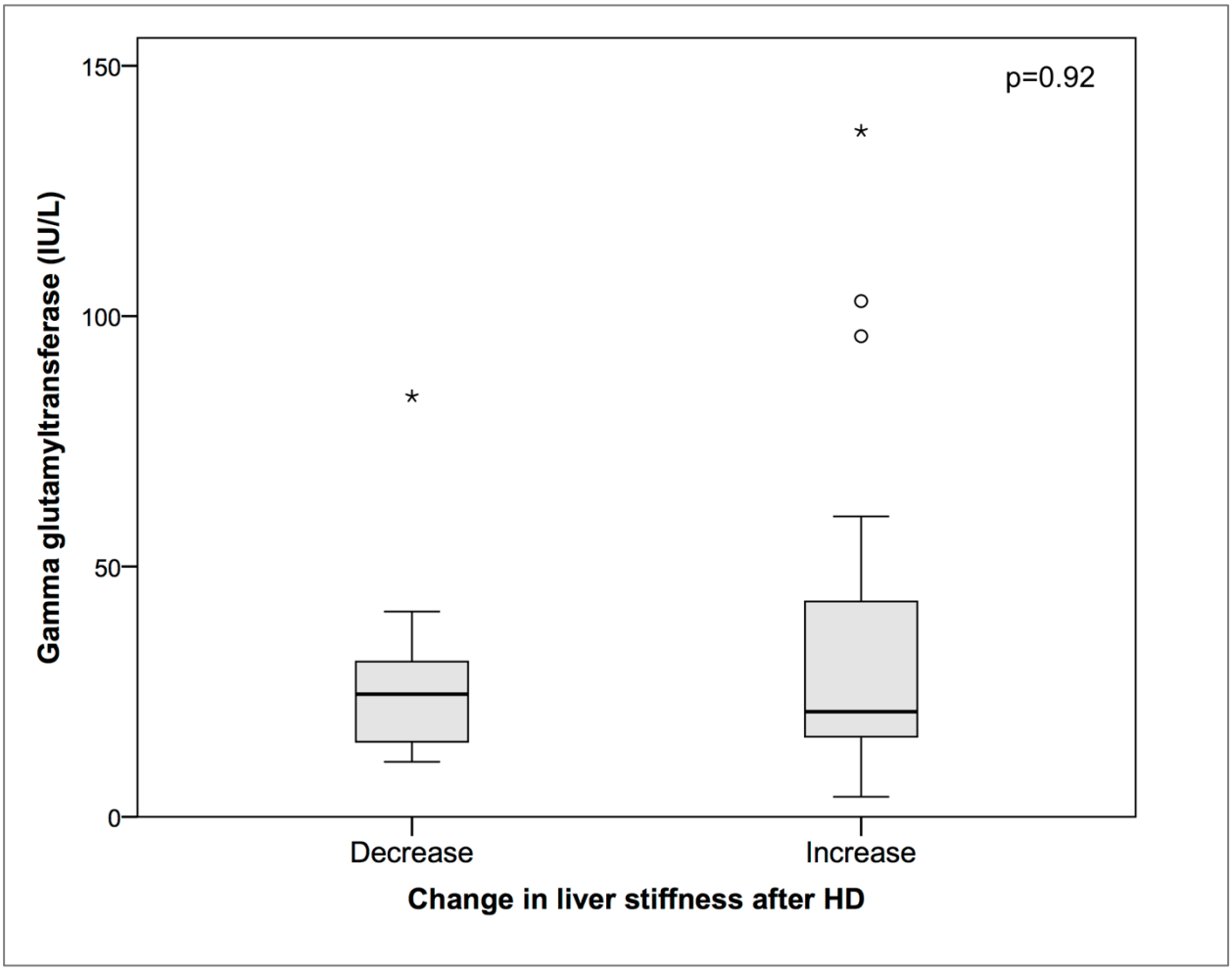
	Pre-dialysis		Post-dialysis	
Marker of Hydration	*R value*	*P value*	*R value*	*P value*
Overhydration (L)	**-0.38**	**0.009**	-0.17	0.28
Total Body Water (L)	-0.15	0.32	-0.14	0.40
Extracellular Water (L)	-0.28	0.07	-0.30	0.05
Intracellular Water (L)	-0.10	0.54	0.06	0.76
ECW/ICW Ratio	-0.21	0.17	-0.20	0.20
Degree over estimated dry weight (kg)	0.24	0.12	0.23	0.16

Figure 20: Pre HD fluid overload is associated with a lower GGT



The magnitude or vector of change in liver stiffness was not associated with pre-dialysis GGT or change in GGT.

Figure 21: Boxplot to demonstrate the lack of relationship between change in liver stiffness and pre-dialysis GGT. Here both dots and asterisks represent outliers.



5. Discussion

Despite its advantages as a rapid, non-invasive bedside tool, these data suggest that transient elastography cannot be used in the clinical setting to determine hydration status or identify significant fluid overload. The impact of hydration status upon liver stiffness appears unpredictable and may be insufficient to eclipse the effects of other variables such as fibrosis, steatosis and heart failure. Nevertheless, there was a significant change in LS after HD. This response was highly variable in terms of direction of change. This variability highlighted the lack of proportional relationship to fluid removal. However, more work is needed to establish if this significant change in LS has significance clinically, either in marking the severity of circulatory stress or in predicting outcomes.

5.1 LS and hydration status

It has been shown that transient elastography is an accurate method to quantify liver fibrosis, when compared to percutaneous liver biopsy, in HD patients at dry-weight [110]. In this study, the majority of subjects had LS within normal limits (<7.5kPa), thereby excluding significant hepatic fibrosis. However, several subjects had elevated LS, although this group did not have clinical evidence of, or clear risk factors for, chronic liver disease, and were not significantly

hypervolaemic. In the whole cohort, pre-dialysis LS did not clearly correlate with clinical or bio-impedance-derived markers of overhydration. There was a weak negative association with E/I ratio which persisted after dialysis, suggesting that those with more severe fluid overload had lower LS, the converse of what was expected to be observed. The reason for this association is unclear but could reflect differing degrees of activation of the venous system to manage excess volume.

5.2. Change in LS after dialysis

Despite this lack of association with hydration, LS significantly changed after a single session of HD. However, this was not a universal decrease, as expected. There was a divergent response with LS increasing in many subjects, yet decreasing considerably in others. Previous studies of liver elastography in HD patients without liver disease have demonstrated similar findings. Kellner et al. reported that liver stiffness increased in 7 out of 17 patients in their study [103]. Khunpakdee et al. reported no statistically significant change in in the distribution of LS values in 36 patients before and after HD, yet 17 patients' LS increased post-dialysis [102].

It was not possible to identify any clear dialysis-related or non-dialysis related factors to conclusively explain this bifid response in study subjects. Pre-dialysis fluid overload or elevated LS did not affect the direction of change in LS after dialysis. Haemodynamic instability during dialysis tended to be associated with an increase in LS.

Acute changes in LS occur through alteration in hepatic haemodynamics. An acute increase in central venous pressure or in afferent blood flow to the liver have both been shown to lead to an acute increase in LS in animal studies [64,111]. Central venous pressure has been shown to fall in dialysis patients, proportionally to ultrafiltration [112,113], therefore this seems an unlikely explanation for the observed increase in LS.

There is relatively little known about changes in afferent blood flow to the liver during HD. In health, an acute reduction in cardiac output triggers vasoconstriction of the post-capillary mesenteric venules leading to the emptying of the splanchnic reservoir of blood into the portal vein and ultimately the systemic circulation, to maintain cardiac preload. Persistent reduction in CO leads to pre-capillary splanchnic vasoconstriction and thus a decrease in afferent flow to the

hepatosplanchnic circulation. In HD patients, it has previously been shown that hepatosplanchnic blood flow tends to decrease throughout dialysis [76]. The observed change in LS might therefore represent differing degrees of activation of the hepatosplanchnic response to the stress of HD.

5.3 Association of GGT with LS

It was noted that GGT levels pre-dialysis correlated with LS. GGT is a transferase enzyme found within the cellular membrane in many tissues, most abundantly within the liver. It acts as a catalyst in amino acid metabolism and in glutathione synthesis and degradation. Serum levels are elevated in both acute and chronic liver disease. However, GGT has recently been recognised as an independent marker of oxidative stress, due to its extracellular catabolism of glutathione [114]. It has been implicated in the formation of atherosclerotic plaque and endothelial dysfunction [115,116]. Serum levels have been shown to predict both all-cause and cardiovascular morbidity and mortality in HD patients [117]. Although our study was not designed to examine the relationship between GGT and LS in detail, our results suggest that the association between GGT and liver stiffness in HD patients is not confined to acute volume status. This relationship may represent sub-clinical chronic liver damage, although

there was no association between LS and the rest of routine liver biochemistry. Another possibility is that both reflect circulatory stress in the HD patient; this hypothesis need further exploration.

5.4 Limitations

A limitation of this study in its exploration of the clinical utility of elastography in HD, is that we did not use a gold-standard method of fluid assessment for comparison, such as stable isotope dilution. Although now widely-used, calculation of body water using bioimpedance analysis requires mathematical assumptions which may introduce error within the dialysis population [118,119].

Furthermore, these observations arise from a single session of dialysis, meaning that we cannot comment on the intra-individual consistency of the relationship between fluid status and liver stiffness. There have been no studies to date which specifically examine this. However, longitudinal observation would avoid the potential confounding effects of fibrosis, steatosis and cardiac dysfunction, as well as potentially providing more insight into the physiology, or pathophysiology, underlying the dynamic change in LS with dialysis. Concomitant assessment of cardiac function might also have aided interpretation of the variability observed. However, echocardiography,

the widely-used bedside test of cardiac function, is itself is subject to the effects of volume status, being variably pre-load dependent [120].

Ingestion of food increases hepato-splanchnic blood flow and thus subjects were asked to fast throughout dialysis. We did not, however, stipulate a prolonged period of fasting prior to HD, nor collect data on the size or content of any recently ingested meal, which has more recently been shown to affect elastography readings in those with liver disease [121]. Any future work will need to address this potentially significant limitation. Furthermore, the effect of fasting upon dynamic liver stiffness is also unknown.

6 Conclusion

This study has shown that there is no proportional relationship between fluid status and liver compliance in a cohort of HD patients either before or after dialysis. There appeared to be a dichotomous change in compliance after dialysis, with an unexpected increase in liver stiffness observed in patients who tended to be less significantly fluid overloaded and at risk of intra-dialytic hypotension. Further work is needed to explore this hepatic response to dialysis, in order to explain the underlying physiology, to examine its relationship to other described hepatosplanchnic effects of dialysis and to assess its clinical significance.

Chapter 3: Liver Congestion and Fluid Removal in HD Patients

Declaration of Contributors

MRI scan acquisition was performed by Dave Reese and Trevor Szekeres.

Quantification of hepatic and peripheral water content was performed by Trevor Wade and Charles McKenzie.

IVC measurements were performed by Trevor Wade.

1 Introduction

1.1 Hypervolemic stress in ESRD

Chronic volume overload is common in dialysis patients. In healthy, non-obese people without kidney disease, 50-60% of body weight is water. In contrast, patients with end-stage renal disease (ESRD) are unable to excrete excess fluid, expanding their extra-cellular space and increasing the percentage of their body weight that is water.

Expanded extra-cellular volume stresses the cardiovascular system. It becomes the main driver for elevated blood pressure in dialysis patients; this relationship has been shown by studies reporting that 90% of hypertension responds to volume control [122,123]. Patients with ESRD develop left ventricular remodelling, both from enlarged left ventricular volume and increased left ventricular wall thickness [124]. Left ventricular hypertrophy (LVH) is associated with increased cardiovascular morbidity and mortality [17]. It is also thought to carry a risk of sudden cardiac death [125].

Chronic volume overload also impacts the gastrointestinal tract and liver. In those with congestive cardiac failure (CCF), it has been demonstrated that volume overload is associated with gut oedema and increased intestinal permeability [126]. This mechanism

contributes to endotoxemia seen in CCF and has been shown to be a driver of chronic inflammation, atherogenesis and mortality [127].

The liver plays a role in the body's response to excess fluid through its capacitance function. Post-sinusoidal venodilatation in the hepatic vascular bed accommodates excess intravascular volume. Furthermore, the liver is also vulnerable to volume overload: congestive hepatopathy is a spectrum of liver damage from chronic passive congestion ranging from mild cholestasis to advanced fibrosis and organ failure. It is unknown whether the hepatic response to volume excess is active in the HD patient. Congestive hepatopathy does not appear to be prevalent unless in the presence of cardiac disease.

The lack of clinical gold-standard measurement and unified definition of volume excess are additional obstacles that contribute to the challenging nature of managing volume overload in dialysis patients. Novel measures such as ultrasound of the inferior vena cava, bioimpedance, and natriuretic peptides have thus far largely failed to replace measurement of weight gain and physical examination to determine volume status to calculate required UF volumes in dialysis units [128].

1.2 Existing tools for measuring volume excess

1.2.1 Physical examination

Measurement of pre-dialysis weight has been a mainstay of fluid assessment in HD patients since the inception of treatment. It is compared to a target "ideal" weight on an individualised basis, which is generally derived from further physical examination of fluid status, including assessment of jugular venous pressure (JVP), the presence of pulmonary and peripheral oedema. However, whilst comparison of pre-dialysis weight with a static target is an easy and rapid measure of volume excess, it is prone to significant error. This arises from several difference sources - the accuracy of measurement, other factors which affect body weight, the influence of hypoalbuminaemia and the impact of co-morbidities such as heart failure.

1.2.2 Bioimpedance analysis

Bioimpedance analysis (BIA) is a non-invasive technique to assess body composition (see Chapter 2, section 2.2.5). It has been shown in non-dialysis patients to correlate with the gold-standard assessment of total body water (TBW) – isotope dilution [129]. In hemodialysis, it has been studied as a tool to assess dry weight . However, BIA cannot discriminate between expanded plasma volume

and tissue oedema, it remains unclear whether it is a useful tool to prevent intra-dialytic organ injury.

1.2.3 Measurement of IVC size

Measurement of the inferior vena cava (IVC) diameter or area has been shown to correlate well with central venous pressure/right atrial pressure [130]. Using ultrasound, IVC measurement is simple and non-invasive, with minimal risk and has been shown to be useful to identify patients with clinically-undetectable hypovolaemia [131]. However, there are several limitations to its use in HD: measurement is affected by right-sided heart failure and/or tricuspid regurgitation; post-dialysis assessment of fluid status is significantly affected by the timing of measurement and finally, it requires specialist skill to perform and is impractical to assess on each patient at each dialysis session.

1.3 Study Rationale

The purpose of this study was to assess the hepatic response to volume excess and fluid removal in HD patients, specifically comparing it with peripheral fluid mobilisation.

Unlike transient elastography, newly developed MRI techniques to assess tissue water content are not subject to confounding from steatosis or fibrosis. This study therefore was designed to utilise MRI to compare hepatic and peripheral tissue water content in patients with ESRD receiving chronic hemodialysis, as well as describing their relationship to other markers of hydration.

We hypothesised that hepatic water content in hemodialysis patients is related to both peripheral and non-invasive measures of hydration pre-dialysis and that hepatic water content falls after a period of ultrafiltration, but to a lesser extent as compared with peripheral fluid content.

1.4 Study Aims

- To compare hepatic and peripheral fluid shifting with HD
- To describe liver water content before and after a session of HD
- To compare liver water content to other measures of hydration in HD patients

2 Methods

2.1 Study Design

A cross-sectional design was used to assess the study objectives. Each subject underwent abdominal and limb MRI and multi-frequency bioimpedance measurements prior to and after a single mid-week routine hemodialysis session. Subjects for the proposed study were drawn from the population of hemodialysis patients who are enrolled in the London Regional Renal Program, Ontario, Canada.

2.2 Ethical Review and Consent

This study was approved by the Health Sciences Research Ethics Board at the University of Western Ontario (HSREB 106868) and conducted according to the GCP guidelines and the Declaration of Helsinki. Potential study candidates were identified by a dialysis nurse. At a routine dialysis visit, the nurse asked the patient whether they were happy to be introduced to the research team. Before the study research team had any interaction with patients, the nurse introduced the research team member to the patient and explained the coordinator's role in the study procedures and assessments. The research team member then explained the study and gave patients an opportunity to ask questions. All patients were given written information to take home and review. All patients were required to

sign and date the informed consent form (ICF) before any study-related assessments were performed.

2.3 Eligibility

To be eligible to participate, patients had to meet all of the inclusion criteria, and none of the exclusion criteria.

Inclusion criteria:

- Patients who receive chronic hemodialysis

Exclusion criteria:

- Patients who have not given consent
- Patients who have known history of liver cirrhosis, hepatitis C or hepatitis B
- Patients who have bilateral leg-amputations
- Patients who have BMI > 30 kg/m^2

2.4 Study Procedures

On their study day, baseline information was obtained from subjects which included: age, gender, height, blood pressure, underlying renal diseases, dialysis information (frequency, blood flow rate, dialysate flow rate and composition, ultrafiltration volume, duration of dialysis

session, Kt/V), most recent residual renal function measurement, and pre- and post-dialysis urea, and creatinine measurements.

Each subject underwent an MRI scan 60 minutes prior to a hemodialysis treatment at University Hospital, London, ON. The MRI scan was conducted at the Robarts Research Institute using the MRI body water measurement protocol (see section 2.5 below). Bioimpedance analysis and clinical assessment of fluid status were performed immediately prior to the MRI scan. The subject then went on to have their routine hemodialysis treatment with ultrafiltration targeting their ideal weight. Once the hemodialysis session was complete, the subject's weight was measured. They were then transferred to have a post-dialysis MRI scan. Bioimpedance analysis was performed approximately 45 to 60 minutes after the hemodialysis treatment. Bloodwork was taken both before and after dialysis for each subject. Subjects fasted throughout their session.

2.5 MRI Data Acquisition

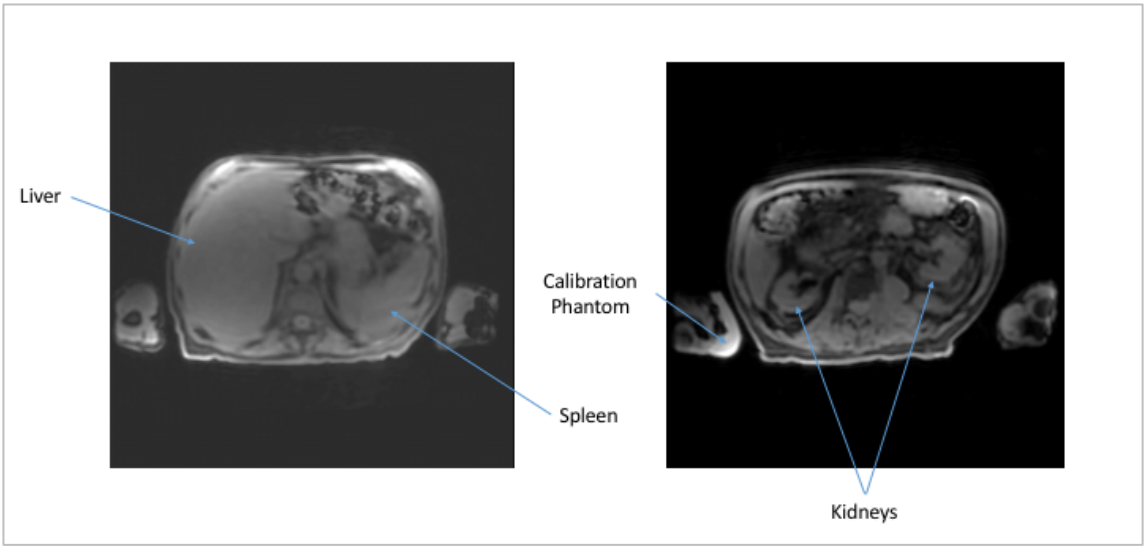
Magnetic resonance imaging (MRI) is widely used as a medical diagnostic imaging tool. Images are formed through the creation of a strong magnetic field that excites hydrogen atoms. When these atoms return to their ground state, the machine detects the emitted radio

frequency signals. These recorded signals form the resultant image. Since the human body primarily consists of water, MRI has become a very effective and useful medical imaging technique. Although MRI is typically used to illustrate physical structures within the body and neuronal activation (fMRI), innovative MRI software has been developed which can be used to measure specific liver water content. This technique has been validated and used in large randomised controlled trials [132–134].

For this study, tissue water content was quantified pre- and post-dialysis using an 3D chemical shift encoded MRI (CSE-MRI) pulse sequence implemented on a 3T MRI scanner (MR 750, GE Healthcare, Waukesha, WI) [132]. Imaging parameters were selected to cover the abdomen with proton density contrast: Field of view (FOV) = 48x34x58 cm^3, matrix = 128x90x72, repetition time (TR) = 4.7 milliseconds (ms), flip angle = 3 deg. The acquisition was accelerated using auto-calibrating reconstruction for Cartesian imaging (ARC) in the phase and slice directions with a 32 channel Torso array for an overall acceleration factor of R=3.9, permitting a complete volume to be acquired in a sub 20s breath hold. The images at the 6 echoes (TE (ms) = [0.37 0.76 1.14 1.152 1.90 2.28]) were receiver sensitivity corrected and processed using IDEAL to give water only and fat only

images. Tissue water content was then evaluated by drawing regions of interest (ROI) in each organ and normalizing by comparing with a water phantom that was included in the field of view [135]. The hepatic ROI was drawn in the right lobe at least 1cm away from the capsule and major vasculature. Unaccelerated, high resolution, 3D CSE-MRI images (FOV = 48x38x48, matrix = 192x160x80) were also acquired in the thigh with otherwise similar parameters and processing, to quantify muscle water content.

Figure 22: Example of MR images acquired in the abdomen pre HD



IVC diameter and area were measured in an axial T2 weighted fast spin echo slice (FOV = 48cm, thickness = 8mm, matrix = 384x160, TE = 80ms) approximately 5 cm below the diaphragm. Measurements were recorded by two readers and averaged.

2.6 Bioimpedance Measurement

Measurements of total body volume status were obtained pre- and post-dialysis using a bioimpedance analyser (Body Composition Monitor®, Fresenius Medical Care, Germany). Measurements were performed using the standard tetrapolar technique, with electrodes placed at both wrists and both ankles after subjects lay supine for at least 10 minutes.

2.7 Blood Sampling

Samples were taken immediately prior to and after dialysis and sent to the London Health Sciences Centre (LHSC) core labs. Full blood count (FBC), serum electrolytes, renal function, liver function tests, calcium, phosphate and clotting function were measured. Test results were obtained by chart review by the research team. Samples analyzed at the LHSC core lab were stored until results were confirmed as per hospital protocol, then destroyed as per routine lab biohazard procedures. No samples were stored as part of this study.

2.8 Statistical Analysis

Data were analyzed using SPSS Version 22 (IBM, Chicago, US). All data were tested for normality using the Shapiro-Wilk test. Parametric data are described using the mean±standard deviation whereas non-

parametric data using the median(IQR). Comparisons of continuous outcomes between two groups were performed using the independent t test for parametric data and Mann Whitney U test for non-parametric data. Comparisons of related outcomes at two different time points were performed using the paired t-test for parametric data and the Wilcoxon signed-rank test for non-parametric data. Bivariate correlation was assessed using Pearson's correlation coefficient for parametric data and Spearman's coefficient for non-parametric data. An alpha error of less than 5% ($p<0.05$) was considered to be statistically significant.

There have been limited studies assessing the effects of hemodialysis hemodynamic changes, including volume congestion and hypoperfusion from fluid removal, on liver stiffness or water content. Therefore, the sample size required to adequately assess these differences was difficult to calculate beforehand. For this initial observational study, a pragmatic target of 20 subjects was selected.

2.9 Data Recording and Storage

Study data were collected and recorded on a Data Collection Form by members of the research team. On all data collection forms and study documents, specific subject data were identified with unique

study identifiers. Any data transferred to electronic storage has been kept on a password protected computer in a secure location in the LHSC Kidney Clinical Research Unit (KCRU) in London, Ontario. A master list that links patient identifying information to the unique identifier is stored separately from any other study documents, in a secure location, on a network drive on the LHSC network. Only study investigators and staff involved in the study have authorization to access study data.

As the study period is complete, all study data will be securely stored in the KCRU storage facilities for 5 years. At the end of the storage period, all study related paper and electronic stored information will be destroyed by a bonded company according to LHSC privacy standards.

3 Results

3.1 Study Population and Dialysis Treatment

Eighteen patients consented to be part of the study from February 2015 to April 2016. Two subjects were excluded due to the presence or development of exclusion criteria. One subject did not complete the study procedures due to intolerance of the MRI procedure. Poor data quality meant that liver MRI data was not available for one subject who completed the study, and peripheral MRI data not obtained for a different two subjects. As liver congestion was the main focus of interest in this study, the 14 subjects for whom liver MRI data was available formed the study population. Subjects in whom peripheral MRI data was not available were excluded from any analyses including this metric.

Characteristics of the study population are shown in Table 9. The majority of patients were male and had either diabetic or hypertensive nephropathy as the underlying aetiology of their renal disease. Other aetiologies included unspecified nephrotic syndrome (2), obstructive uropathy (1), lupus nephritis (1), Prune-Belly syndrome (1) and IgA nephropathy (1). Eight out of the 14 patients had been receiving dialysis for less than one year, five of the remaining patients between 1-2 years and one patient had been on

dialysis for over 14 years. The majority of patients (86%) were dialysed via central catheter access rather than peripheral fistula access.

All patients had normal liver function tests (LFTs) – specifically bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) prior to dialysis.

Table 9: Patient Characteristics (n=14)

Age (yrs)	58.6±18
Gender (M/F)	13/1
BMI (g/m^2)	23.8±2.6
Diabetes (Y/N)	5/9
Known CAD (Y/N)	5/9
Aetiology of ESRD (DM/HTN/Other)	5/3/6
ACEi/ARB (Y/N)	5/9
Calcium channel blocker (Y/N)	10/4
Beta blocker (Y/N)	10/4
Alpha blocker (Y/N)	2/12
Diuretic (Y/N)	6/8
Time on dialysis (months)	9 (1-172)
Time receiving HD (months)	6 (1-172)
Previous transplant (Y/N)	4/10
Current HD access (CVC/AVF)	12/2

M=male; F=female; Y=yes; N=no; CAD=coronary artery disease; ESRD=end-stage renal failure; HTN=hypertension; DM=diabetes; ACEi= angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CVC= central venous catheter; AVF=arteriovenous fistula

3.2 Pre-dialysis hydration and organ congestion

As expected, both clinical and bioimpedance measures pre-dialysis suggested fluid overload in the majority of patients. However, one subject was 3kg under their target weight prior to dialysis and bioimpedance measured excess fluid was -1.2L. Full bioimpedance analysis was unobtainable in one subject who demonstrated clinically significant fluid overload, being 6kg over their target weight with profound peripheral oedema.

Figure 23: The positive association between weight and bioimpedance-derived measure of excess fluid (n=14) kg = kilograms; L=litres

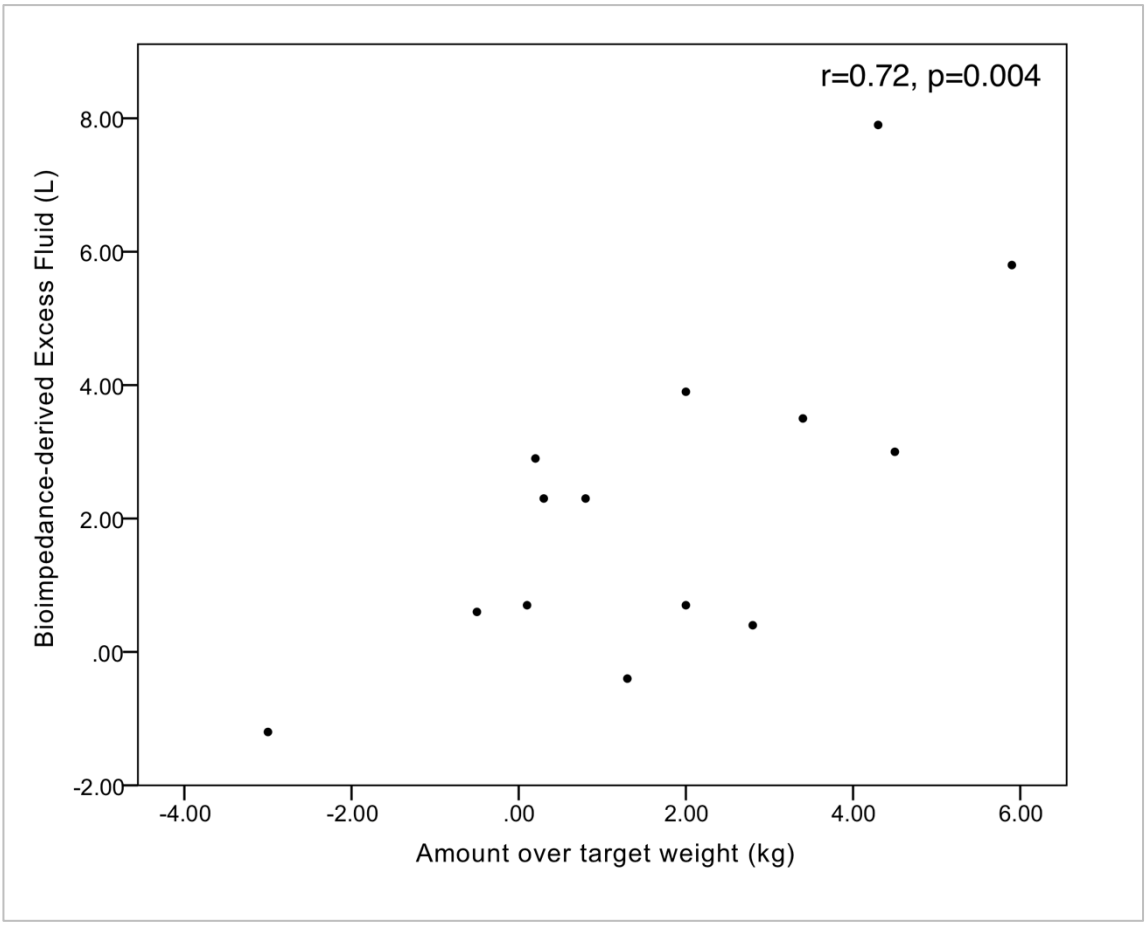


Table 10: Pre-dialysis characteristics including measures of hydration

Characteristic	n=14	Normal Range (if applicable)
Systolic BP (mmHg)	150±18	
Diastolic BP (mmHg)	71.4±15	
Amount over target weight (kg)	1.72±2.3	
Presence of oedema (Y/N)	9/5	
Excess Fluid (L)	2.31±2.5	
Total Body Water (L)	40.0±9.4	
Extracellular Water (L)	18.2±3.2	
E/I Ratio	0.92±0.2	
Haemoglobin (g/L)	107±13	135-155
Haematocrit	0.324±0.04	
Sodium (mmol/L)	136±3.9	135-145
Potassium (mmol/L)	4.31±1.0	3.4-5.4
Albumin (g/L)	38.5±6.2	35-45
Bilirubin (μmol/L)	4.19±1.2	

mmHg=millimetres of mercury; kg=kilograms; Y=yes; N=no; L=litres; E/I=extracellular water/intracellular water; g/L=grams per litre; mmol/L=millimoles per litre; μmol/L=micromoles per litre)

Pre-dialysis MRI-measured limb (thigh) water content was associated with a higher total body water (r=0.59, p=0.06) (Figure 24), a higher diastolic BP (r=0.64, p=0.02) (Figure 25) and higher intracellular water volume (r=0.70, p=0.02) (Figure 26). It did not correlate significantly with excess fluid measurement, extracellular water or with weight.

Figure 24: The relationship between pre-dialysis thigh water content and total body water (n=11), demonstrating a non-significant trend towards higher peripheral water content with higher TBW. L= litres; g/mL = grams per millilitre)

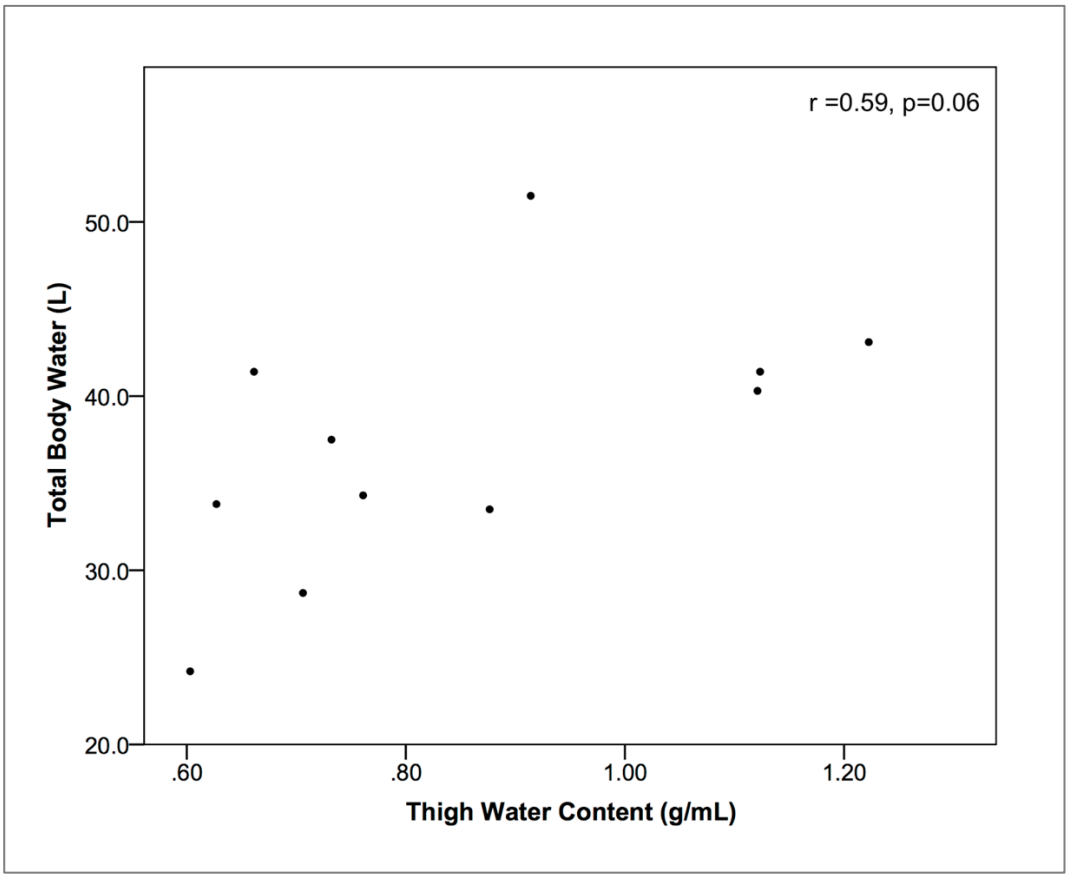


Figure 25: The relationship between pre-dialysis thigh water content and diastolic BP, demonstrating significantly higher DBP with higher peripheral water content (mmHg = millimetres mercury; g/mL = grams per millilitre)

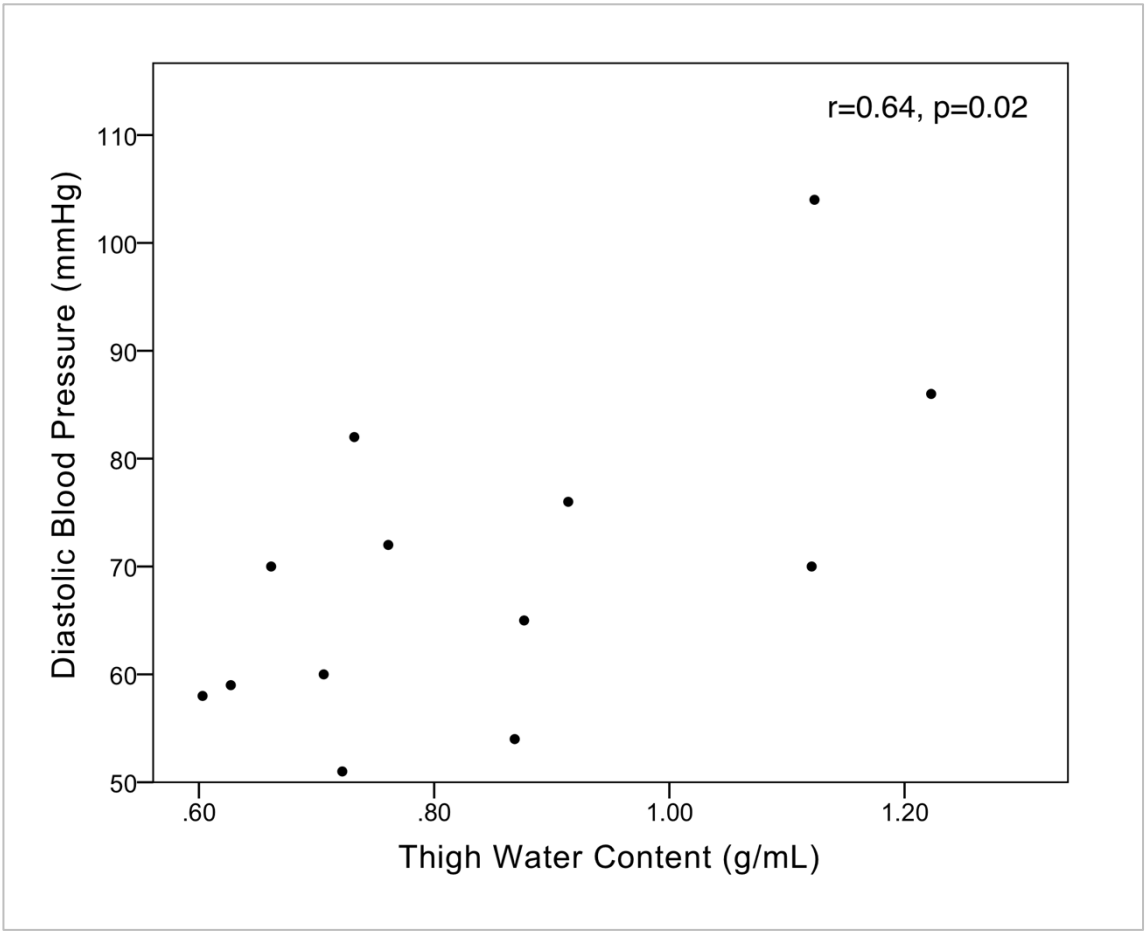
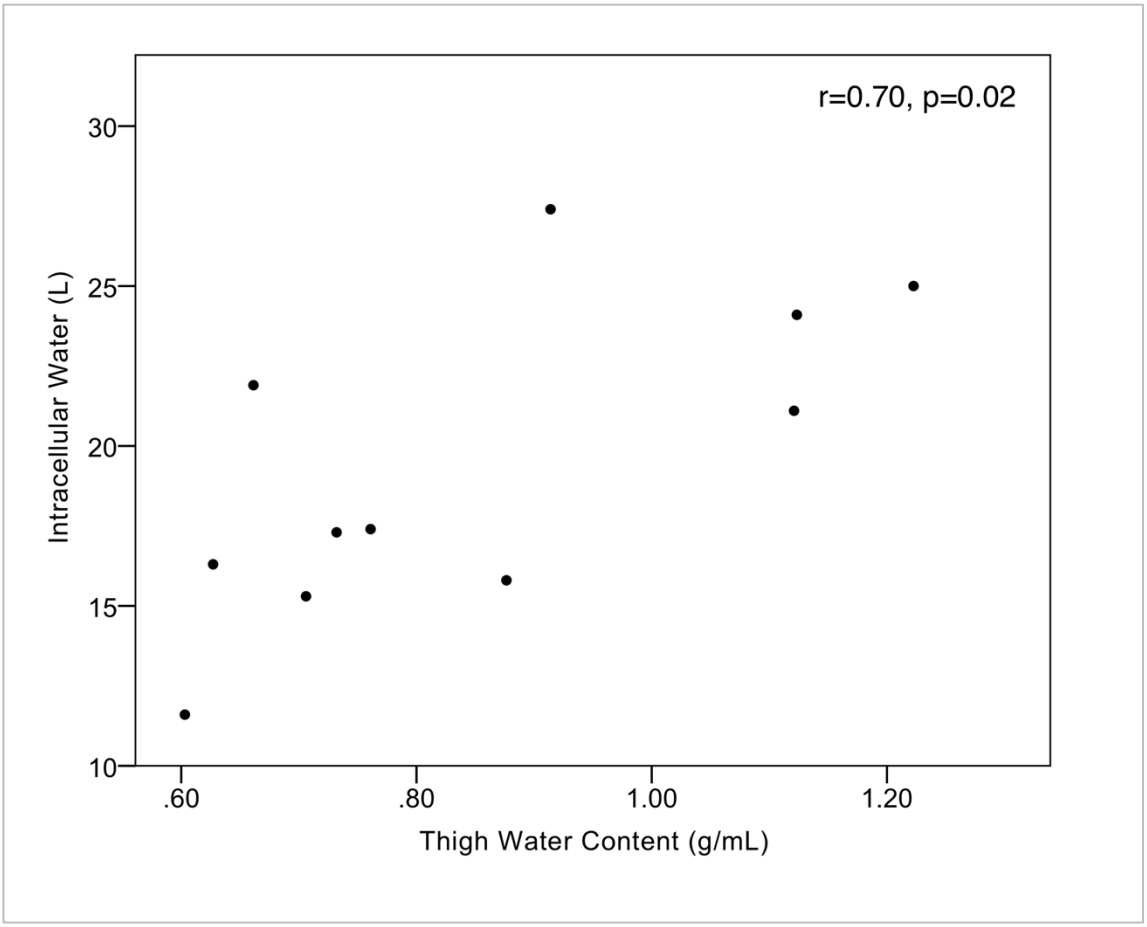
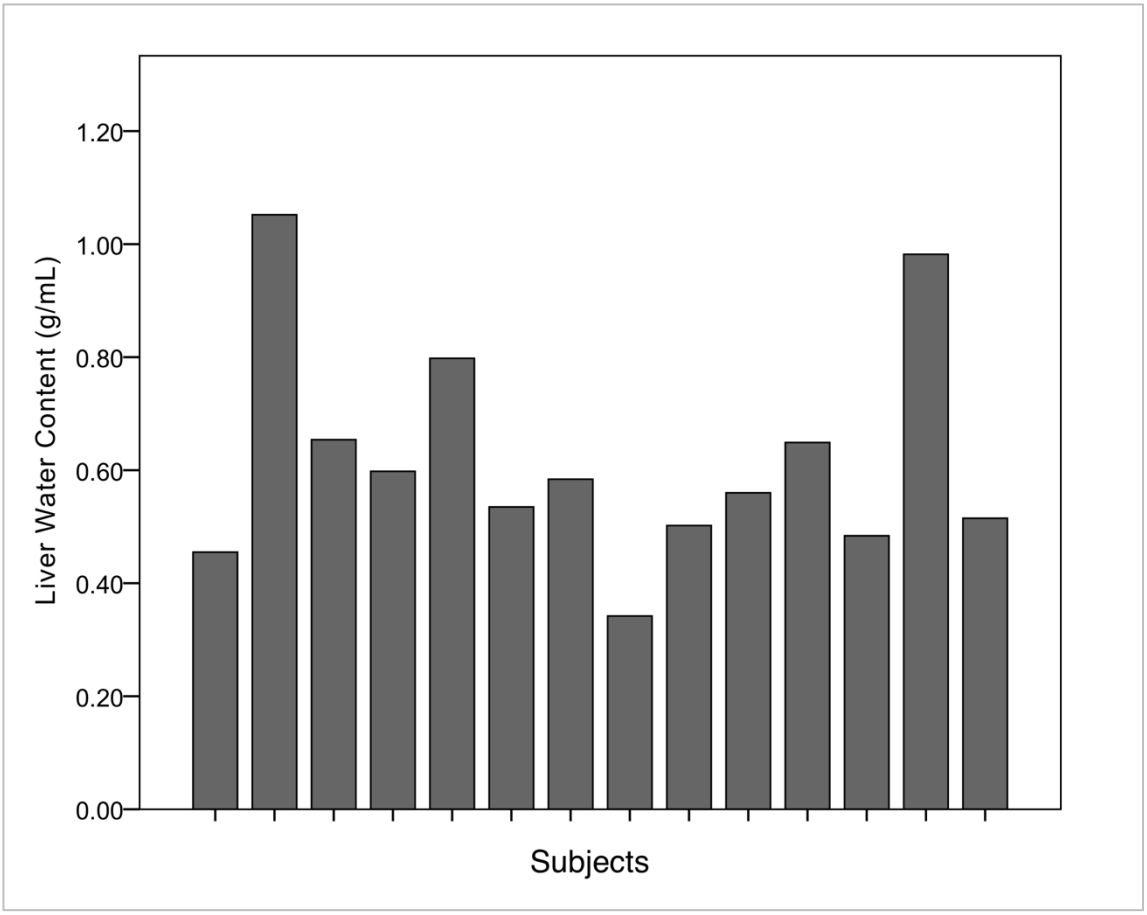


Figure 26: The relationship between pre-dialysis thigh water content and intracellular water content, demonstrating a significant association between higher thigh water content and intracellular water. L= litres; g/mL = grams per millilitre)



The mean pre-dialysis hepatic water content was 0.63 ± 0.19 g/mL (range 0.34 g/mL to 1.05g/mL) (Figure 27).

Figure 27: Plot showing the variability in pre-dialysis hepatic water content. Each bar represents a separate subject. g/mL = grams per millilitre



There was no association between hepatic water and clinically or bioimpedance-derived measures of overhydration (Table 11).

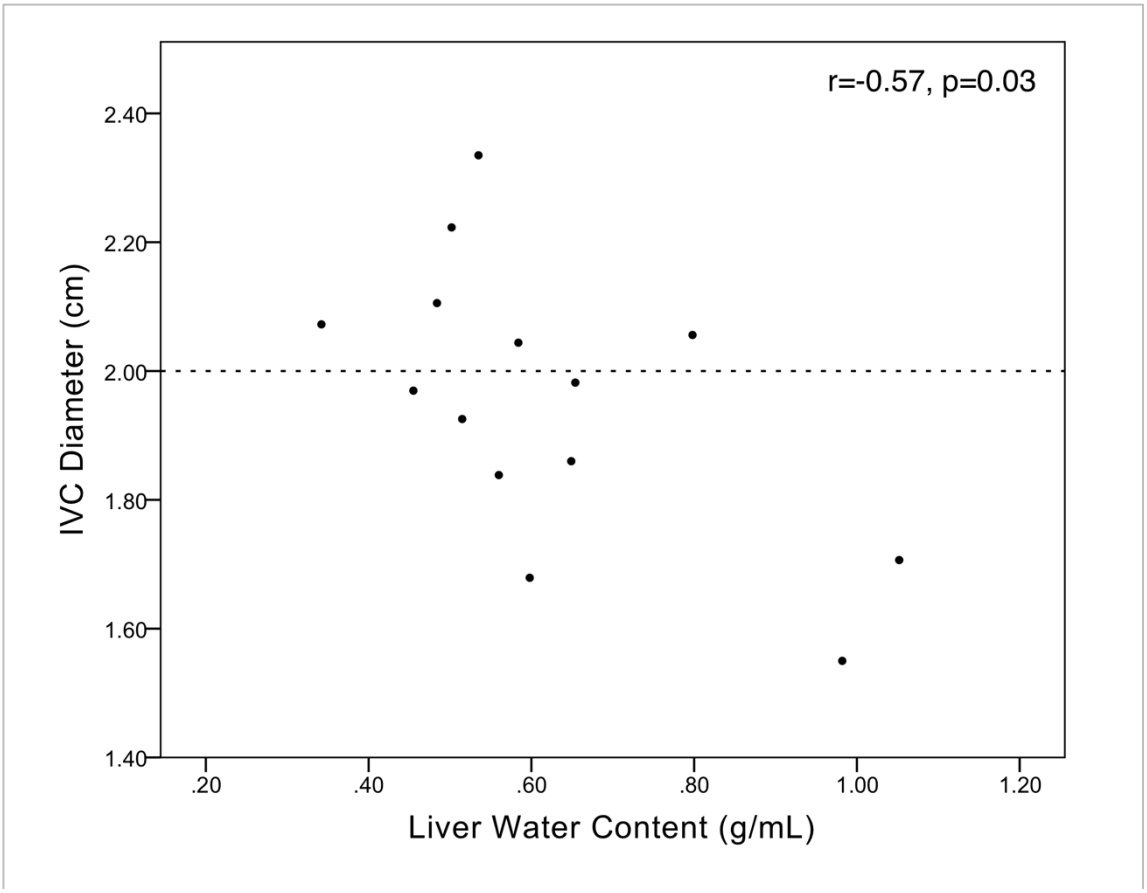
Table 11: Relationship between pre-dialysis liver water content and measures of hydration

	Correlation Statistic	P value
Amount over target weight (kg)	0.22	0.44
Excess fluid (L)	-0.15	0.60
Total body water (L)	-0.41	0.18
Intracellular water (L)	-0.42	0.18
Diastolic BP (mmHg)	-0.06	0.85
Thigh water content (g/mL)	-0.47	0.11

kg=kilograms; L=litres; mmHg=millimetres of mercury; g/mL= grams per millilitre

Higher hepatic water content was associated with a smaller IVC diameter (r=-0.57, p=0.03) (Figure 28).

Figure 28: The relationship between pre-dialysis liver water content and IVC diameter, demonstrating that those with higher IVD diameter had a significantly lower hepatic water content. The dotted line represents an IVC diameter above 2cm, typically used as a marker for significant volume overload.



3.3 Dialysis-related changes in organ congestion and hydration markers

All subjects underwent their prescribed length of dialysis session. Ultrafiltration volumes ranged from 0 to 4090mL (mean 29.5±17mL/kg). Two patients experienced symptomatic intra-dialytic hypotension, with dizziness or severe cramps. Three subjects had IDH defined as a nadir systolic BP of less than 100mHg, whilst six subjects experienced a fall in systolic BP of 40mmHg. None of the sessions were discontinued due to adverse symptoms.

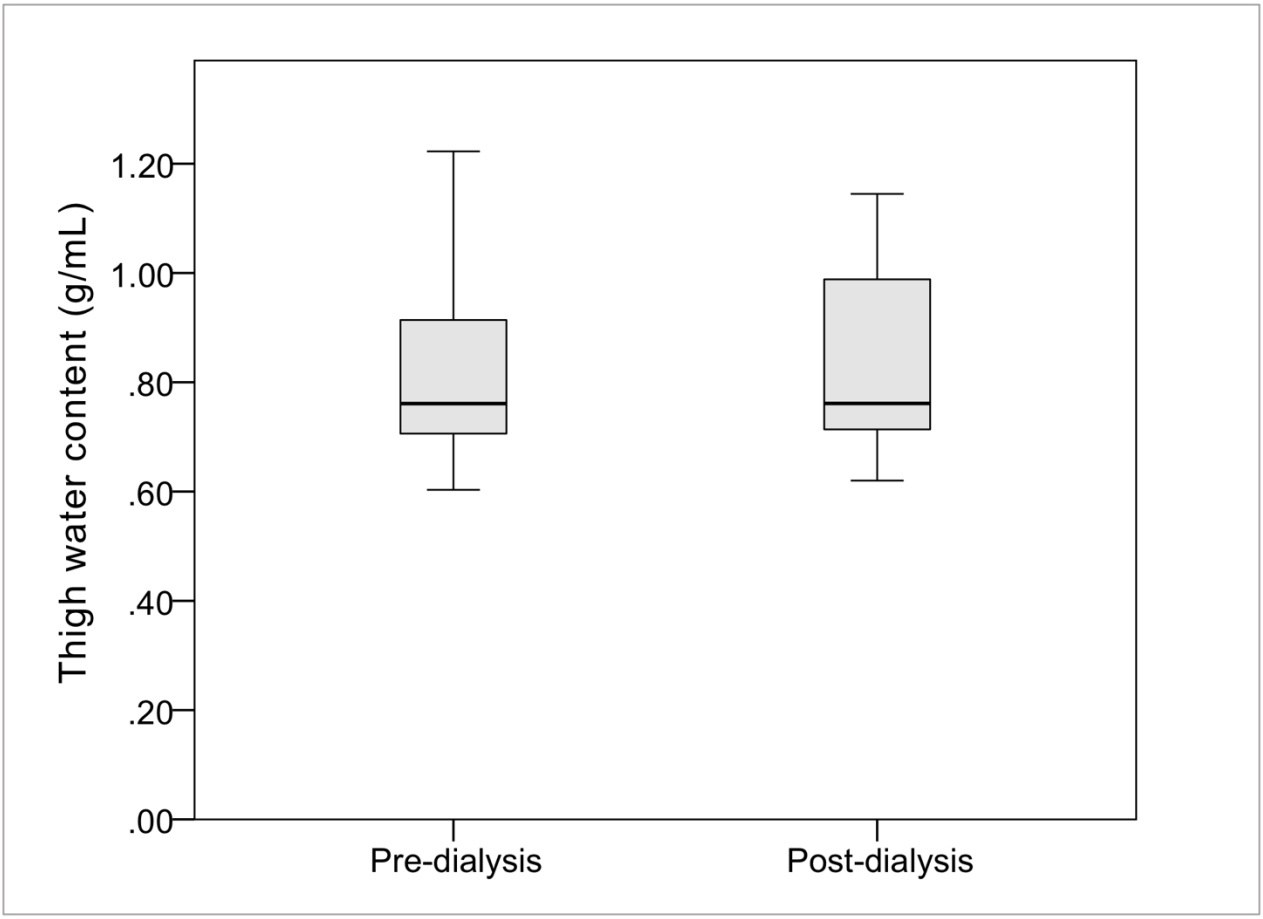
Table 12: Session Characteristics

Session Length (180/210/240 mins)	2/4/8
Total UF (mL)	2140±1200
UF by weight (mL/kg)	29.5±17
Averaged UF rate (mL/min)	9.86±6.0
Symptomatic IDH (n (%))	2 (14.3)
Nadir <100mmHg (n (%))	3 (21.4)
Fall in SBP >40mmHg (n (%))	6 (42.9)
Fall in SBP >20mHg (n (%))	6 (42.9)

Mins=minutes; mL=millilitres; mL/min=millilitres per minute; n=number

Total body water fell by a mean of 1.59L to 37.4±8.4L. Peripherally MR-measured water content also decreased from 0.85±0.21g/mL to 0.83±0.18g/mL, although this was not a statistically significant change (p=0.55).

Figure 29: Boxplots to demonstrate lack of significant change in peripheral muscle water content with dialysis (n=11, p=0.55; g/mL = grams per millilitre)



The mean liver water content trended upward instead of decreasing after dialysis (from 0.63±0.2g/mL to 0.70±0.2g/mL), although this was not statistically significant (p=0.34). This trend was not universal with liver water content increasing in 8 out of 14 subjects, but falling in the remaining 6 subjects. The magnitude of the change in liver water content pre- and post- hemodialysis was not associated with ultrafiltration volume (r=-0.04, p=0.88) (Figure 30) or the pre HD IVC diameter (r=0.10, p=0.97) (Figure 31).

Figure 30: The lack of relationship between UF volume and change in liver water content (n=14). The dotted line indicates where there is no change in liver water content.

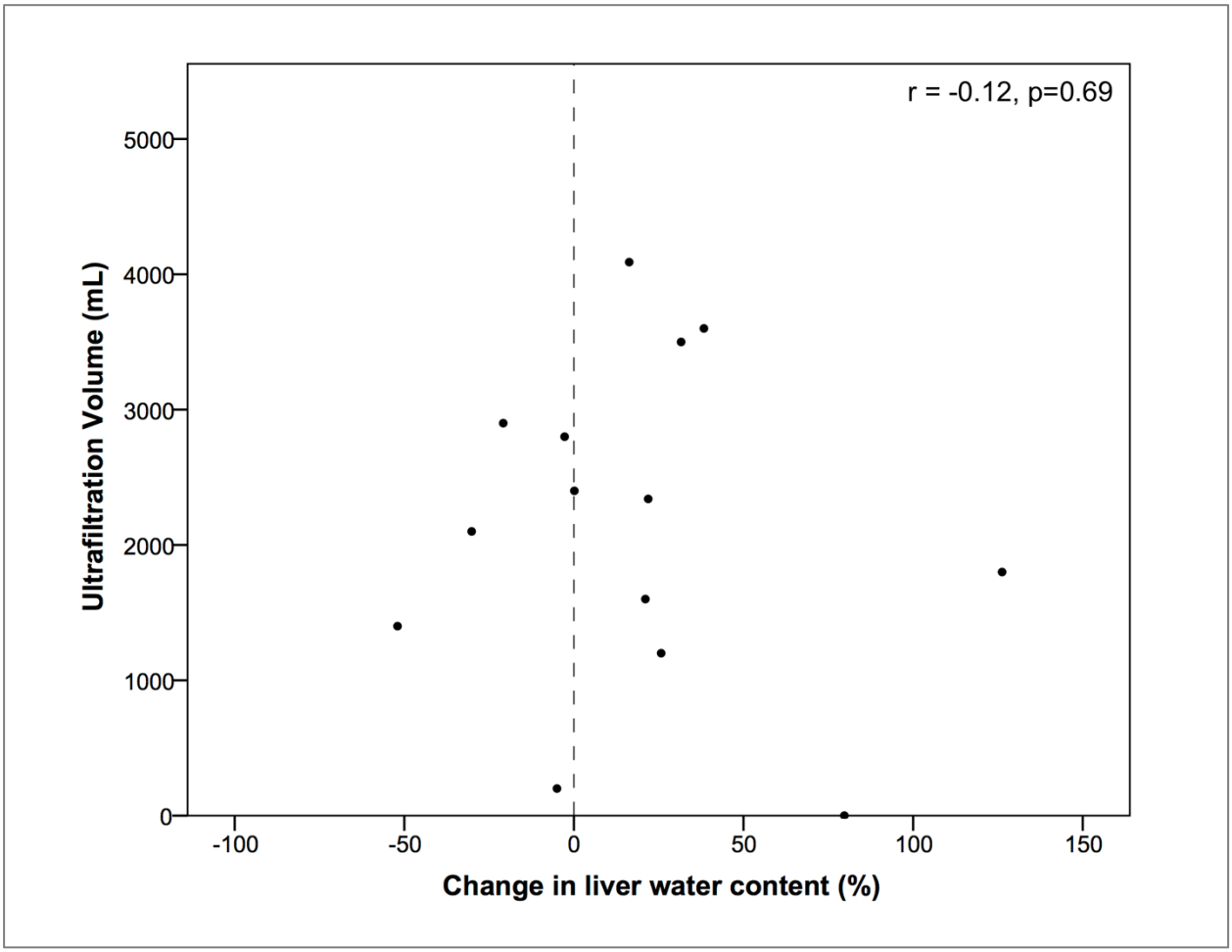
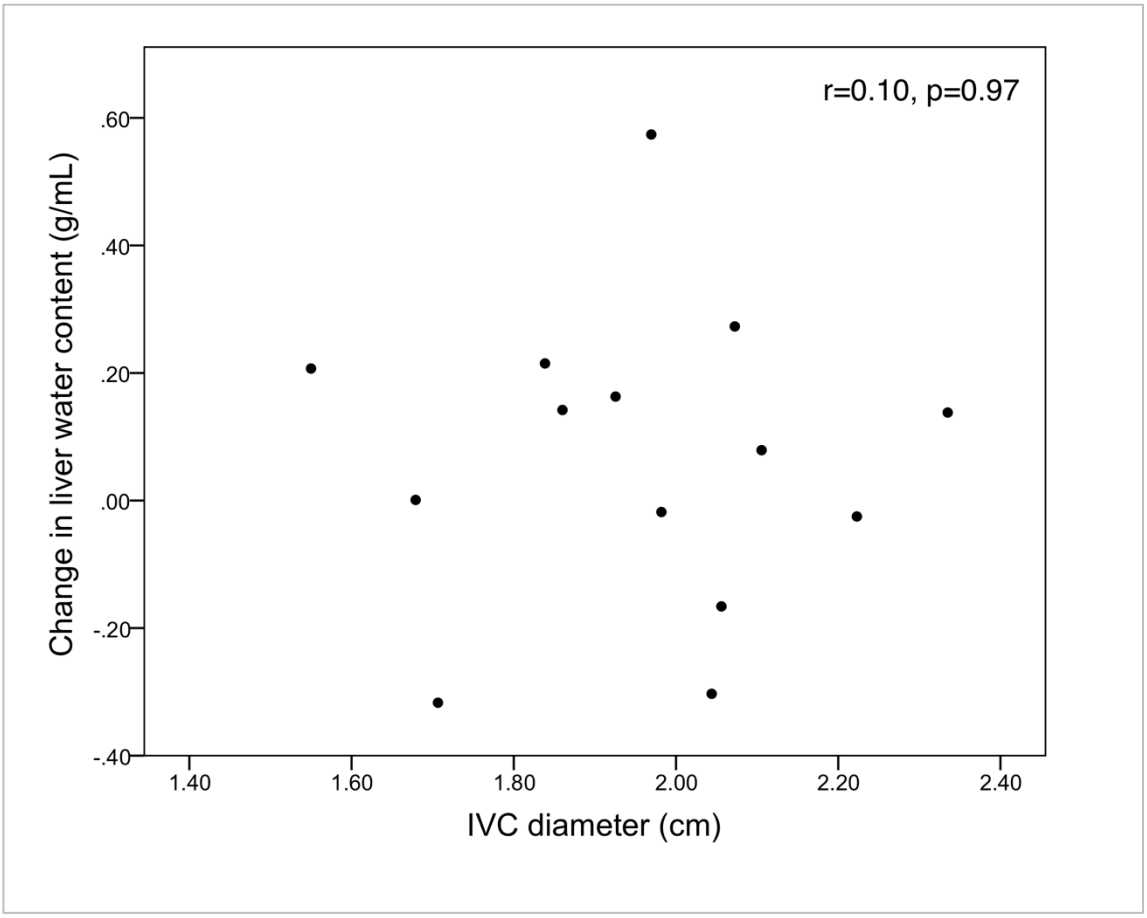


Figure 31: The lack of relationship between pre HD IVC diameter and change in liver water content after HD. n=14, cm = centimetres, g/mL = grams per millilitre.



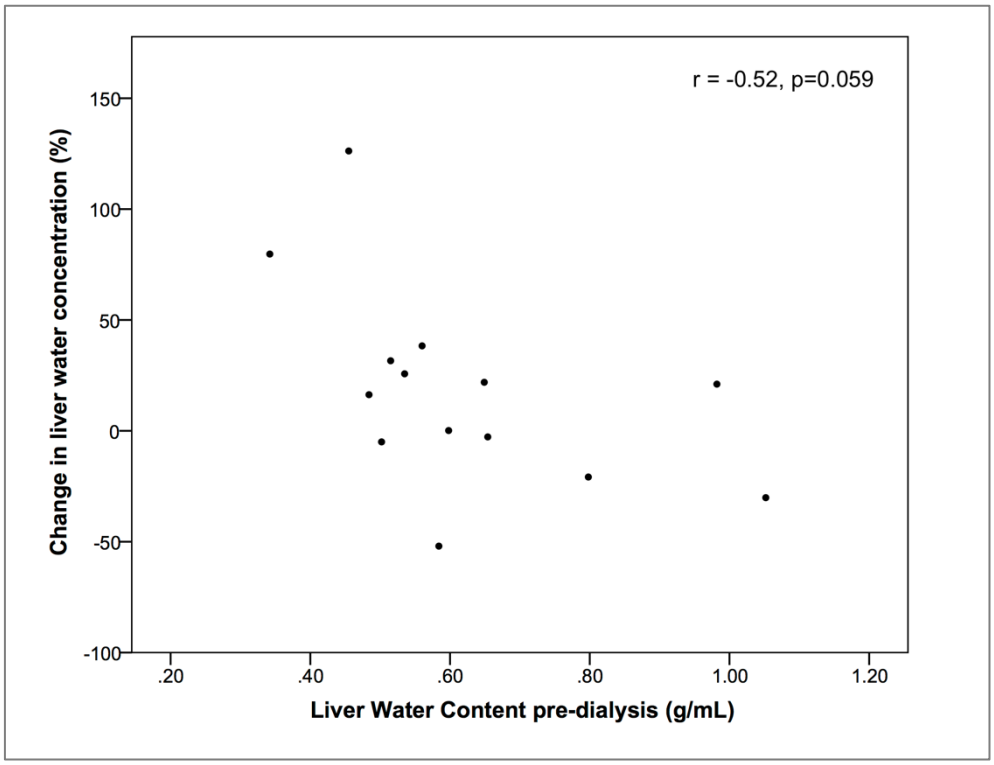
There were no clear clinical factors which associated with the direction of change in liver water content, including age, co-morbidity, hydration status before dialysis and UF volume (see Table 13). However, those subjects with lower pre-dialysis liver water content tended to show the biggest increase after dialysis (Figure 32).

Table 13: Characteristics of those who demonstrate increased liver water content with ultrafiltration

	Up (n=8)	Down (n=6)	P value
Age (yrs)	54.3±19	64.3±17	0.33
Time on hemodialysis (months)	4.5(12)	8.5(11)	0.57
CHF (%)	25%	17%	0.62
Diabetes (%)	38%	33%	0.66
Pre-dialysis SBP (mmHg)	151±23	148±12	0.78
Pre-dialysis DBP (mmHg)	73.1±19	69.2±10	0.65
Pre-dialysis Over TW (kg)	2.41±2.3	0.80±2.2	0.21
Pre-dialysis TBW (L)	41.8±11	36.2±7.0	0.32
Pre-dialysis Excess Fluid (L)	2.81±2.8	1.65±1.9	0.41
Pre-dialysis ECW (L)	19.1±3.9	17.3±2.5	0.36
Pre-dialysis E/I ratio	0.90±0.2	0.94±0.1	0.65
Pre-dialysis thigh water content (g/mL)	0.85±0.1	0.83±0.3	0.89
Pre-dialysis IVC diameter (cm)	1.96±0.2	1.94±0.2	0.94
SBP <100 during HD (%)	25%	17%	0.62
Ultrafiltration volume (mL/kg)	31.1±19	27.2±15	0.68
Reduction in TBW (L)	1.80±5.1	1.38±1.4	0.85

yrs=years; CHF=chronic heart failure; SBP=systolic blood pressure; DBP=diastolic blood pressure; mmHg=millimetres of mercury; TW=target weight; kg=kilograms; TBW=total body water; L=litres; ECW= extracellular water; E/I ratio=extracellular/intracellular water ratio; g/mL=grams per millilitre; IVC=inferior vena cava; cm=centimetres; mL/kg=millilitres per kilogram

Figure 32: Relationship between pre-dialysis liver water concentration and change post-dialysis



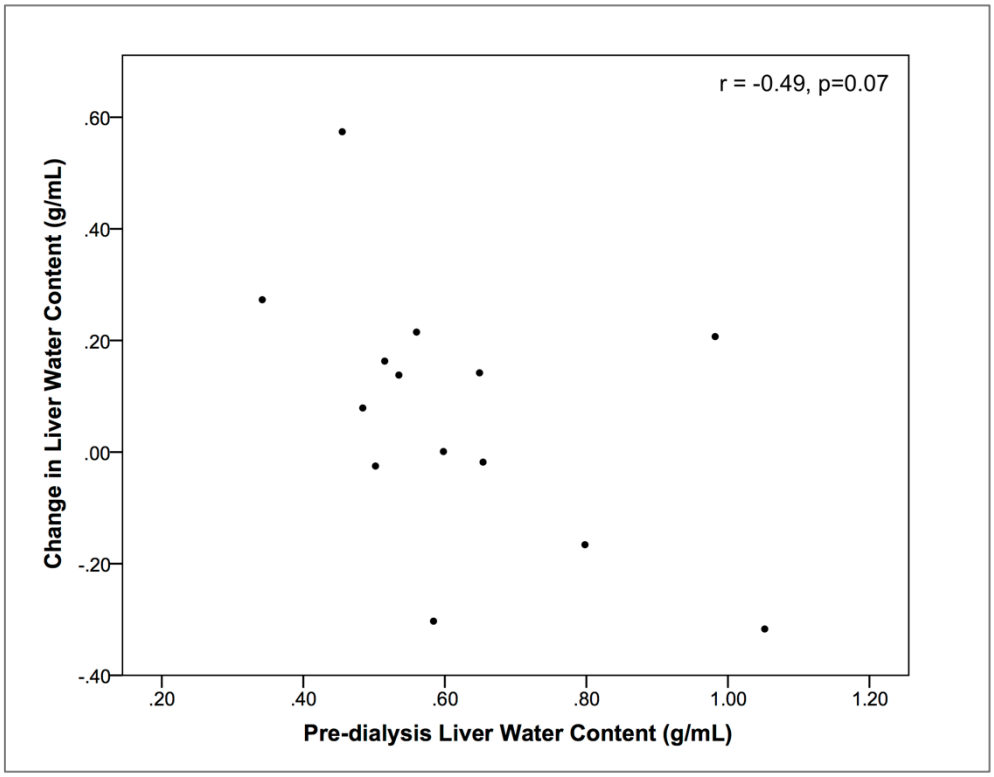


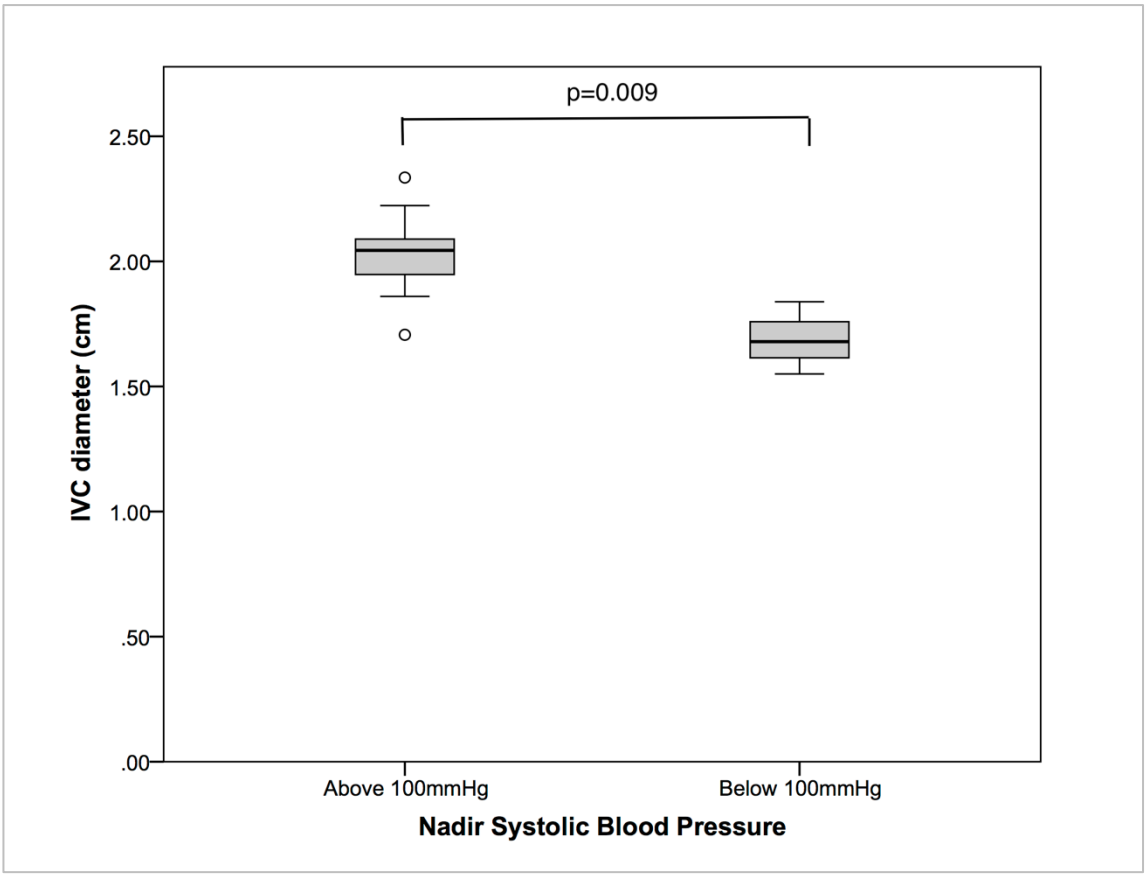
Table 14: UF volume, peripheral and hepatic water content

Patient	UF volume (mL)	Change in thigh water content (g/mL)	Change in liver water content (g/mL)
1	0	-.14	.27
2	200	-.13	-.03
3	1200	n/a	.14
4	1400	-.01	-.30
5	1600	-.01	.21
6	1800	.09	.57
7	2100	-.04	-.32
8	2340	.02	.14
9	2400	.12	.00
10	2800	-.35	-.02
11	2900	.02	-.17
12	3500	.14	.16
13	3600	.00	.22
14	4090	n/a	.08

IVC diameter did not change significantly after dialysis. Nevertheless, the correlation between liver water content and IVC diameter persisted after dialysis ($r=-0.60$, $p=0.03$). Furthermore, pre-dialysis IVC diameter was lower in those who suffered IDH, with a nadir blood

pressure <100mHg (2.02 ± 0.17cm vs 1.69 ± 0.14cm, p=0.009) (Figure 33).

Figure 33: The relationship between nadir BP and IVC diameter, demonstrating that those with higher IVC diameter pre HD were significantly less likely to suffer IDH (n=14).



4 Discussion

This is the first study to specifically compare the effects of HD with ultrafiltration upon liver water content with peripheral and total body water. We have shown that although peripheral fluid content tends to be associate with traditional measures of fluid status, liver water content bears no clear relationship to measures of overall hydration. Furthermore, in a manner similar to that observed with elastography, there was a variable response in MRI-measured hepatic water content to HD which often increased after HD.

4.1 Liver water content and hydration markers

Whilst MR-measured peripheral water content and commonly-used markers of overall hydration tended to correlate with each other, there was no demonstrable relationship between hepatic water content and the majority of hydration markers. There was also marked variation in liver water content pre-dialysis.

Liver water was measured using an MR technique initially designed to assess hepatic steatosis, by separating it from water content. This technique has been validated in multiple animal studies and employed in large, randomized controlled trials. The value reported represents water concentration. In the liver, as there is no significant

extravascular, extracellular space, this is a composite of intracellular fluid, bile and largely intravascular volume. Increased hepatic congestion is expected in the presence of fluid overload. Both passive distension and active venodilatation occur in the face of hypervolaemia to accommodate additional circulatory volume. Chronic congestion may lead to increased hepatic size, but also leads to hepatic injury and fibrosis, limiting the distensibility of liver tissue. It is unclear whether patients with end-stage renal failure undergoing regular dialysis develop chronic hepatic congestion. In the subjects in this study, liver biochemistry was within the normal range, making advanced fibrosis unlikely. Water concentration was nevertheless highly variable but with no clear relationship to MR-measured, bioimpedance-derived or clinical assessments of hydration. One subject clinically appeared to be significantly volume overloaded pre-dialysis, yet their liver water concentration was not elevated.

Such results suggest that liver water concentration does not accurately reflect hepatic congestion in dialysis patients. However, liver water concentration was inversely associated with one marker of hydration: IVC diameter. IVC size reflects the central venous pressure and is used as a marker of fluid status, although is subject to confounding from cardiac dysfunction. A higher IVC diameter is

reflective of circulatory volume overload, therefore one would expect the relationship with liver water concentration to be the inverse of what was observed. This finding cannot be conclusively explained from the data obtained as part of this study. However, it could reflect a failure of activation of the hepatic response to volume excess.

4.2 Change in liver water with HD

Changes in liver water concentration with ultrafiltration were varied and not proportional to the volume of fluid removed. Moreover, it was not only observed that liver water content reduced to a lesser extent than peripheral fluid, in many patients it increased.

In health, in response to a reduction in circulating volume, there is immediate contraction of liver volume and expulsion of pooled blood. An increase in liver water concentration may therefore represent a physiological contraction of liver volume to support the circulation and vital organ perfusion. This is supported by the observation of lower liver water concentration (i.e. possibly a larger, more congested liver) being associated with a greater increase post-ultrafiltration. The preservation of the relationship of IVC diameter and water concentration post-dialysis and the association of IVC diameter with IDH additionally strengthen this hypothesis.

The variability observed, however, suggests this physiological reaction does not occur in all patients undergoing dialysis with ultrafiltration. This may have arisen due to differing levels of circulatory stress endured, with some subjects not meeting the requirements for activation of hepatic venoconstriction. Another explanation is that some subjects exhibited an abnormal hepatic response to the circulatory stress of dialysis. In another study examining hepato-splanchnic flow in dialysis, it was shown that diabetic subjects exhibit a more pronounced splanchnic haemodynamic response when compared to non-diabetic subjects [77]. The severity of cardiac and baseline autonomic dysfunction are also highly variable in HD patients [12,14].

4.3 Limitations

This small observational study was designed to explore the effects of HD upon hepatic and peripheral fluid shifting and liver congestion and as such was not powered to conclusively establish trends, therefore these results must be interpreted with a degree of caution. There are several limitations to consider which would be useful to address when designing future larger studies. Subjects were not starved for a specific time prior to dialysis; ingestion of a large or lipid-rich meal

immediately prior to dialysis may have affected individual results. We did not measure cardiac function as part of the study and knowledge of right-heart function, specifically, would aid interpretation of the relationship between IVC measurements, haemodynamic stability and liver water content. However, static measures of cardiac function are affected by volume status. With regards to MR imaging, it remains to be established whether water content is homogeneous within the liver. Although we selected as large a region of interest as possible, excluding major vessels, whole organ coverage (as well as assessment of liver volume) may be necessary for accurate quantification, although this is technically more challenging.

5 Conclusion

In conclusion, we have shown that liver water content does not reflect volume status in the hemodialysis patient, nor does it reduce with ultrafiltration, unlike peripherally-held volume. Instead, there appears to be a complex and variable haemodynamic response within the liver during dialysis which requires further exploration. This observation emphasises the limitations of current available methods to measure hydration in reflecting central volume status.

Chapter 4: Evaluation of the effects of HD upon hepatic perfusion and function

Declaration of Contributors

CT scan acquisition was performed by Tony Wales, Jennifer Hadway Ranaan Marants and Ting-Yim Lee.

CT perfusion mapping and calculation of hepatic blood flow parameters was performed by Ranaan Marants.

ICG clearance was calculated by Ranaan Marants and Ting-Yim Lee.

Uraemic toxin measurements were performed by Nicolas Tonial, Tom Velenosi and Brad Urquhart.

1 Introduction

End stage renal disease is characteristically associated with a high symptom burden due to the systemic accumulation of toxic substances. In HD patients, there is often poor correlation between the severity of uraemic symptoms and measured dialysis adequacy (based on small molecule clearance). Many symptoms are refractory to conventional approaches to increase toxin clearance. However, systemic toxin exposure is also determined by toxin delivery into the circulation from the gut via the liver, a process which is increasingly recognized to be a key target to attenuate the effects of uraemic toxicity [136,137].

Increased translocation of toxins from the gut into the portal circulation occurs in end-stage kidney disease, as a consequence of increased intestinal putrefaction, gastrointestinal dysbiosis and a chronically dysfunctional intestinal barrier [138]. The liver receives the toxin-laden portal blood and acts as the final functional barrier to the systemic circulation [139]. The integrity of the intestinal barrier and hepatic metabolic function therefore must act as key predicators of uraemic toxin transfer into the circulation. There is a growing body of evidence that suggests the gut is acutely vulnerable to the circulatory stress of dialysis [76–78], in a manner comparable to

ischemic injury seen in the heart and brain [21,22]. The effects of HD-induced circulatory stress upon the liver itself are unknown. Many uraemic toxins directly affect liver cell metabolism [140,141] and hepatic dysfunction is potentiated by heart failure [66], additional hypoxic injury [82], venous congestion, increased myocardial demand [66], endotoxemia [84] and microcirculatory dysfunction [85] - all of which are present during HD [8].

We hypothesised that the circulatory stress of HD acutely affects hepatic and splanchnic perfusion, leading to increased proportion of portal-derived blood and temporary functional compromise, increasing uraemic toxin transfer into the circulation. An initial study was therefore conducted to comprehensively describe the effects of HD upon the hepato-splanchnic circulation and to begin to evaluate the impact upon hepatic excretory function and systemic toxin exposure. Novel, dynamic CT imaging methodology, allowed detailed assessment of multi-organ perfusion simultaneously, during dialysis treatment sessions. Functional assessment of the impact of circulatory stress was achieved through measurement of the clearance of hepatically-excreted dye and the quantification of pre and post-dialysis levels of a range of commonly-studied uraemic toxins.

2 Methods

2.1 Summary

The study was approved by the Western University Health Sciences Research Ethics Board and conducted according to International Council for Harmonisation Good Clinical Practice (ICH GCP) standards. 15 chronic HD patients without known gastrointestinal or liver disease from the London Regional Dialysis Program (Ontario, Canada) underwent detailed serial assessment of liver perfusion during dialysis using dynamic-contrast imaging with a latest generation 256-slice CT scanner [GE Revolution] which allows total organ coverage. Total liver (LBF), portal (PV) and hepatic arterial blood flow were directly quantified using a validated technique [99] pre-dialysis, one and three hours into dialysis and 15 minutes post-dialysis. Heterogeneity of perfusion throughout the liver was also assessed. Intestinal perfusion was serially measured using the same imaging modality. Matched hepatic excretory function was assessed by repeatedly measuring indocyanine green (ICG) clearance. HD-induced myocardial stunning was detected with strain-based measurement of regional wall contractile function using serial echocardiography. Mass spectrometry was utilized to measure plasma levels of a range of protein-bound and non-protein bound uraemic toxins. Patients also

completed a range of health questionnaires to assess the severity of uraemic symptom experience.

2.2 Study Population

Patients were recruited from the prevalent HD population of the London Regional Renal Program (Ontario, Canada). For inclusion, patients had to have been treatment with chronic HD for at least 3 months and have minimal residual renal function (defined as less than 100mL of urine/24hours). Exclusion criteria included the presence of liver disease or chronic gastrointestinal disease, allergy to iodinated contrast, active infection or malignancy, contraindication to fasting or hypoglycaemia during dialysis.

2.3 Dialysis Treatments

Clinical examination, assessment of weight and blood pressure was performed prior to all treatments. Body composition analysis using bioimpedance was performed pre-HD in nine subjects.

Dialysis treatments were delivered using a Fresenius 5008 dialysis machine using high flux polysulfone dialyzers as per the patient's usual prescription. Treatment times were either 210 or 240 minutes. For treatments, dialysate composition was programmed individually

according to their usual prescription: this composed of a set sodium of 140mmmol/L for all subjects except one who was 138mmol/L; four patients had 1.5mmol/L potassium dialysate and the rest 3.0mmol/L; all subjects had a dialysate calcium of 1.25mmol/L and bicarbonate ranged from 34-40mmol/L. Anticoagulation was achieved using low-molecular weight heparin (dalteparin) except for one subject who received unfractionated heparin during their dialysis treatment, three subjects were anticoagulated with warfarin. Dialysate flow was 500mL/min and temperature was set at 36.5ºC. For each session, net fluid removal was set on an individual basis according to ideal dry weight. Blood pump speed varied between 320 and 450 ml/min depending on vascular access. Access was via arteriovenous fistula in seven patients and via central venous catheter in the remaining eight patients.

2.4 CT Assessment of Liver Perfusion

2.4.1 Basic Principles of CT perfusion imaging

Computerised tomography (CT) describes a process in which detailed multi-dimensional images of the body are generated from a series of single-axis X-rays. CT is widely used in clinical practice for assessing the anatomy of organs and the musculoskeletal system. Iodinated contrast agents are often used to delineate vasculature and tissues

more precisely. Such agents attenuate X-rays proportionally to iodine content in tissue. Therefore, measurable tissue attenuation, expressed in Hounsfield Units (HU) is directly proportional to the local concentration of contrast agent within the tissue of interest.

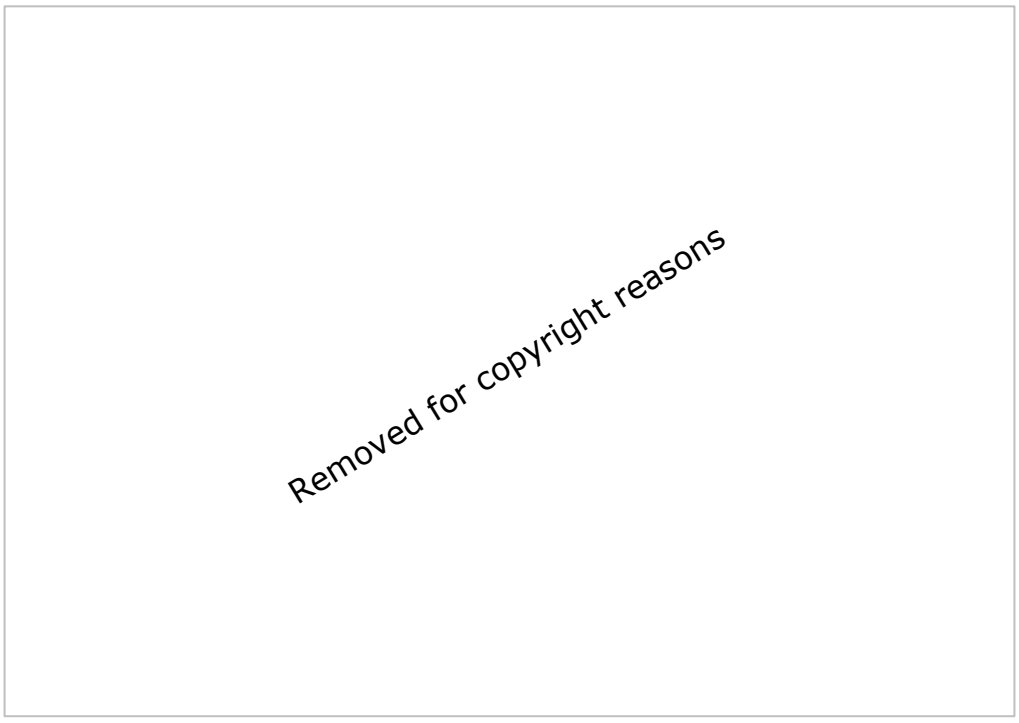
CT perfusion imaging describes the process of measurement of tissue blood flow through dynamic image acquisition following contrast administration. It is based on the increase and subsequent decrease of contrast agent within a tissue over a period of time. Rapid sequential scans before, during and after contrast administration allow the creation of a time-intensity (or density) curve which represents both the amount of contrast within the intravascular portion of a given volume of tissue and the amount of contrast which has passed into the extravascular spaces. The curve is then referenced against a large vessel such as the aorta to allow modelling of arterial input and thus quantify tissue perfusion.

This imaging technique has been widely applied in research settings to the study of cerebral perfusion [142] and more recently to myocardial perfusion [143].

2.4.2 CT perfusion imaging in the liver

The liver is a challenging organ to assess with CT perfusion imaging due to its unique dual blood supply as well as its movement during respiration. Dual capture of blood inflow from both the aorta and the portal vein is necessary to calculate perfusion within hepatic tissue. Methods to allow motion artefact to be overcome prior to quantifying perfusion have been validated [144].

Figure 34: From Stewart et al.[99], Time density curves generated from the aorta, portal vein and liver tissue

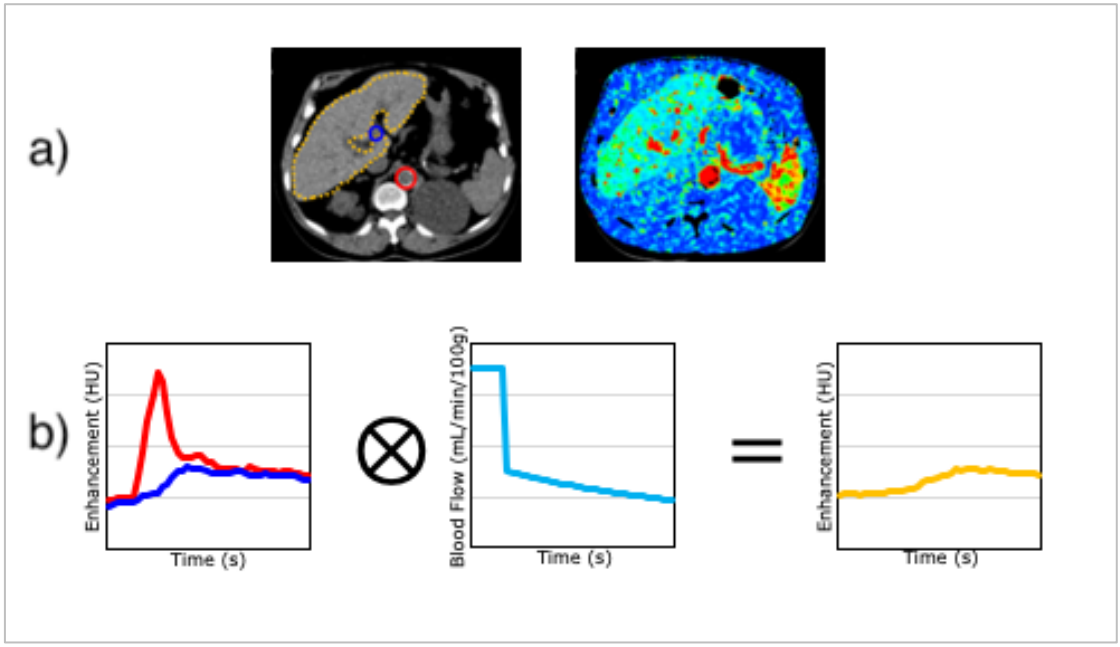


A variety of theoretical models exist to calculate hepatic tissue perfusion and are described elsewhere [99,145,146]. For this study, a method validated in animal studies [99] was used. This was developed by one of the co-investigators (Professor Ting-Yim Lee) and is licensed to GE Healthcare (Waukesha, WI) and is outlined below.

For tissues with a single-source of inflow, the concentration of contrast in tissue, Q(t), depends on arterial contrast delivery, $C_a(t)$, and the inherent hemodynamic properties of the tissue, represented by the blood flow-scaled impulse residue function, $R_F(t)$, a mathematical tracer kinetic model of contrast remaining in tissue following an instantaneous bolus administration. This relationship is given as: $Q(t) = C_a(t) \otimes R_F(t)$, where $\otimes$ is the convolution operator. A CT perfusion scan allows for the measurement of $C_a(t)$ and Q(t), and functional parameters such as blood flow, blood volume and mean transit time can be obtained from $R_F(t)$ using *de*convolution: $R_F(t) = Q(t) \otimes^{-1} C_a(t)$. In the case of the liver, which has a dual vascular supply, contrast delivery is separated into hepatic arterial ($C_h(t)$) and portal venous ($C_p(t)$) components: $C_a(t) = \alpha \cdot C_h(t) + (1 - \alpha) \cdot C_p(t)$, where α is the hepatic arterial fraction. The CT perfusion operational

equation for the liver then becomes: $Q(t) = \left[\alpha \cdot C_h(t) + (1-\alpha) \cdot C_p(t)\right] \otimes R_F(t)$.

Figure 35: Pictorial representation of deconvolution-based CT perfusion. (a) Dynamic contrast-enhanced CT scan with aorta (arterial input), portal vein (venous input) and liver parenchyma outlined in red, blue and orange, respectively. (b) Deconvolution equation



CT perfusion imaging of the liver has typically been employed to assess liver tumours in clinical research to date. However, the newest generation of multi-slice CT scanners allow whole organ capture and thus allow near total-organ coverage with a lower radiation exposure.

2.4.3 Image acquisition for this study

CT scanning was performed using a 256-slice GE scanner (GE, Soningen, Germany). Scans were completed pre-dialysis, at one hour and three hours into dialysis as well as 15 minutes after cessation. For the scans during HD, subjects moved from a chair next to the CT scanner onto to scan bed without any interruption to HD.

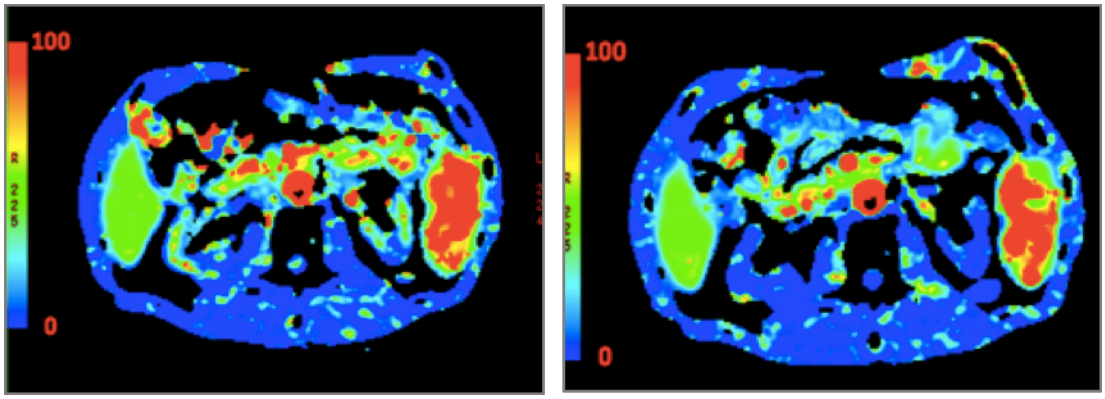
Figure 36: Simultaneous CT perfusion imaging and HD (image shared with the subject's permission)



For the baseline perfusion study, a low radiation dose localization scan was performed from mid-chest to mid-pelvis to determine the scan locations for the subsequent liver perfusion studies. Each

perfusion study scanned the entire liver thirty-three times using 32 x 5mm thick slices with intravenous contrast injection. Each scan used 120kilovolts (kV), 32milliamps (mA) and one second rotation time. The timing of these repetitive scans was as follows: 20 at 2 second (s) intervals, 5 at 3s, 3 at 5s and 3 at 15s for a total time of 120s for each perfusion study. Iodinated contrast (Omnipaque 300, GE Healthcare, Waukesha, WI) at a dose of 1mL/kg was injected via a forearm vein at a rate of 3-4 mL/s five seconds prior to start of scanning.

Figure 37: CT perfusion imaging of the abdomen using dynamic contrast enhancement



The acquired images were anonymized, transferred to a secured workstation (GE Healthcare) for offline analysis and registered using non-rigid registration to minimize breathing motion [144]. After registration, the images were analyzed using the CT Perfusion

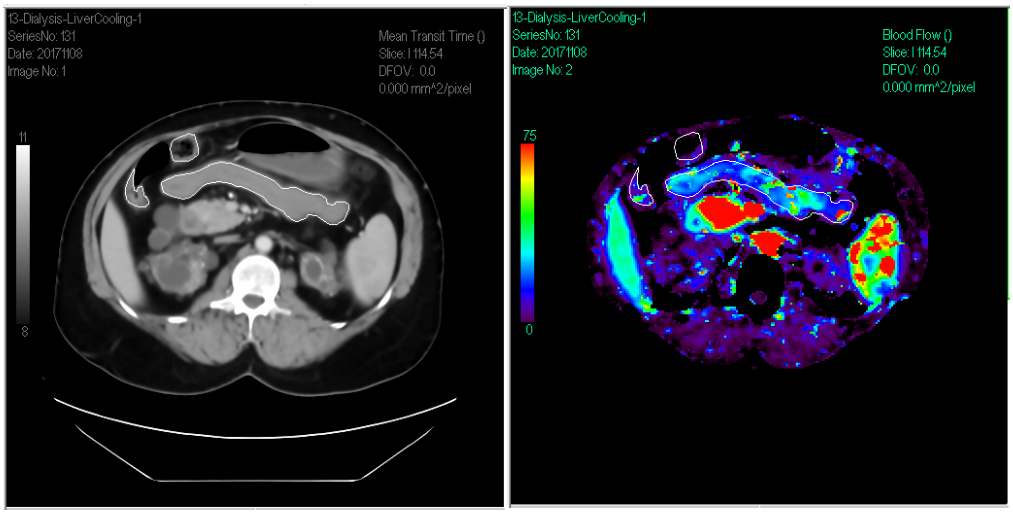
software (GE Healthcare). Regions of interest (ROIs) were placed in the aorta and portal vein within the hepatic hilum to generate the arterial and portal time density curve (TDC) respectively. These TDCs were then used to calculate the functional maps of arterial blood flow (ABF), portal blood flow (PBF), total blood flow (TBF), total blood volume (TBV), mean transit time (MTT) (where TBF= TBV/MTT) and portal fraction of blood flow (PFF=PBF/TBF) for all CT slices of the liver using CT Perfusion software, which was developed by one of the co-investigators (Professor Ting-Yim Lee), validated in prior studies [99] and licensed to GE Healthcare. From all the functional maps of the different CT slices of the liver, the global hepatic arterial blood flow (HABF), portal blood flow (PBF), total liver blood flow (LBF), total blood volume (TBV) and mean transit time (MTT) were calculated as the volume-weighted average of the mean parameter for each functional map.

Perfusion heterogeneity was assessed by analysing perfusion data from all functional maps (adjusted for area) to illustrate the variety of perfusion throughout the liver. The distribution of data from each timepoint was compared to pre-HD values using the Mann Whitney U test.

2.5 CT Assessment of Intestinal perfusion

Intestinal perfusion parameters were derived from the same perfusion maps created in assessment of liver perfusion. Specific licensed software to assess organ perfusion using dynamic contrast-enhanced CT was utilized (CT Perfusion 5, GE Healthcare). To measure intestinal perfusion, a loop of bowel was identified on all possible slices of time-averaged contrast-enhanced scans (i.e. average maps) at the four study time points. An ROI encompassing the identified loop of bowel was drawn on each slice, and these ROIs were used to calculate the volume-weighted average of the mean perfusion of the blood flow maps. Image slices were excluded from intestinal perfusion analysis if excessive bowel movement resulted in either poor image quality or inconsistent loop of bowel identification between time points.

Figure 38: Example of bowel ROI and generated perfusion map



2.6 Measurement of ICG Clearance

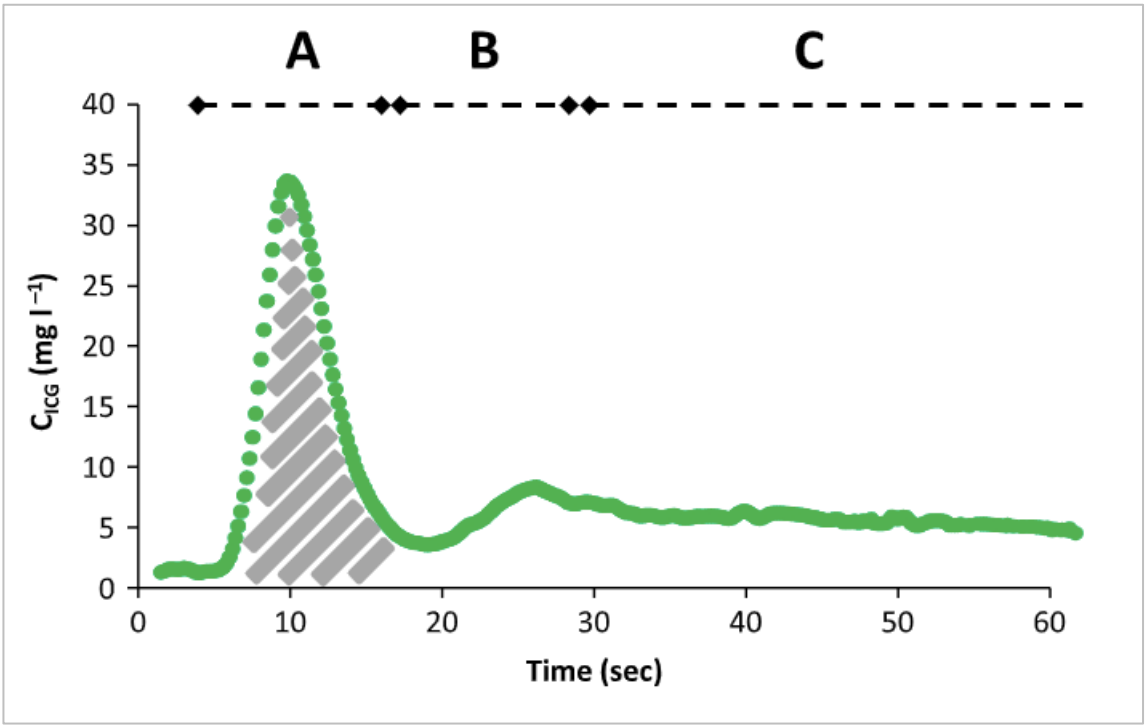
Hepatic clearance of a hepatically-eliminated substance can be defined as a product of liver blood flow and the extraction capacity of the liver. Where, liver blood flow is relatively constant, clearance of highly extracted substances can therefore be used as a marker of hepatic function.

In practice, measurement of ICG clearance is a widely used method to assess liver function and has been validated in several disease states and even during acute hemodynamic changes such as those associated with orthotopic liver transplantation [147,148]. It has been in use as a marker of hepatic function since the 1960s [149].

ICG is an inert, water-soluble, fluorescent substance which is highly protein-bound and has an hepatic extraction rate of over 70% in healthy individuals. After intravenous injection, it is bound almost completely to plasma proteins and distributed rapidly in the blood. It is exclusively extracted from the circulation by the liver and is excreted unchanged into bile. It is not subject to enterohepatic re-circulation. A typical dye disappearance curve (Figure 39) shows a rapid plasma peak shortly after injection, thereafter a second, much lower, peak reflects re-circulation followed by a progressive decline,

reflecting elimination from the circulation. Clearance is expressed as the rate of disappearance of ICG from the plasma.

Figure 39: From Vos et al. [150]. Example of a typical ICG indicator-dilution curve. ICG = indocyanine green; CICG = ICG blood concentration. A: primary peak, B: secondary peak (re-circulation phase), C: (hepatic) elimination phase. The shaded grey area represents the area under the primary curve, allowing cardiac output calculations



Traditionally, measurement of ICG clearance required invasive arterial sampling at regular intervals, followed by ex vivo analysis of ICG concentration using liquid chromatography. However, a non-invasive measurement method using pulse-dye densitometry is now widely

used. This method uses specific wavelengths of light in combination with pulse oximetry to compute the blood concentration of ICG in relation to haemoglobin and has been validated against the original method of measurement in healthy individuals [151].

For this study, measurement of ICG clearance was performed using this non-invasive method. ICG dye at a dose of 0.2mg/kg was injected immediately after each CT scan. Real-time monitoring of ICG clearance was measured for approximately 15 minutes using an optical probe from the DDG unit (Nihon Kohden, Tokyo, Japan) attached to the subject's finger. The ICG clearance rate, defined as the volume of plasma or interstitial fluid that is completely cleared of ICG per minute, was calculated from the parameters of a biexponential fit to the measured ICG concentration versus time curve. The fit is based on a formulated open two-compartment kinetic model of ICG uptake and excretion [152] The ICG clearance rate was computed as follows:

$$K_E = \frac{Q\alpha\beta}{C_p(0) \cdot (A\beta + B\alpha)}$$

Here, K_E is the ICG clearance rate (mL/min), Q is the amount of injected ICG (mg), $C_p(0)$ is the initial plasma concentration of ICG

(mg/mL), and A, B, α, β are biexponential fit parameters (i.e., $Ae^{-\alpha} + Be^{-\beta}$).

Figure 40: DDG-2001 Pulse Dye Densitometer (Nihon Kohden, Tokyo Japan)

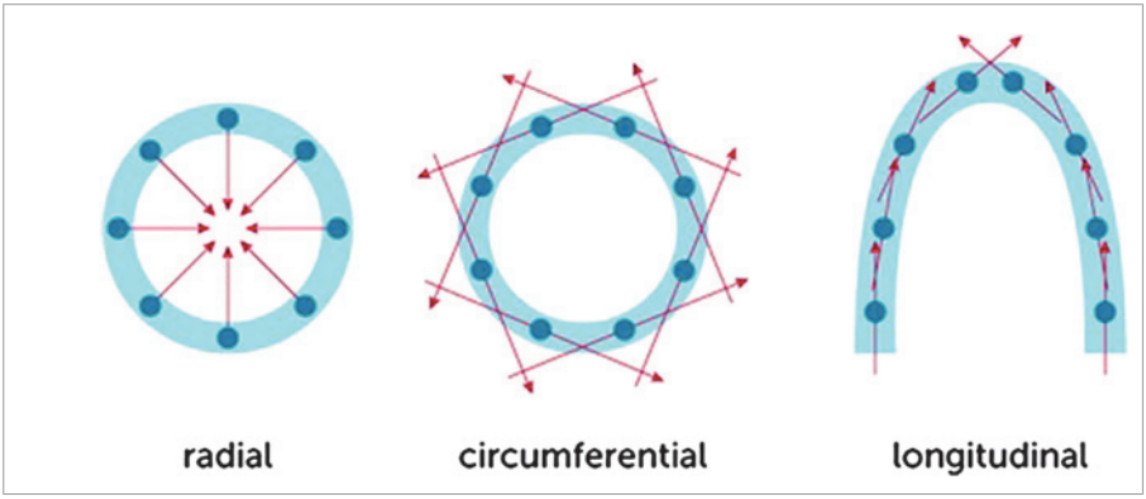


2.7 Echocardiography

Echocardiography is a commonly used bedside tool to assess cardiac structure and function. It was reported as a feasible research tool to detect reversible intra-dialytic cardiac injury through the detection of regional wall motion abnormalities (RWMAs) in 2009 [25] and has since been widely used in the dialysis population for this purpose. More recently, echocardiographic software to detect more sophisticated measures of myocardial deformation, including strain has been applied in the HD population [153].

Longitudinal strain describes the change in length from the base to the apex of the heart. It is expressed as a percentage, with negative values indicating contraction and positive values indicating lengthening. A reduction in contraction (i.e. less negative strain value) therefore represents a measure of impaired contractility. It can be expressed for each ventricular segment or globally for the left ventricle.

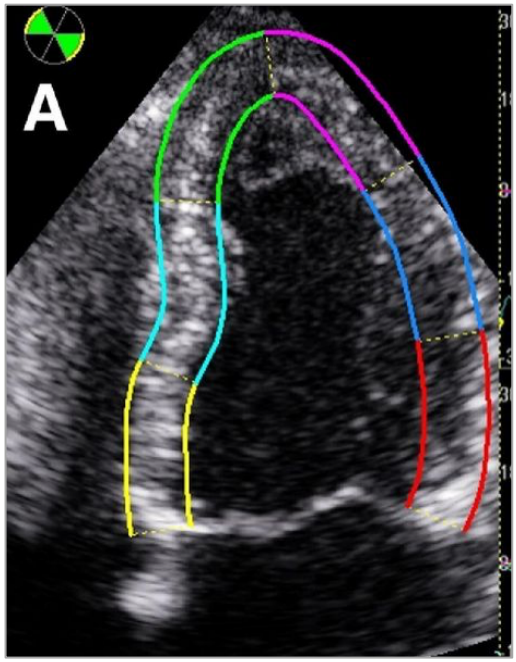
Figure 41: Types of left ventricular myocardial wall strains, from Blessberger and Binder, 2010 [154]



Speckle-tracking echocardiography (STE) is an offline method for strain measurement which uses the detection of speckle artefacts in the generated images and tracks them throughout the cardiac cycle. Detection of movement of these speckles allows calculation of strain values. This software has been validated and generated references for

the normal population for both global and segmental longitudinal strain [154].

Figure 42: Example of speckle-tracking of the left ventricle to allow calculation of segmental longitudinal strain (from Marwick et al. [155])



For this study, echocardiography and analysis were performed by a trained investigator (CG) prior to commencing dialysis and 15 minutes prior to the cessation using commercially available equipment (1.5-3.6MHz M4S probe, Vivid-q, GE Medical Systems, Soningen, Germany). Standard apical and parasternal 2 and 4-chamber views were recorded for off-line digital analysis with a semi-automated computer program (EchoPac, GE Healthcare) using 2D speckle tracking software. Images were anonymized and analyzed in

a random order. Three cardiac cycles were analyzed for each time point and values derived for segmental (12 left ventricular segments) and global longitudinal strain. Basic indices of cardiac function such as ejection fraction were also determined using this software. The presence of myocardial stunning was defined as a reduction in longitudinal strain of more than 20% in two or more segments of the left ventricle. The number of left ventricular segments exhibiting a reduction in strain of more than 20% was also recorded.

2.8 Blood sampling

Blood samples were collected pre and post dialysis for measurement of standard hematological and biochemical parameters. Blood was also collected in EDTA at these times and 2 hours into dialysis for storage as plasma and batch analysis at the end of the study. For storage, blood was centrifuged at 1500rpm for 5 minutes with plasma collected and stored at -80ºC.

2.9 Uraemic Toxin Measurement

Quantification of uraemic toxin concentrations in plasma using mass spectrometry was carried out by colleagues in Schulich School of Medicine and Dentistry, Western University. A selection of uraemic toxins were chosen for this study, based upon a range of protein-

binding affinity as well as their known association with cardiovascular outcomes in the HD population [86,156,157]. These were indoxyl sulphate, p-cresyl sulphate, phenyl sulphate, hippuric acid and p-cresyl glucuronide.

Mass spectrometry (MS) is a technique which uses the ionisation of chemical species to determine their mass-to-charge ratio (m/z) and thus determine the mass spectrum of a given sample. It can be coupled with chromatographic techniques to enable visual detection of the mass spectrum. Time of Flight mass spectrometry (TOF-MS) describes a refinement of the technique whereby a consistent charge is applied to the substrate of ions and mass calculated by the velocity of the various compounds. Quantification of compounds within a sample can then be achieved through comparison with known parameters. Liquid-chromatography mass spectrometry (LC-MS) has been commonly used in the detection of uraemic toxins and is considered to be an accurate methods for quantification of a range of relevant compounds [158].

For this study, indoxyl sulphate was obtained from Gold Biotechnology (Olivette, MO). P-cresyl glucuronide was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Isatin was

purchased from Alfa Aesar (Ward Hill, MA). Hippuric acid and chloropropamide were purchased from Sigma-Aldrich (St. Louis, MO). P-cresyl sulphate and phenyl sulphate were synthesized as previously described [159].

Plasma protein was precipitated with acetonitrile (ACN) containing internal standards, chloropropamide (2.5 μM) and isatin (5μg/mL), in a 3:1 ratio of ACN to plasma. The samples containing ACN were incubated at -20°C for 20 minutes. Samples were centrifuged at 14,000xg for 10 minutes and 150μL of the clear supernatant was transferred to a Ultra Performance Liquid Chromatography (UPLC) vial for quantification.

Concentration of indoxyl sulphate (m/z 212.0018), p-cresyl sulphate (m/z 187.01433), hippuric acid (m/z 178.0504), p-cresyl glucuronide (m/z 283.0818), and phenyl sulphate (m/z 171.9836) were determined by Ultra Performance Liquid Chromatography (UPLC) coupled to Quadrupole Time of Flight (QTOF) mass spectrometry using chloropropamide (m/z 275.0257) as an internal standard. The chromatograph was an Acquity I-Class UPLC system coupled to a Xevo-G2-S QTOF mass spectrometer (Waters Corporation, Milford, MA). The uremic toxins were separated using a CORTECS UPLC C18

2.1 X 50 mm, 1.6µm column (Waters Corporation, Milford, MA) maintained at 40°C. The mobile phases were water with 0.1% formic acid (pump A) and acetonitrile with 0.1% formic acid (pump B). The flow rate was maintained at 0.6 mL/min and the gradient was as follows, 0% B for 0.5 minutes followed by a linear increase to 60% B over the next 2 minutes followed by a 1-minute wash (80% B) and a 1-minute re-equilibration (0% B). The mass spectrometer was operated in negative ionization mode with the following settings: capillary voltage, 0.5 kV; desolvation gas (nitrogen) flow and temperature of 1200L/hr and 600°C; source temperature, 150°C; cone gas flow, 50 L/hr; sampling cone voltage of 40 V and a data acquisition rate of 0.1 seconds per scan. The full mass detection range of m/z 50-1200 was used and the lock-mass spray of leucine-enkephalin (500picograms/µL) was utilized to ensure mass accuracy throughout the run.

Quantification by accurate mass was determined by comparing peaks to a standard curve of indoxyl sulfate (0 to 500µM), p-cresyl sulfate (0 to 2000µM), hippuric acid (0 to 2000µM), p-cresyl glucuronide (0 to 125 µM) and phenyl sulfate (0 to 250µM). Quality control samples consisted of each uremic toxin at 100µM and were injected every 9 samples. Quality control samples were used to calculate both

accuracy and relative standard deviation respectively for each toxin: indoxyl sulfate (106.3%; 6.54%), p-cresyl sulfate (109.8%; 5.37%), hippuric acid (108.6%; 4.58%), p-cresyl glucuronide (99.97%; 7.09%), and phenyl sulfate (107.0%; 5.37%).

Reduction ratios were calculated as follows:

$$Reduction\ ratio\ = \frac{post - HD\ toxin\ concentration - pre - HD\ toxin\ concentration}{pre - HD\ toxin\ concentration}$$

2.10 Assessment of Symptom Burden

Symptom burden was assessed using validated health questionnaires examining specific symptoms which have been shown to be highly prevalent in the ESRD population [160]. Gastrointestinal symptoms were a particular focus of interest for the study of the acute effects of HD upon the gut and liver. Each questionnaire was chosen due to its proven validity in clinical practice and for the ease and rapidity of completion. These were administered prior to the start of dialysis.

To assess general health, the Short Form 36 (SF36) health status questionnaire was used. The questionnaire is a non-disease-specific instrument, validated in the comparison of overall quality of life between patients with different conditions and those with no known

disease, as well as the dialysis population [161]. The questionnaire assesses eight areas of physical and mental health: physical and social functioning, role limitations due to physical problems and emotional problems, mental health, energy and vitality, pain, and general perception of health.

To assess pruritus, the 5D Itch questionnaire was employed. This questionnaire was based upon a pain questionnaire and has been validated in the uraemic population [162]. It examines the domains of sleep quality, quality of life, effect on daily activities and coping abilities.

To assess mental health, the Hospital Anxiety and Depression Scale (HADS) was employed. This self-assessment scale was developed as a screening device for measuring anxiety and depression severity and has been found to be a reliable instrument for the general population as well as in ESRD [163]. Patients choose one of four responses that best describes their current feelings for each of 14 statements. These are reviewed, using a four point (0–3) response rating, and combined, so possible scores range from 0 to 21 for both anxiety and for depression. The HADS takes 2 - 5 minutes to complete.

For assessment of appetite and gastrointestinal symptoms, the Simplified Nutritional Assessment Questionnaire (SNAQ) was used. This questionnaire is a 4 item questionnaire which has been validated in assessment of appetite and nutrition in groups at risk of malnutrition including the elderly population [164].

2.11 Statistical Analysis

Data were analyzed using SPSS Version 22 (IBM, Chicago). All data were tested for normality using the Shapiro-Wilk test. Comparison of continuous outcomes between two groups was done using the independent t test for parametric data and Mann Whitney U test for non-parametric data. Comparisons of related outcomes at two different time points were performed using the paired t-test for parametric data and the Wilcoxon signed-rank test for non-parametric data. Bivariate correlation was assessed using Pearson's correlation coefficient for parametric data and Spearman's coefficient for non-parametric data. An alpha error of less than 5% ($p<0.05$) was considered to be statistically significant.

3 Results

3.1 Study Population and Dialysis Treatment

Fifteen patients aged 34-82, six of whom were diabetic, underwent a single session of conventional HD whilst serial imaging was performed. Detailed demographic and medical background information of the patients are shown in Table 15. Inter-dialytic weight gain (IDWG) ranged from 0.7kg to 4.2kg (median 2.1kg) with delivered median±IQR UF volumes of 22.8±9.1ml/kg. Delivery of dialysis therapy during the study is summarized in Table 16.

Two patients experienced symptomatic intra-dialytic hypotension with systolic blood pressures of <100 mmHg. All patients arrived fasted overnight and 13 fasted throughout dialysis. Two patients required to eat during dialysis, due to unforeseen diabetic requirements. These subjects received a small snack and were not excluded after the time of eating, although a separate analysis was undertaken to examine the effect of these subjects upon the overall results (see Section 3.7).

Table 15: Demographic Information and Medical History of Study Subjects (n=15)

Age	64 (34-82)
Gender (M/F)	10/5
Diabetes (Y/N)	6/9
History of CHF (Y/N)	4/11
History of CAD (Y/N)	5/10
ACEi/ARB (Y/N)	5/10
Beta blocker (Y/N)	11/4
Statin therapy (Y/N)	5/10
Duration of HD (months)	53 (5-547)
Previous transplant (Y/N)	5/10
Systolic BP (mmHg)	154±21.0
Diastolic BP (mmHg)	71.5±25.4
Hemoglobin (g/L)	109±23.7
Thrombocyte count	177±61.5
Urea (mmol/L)	19.5±4.42
Creatinine (mmol/L)	807±157
Sodium (mmol/L)	135±3.53
Albumin (g/L)	38.3±2.46

All biological parameters represented as pre-dialysis values. CHF=chronic heart failure; CAD=coronary artery disease; ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker; Haemodialysis=HD; BP=blood pressure

Table 16: Summary of delivered dialysis therapy

Patient	IDWG (kg)	Dialysis Duration (mins)	UF volume (mL)	Max UF rate (mL/hr)	Dialysate Sodium	Access used	IDH? (SBP <100mmHg)	Anticoagulation Used
1	4.2	240	3400	1000	140	AVF	N	LMWH
2	1.8	210	1600	679	137	AVF	N	LMWH
3	2.6	210	1800	715	140	CVC	N	LMWH
4	1.4	180	2000	667	140	CVC	N	LMWH
5	2.2	240	1860	625	137	CVC	N	LMWH
6	1.3	210	1300	429	140	CVC	N	None
7	-0.7	240	1700	425	140	CVC	N	Minimal heparin
8	2.1	210	2000	522	140	AVF	N	Minimal heparin
9	2.1	210	2070	715	140	CVC	Y	LMWH
10	3	240	1950	750	138	AVF	N	LMWH
11	0.6	210	0	0	140	CVC	Y	None
12	3.4	210	2500	858	140	AVF	N	LMWH
13	3.1	240	2100	829	140	CVC	N	LMWH
14	2.1	240	1400	400	140	AVF	N	LMWH
15	2.3	240	2600	650	140	AVF	N	None

IDWG=intra-dialytic weight gain; kg=kilograms; UF=ultrafiltration; IDH=intra-dialytic hypotension, SBP=systolic blood pressure; AVF=arterio-venous fistula; CVC=central venous catheter; LMWH= low molecular weight heparin.

3.2 Hepatic Perfusion and Function during Dialysis

Pre-dialysis liver blood flow (LBF) ranged from 65.2mL/min/100g to 119mL/min/100g (mean±SD 85.0±15mL/min/100g). There was no association with age, dialysis vintage, liver function tests, markers of volume status or cardiac index. Hepatic blood volume ranged from 32.7mL/100g to 40.5mL/100g (median 36.1mL/100g) and also was not associated with clinical, echocardiographic and bioimpedance derived markers of volume status (Table 17).

Table 17: Relationship between volume status and pre-HD hepatic blood volume

Variable	R value	P value
Weight over target (kg)	0.12	0.68
Systolic blood pressure (mmHg)	0.16	0.57
Diastolic blood pressure (mmHg)	0.21	0.45
Left atrial volume (mL)	-0.24	0.39
Total Body Water (L) (n=9)	-0.41	0.28
Extracellular Water (L) (n=9)	-0.39	0.29

Liver blood flow was maintained over the course of dialysis (p=0.66 at three hours, p=0.79 post-dialysis). However, hepatic blood volume (HBV) (Figure 43) and mean transit time (MTT) of blood through the

liver (Figure 44) significantly decreased by the end of HD ($p=0.001$ and $p=0.009$ respectively).

Table 18: Total liver blood flow before, during and after HD. LBF= liver blood flow. Shaded subjects ate after 1 hour but before 3 hours of dialysis.

Subject	Pre HD LBF (mL/min/100g)	1 hr LBF (mL/min/100g)	3hrs (mL/min/100g)	Post HD (mL/min/100g)	**Change in 1 hr LBF (%)**	**Change in 3 hr LBF (%)**	**Change in post HD LBF (%)**
1	99.3	91.0	115	105	**91.6**	**116**	**106**
2	69.1	82.1	74.4	70.1	**119**	**108**	**101**
3	76.4	86.8	89.2	78.9	**114**	**117**	**103**
4	106	82.8	n/d	87.6	**78.2**	**n/d**	**82.8**
5	119	125	117	115	**105**	**98.8**	**97.0**
6	86.4	79.1	82.0	84.3	**91.6**	**94.9**	**97.6**
7	65.2	64.4	59.7	67.4	**98.8**	**91.6**	**103**
8	94.7	89.5	93.5	94.9	**94.5**	**98.7**	**100**
9	84.8	n/d	88.9	88.3	**n/d**	**105**	**104**
10	77.2	89.6	89.9	94.5	**116**	**116**	**122**
11	69.0	72.3	69.1	69.0	**105**	**100**	**100**
12	85.8	107	105	94.0	**125**	**123**	**110**
13	86.1	70.3	70.8	91.7	**81.7**	**82.2**	**107**
14	85.0	77.4	71.4	77.6	**91.0**	**84.0**	**91.3**
15	71.7	62.0	60.7	64.9	**86.5**	**84.7**	**90.6**

HD=haemodialysis; LBF=liver blood flow; hrs = hours; g= grams

Figure 43: Boxplot to demonstrate the change in hepatic blood volume throughout dialysis, demonstrating a significant fall after 3 hours and post HD. Here dots and asterisks represent outliers (n=15)

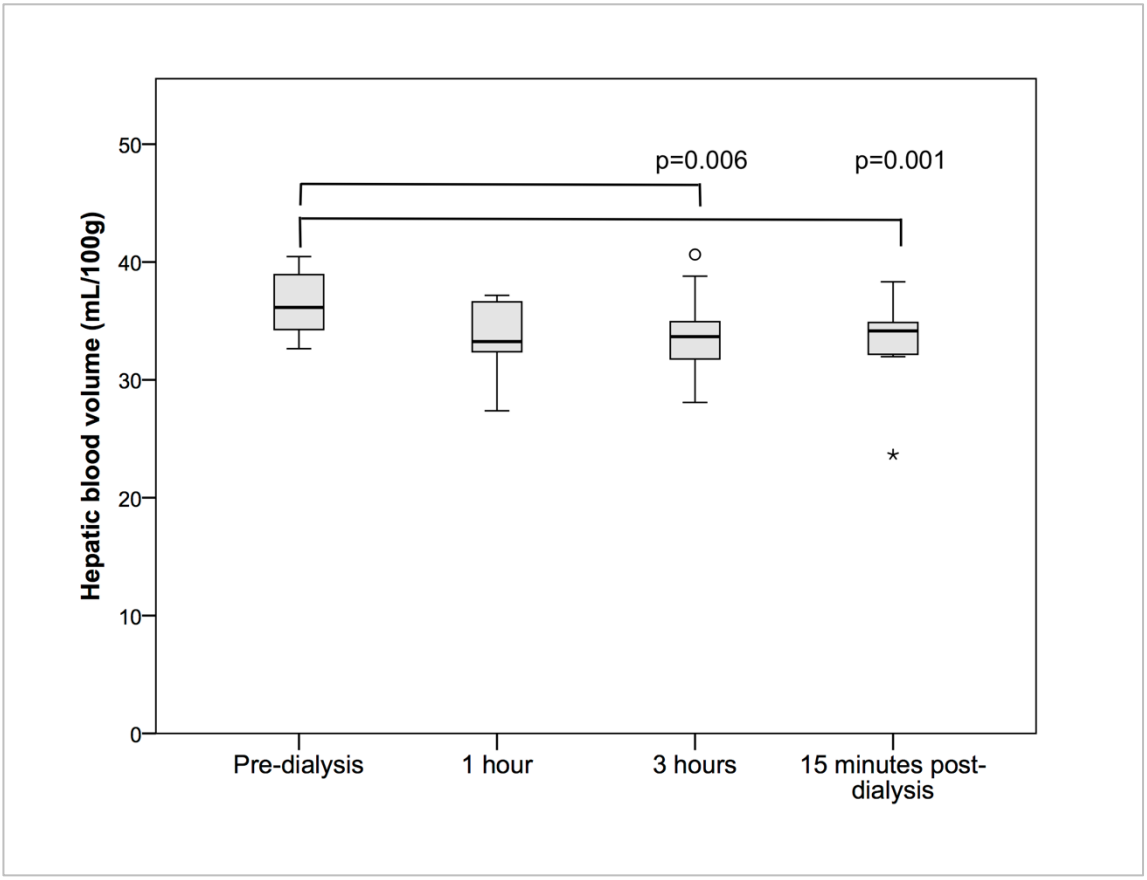
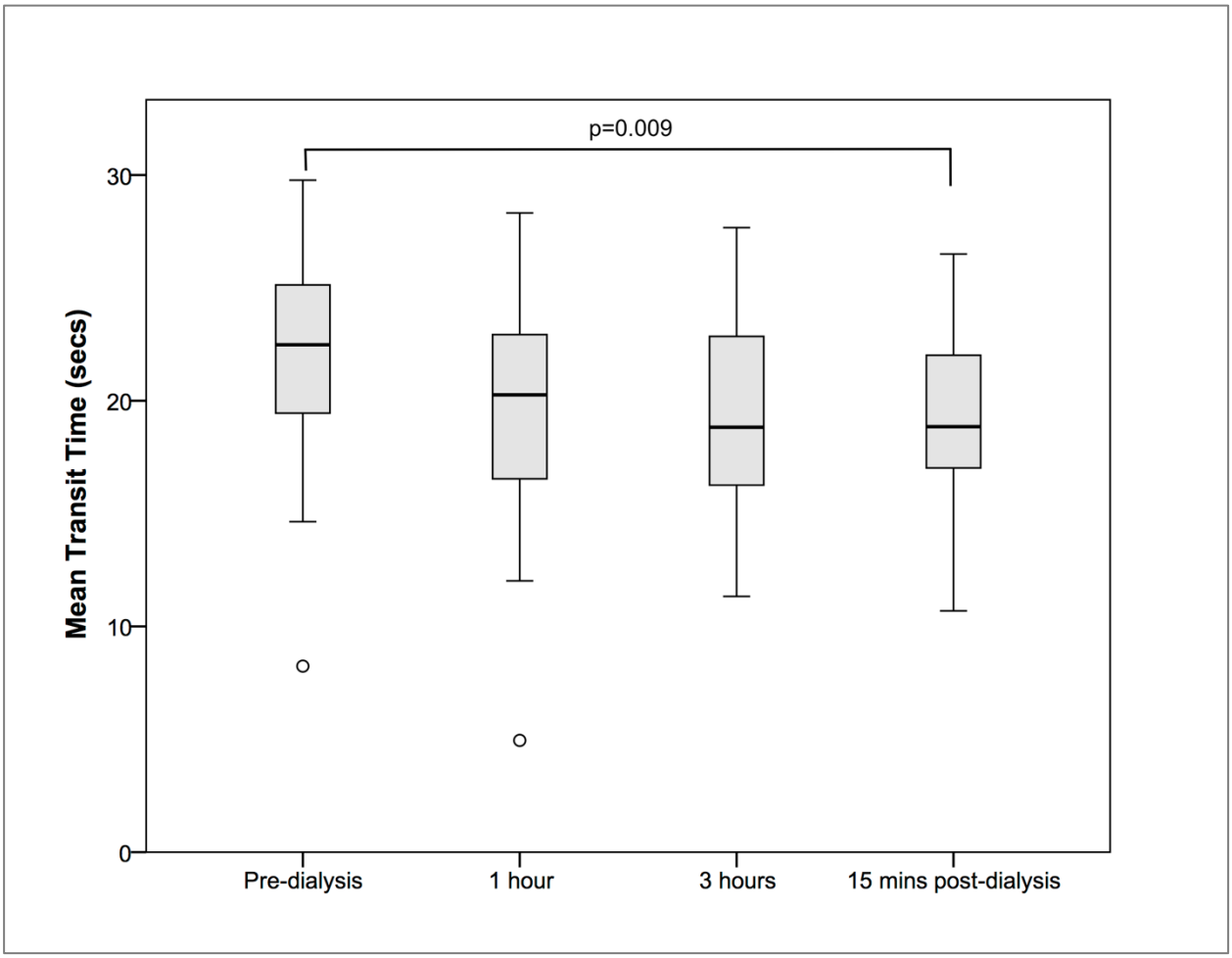


Figure 44: Boxplot to demonstrate the change in mean transit of hepatic blood during dialysis, again demonstrating a fall throughout dialysis, although only to a statistically significant level post HD. Dots represent outliers.(n=15)



Additionally, HD induced heterogeneity of perfusion throughout the liver, (see Figures 45-48). There was a significant change in the distribution of total tissue perfusion at 3 hours and post dialysis. The distribution of portally-derived perfusion did not significantly alter at any timepoint whereas there was a significant difference in the distribution of arterially-derived perfusion at all timepoints (Table 19).

Table 19: Statistical comparison of the distributions of overall, arterial and portal-derived perfusion compared to pre HD (all available ROIs included)

	Overall	Hepatic artery	Portal vein
1 hour (n=272)	p=0.075	p=0.001	p=0.361
3 hours (n=276)	p=0.036	p<0.001	p=0.428
Post HD (n=295)	p=0.020	p=0.001	p=0.137

Figure 45: Each histogram outlines the distribution of hepatic perfusion for each imaging timepoint (all available ROIs) Top left=pre HD, top right=1 hour, bottom left=3 hours, bottom right=post HD)

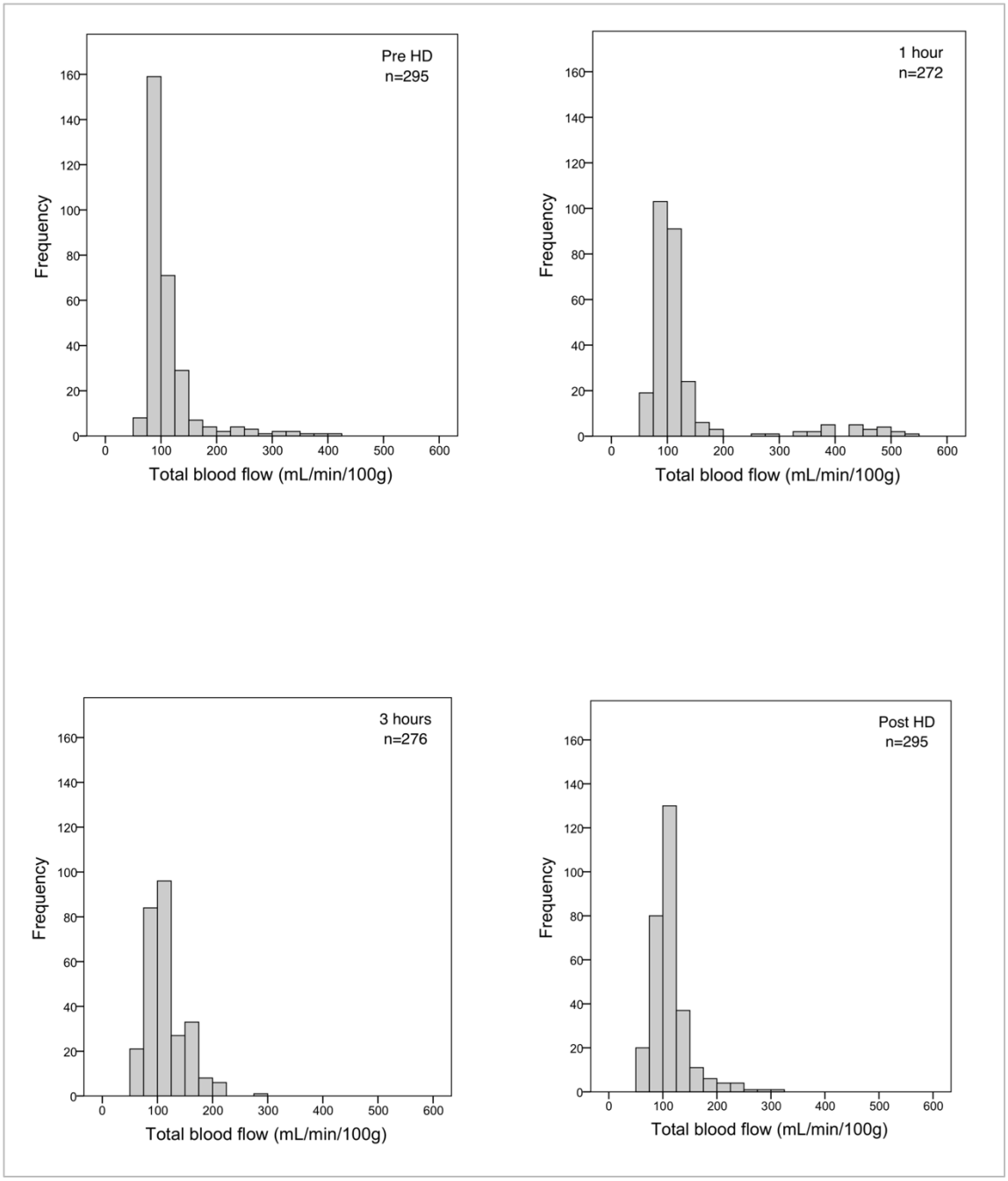


Figure 46: Each histogram demonstrates the distribution of PV-derived perfusion for each imaging timepoint (all available ROIs). Top left=pre HD, top right=1 hour, bottom left=3 hours, bottom right=post HD There was no significant difference between the timepoints

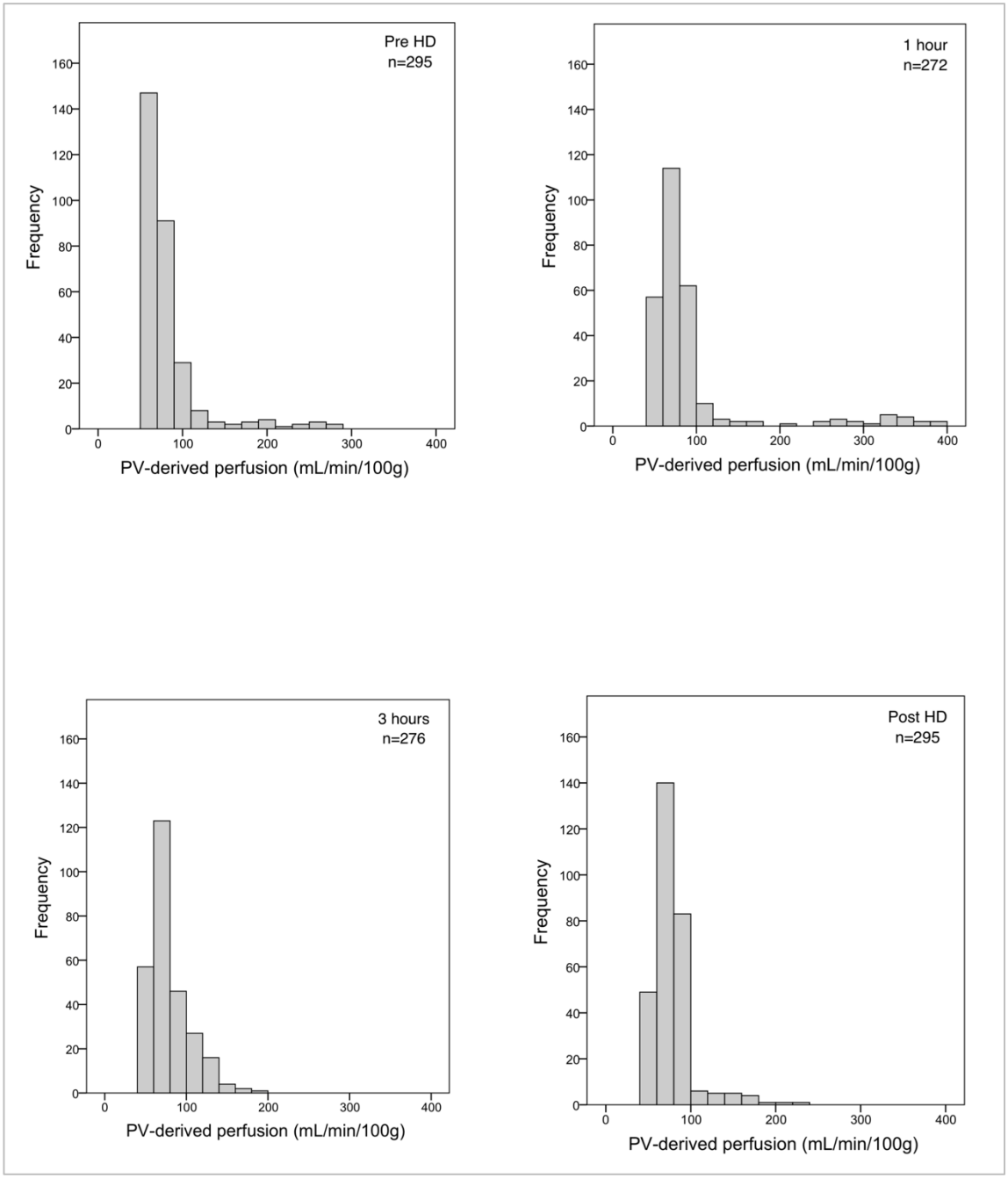


Figure 47: Each histogram shows the distribution of HA-derived perfusion for each timepoint (all available ROIs) (top left=pre-HD, top right=1 hour, bottom left=3 hours, bottom right=post HD)

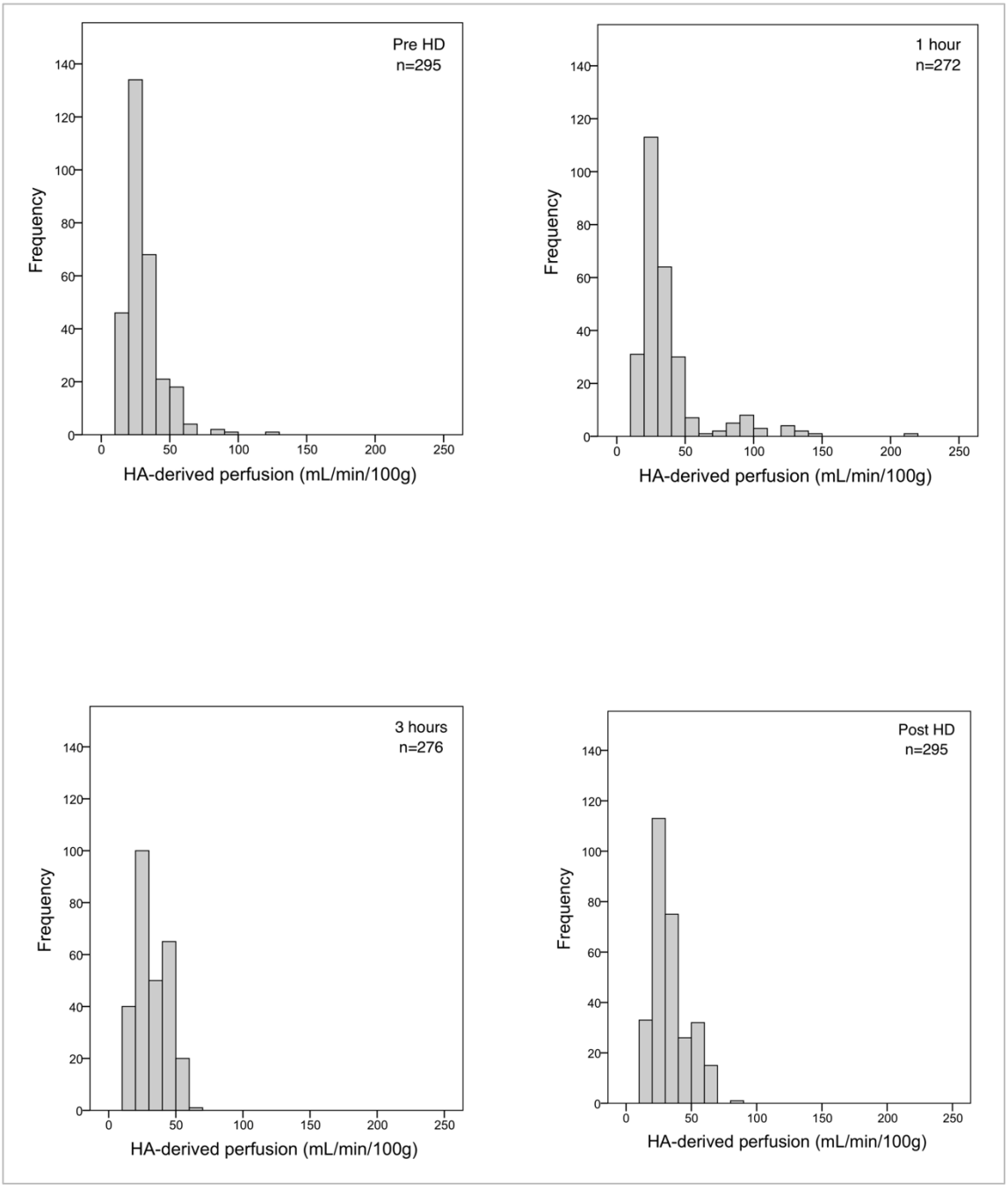
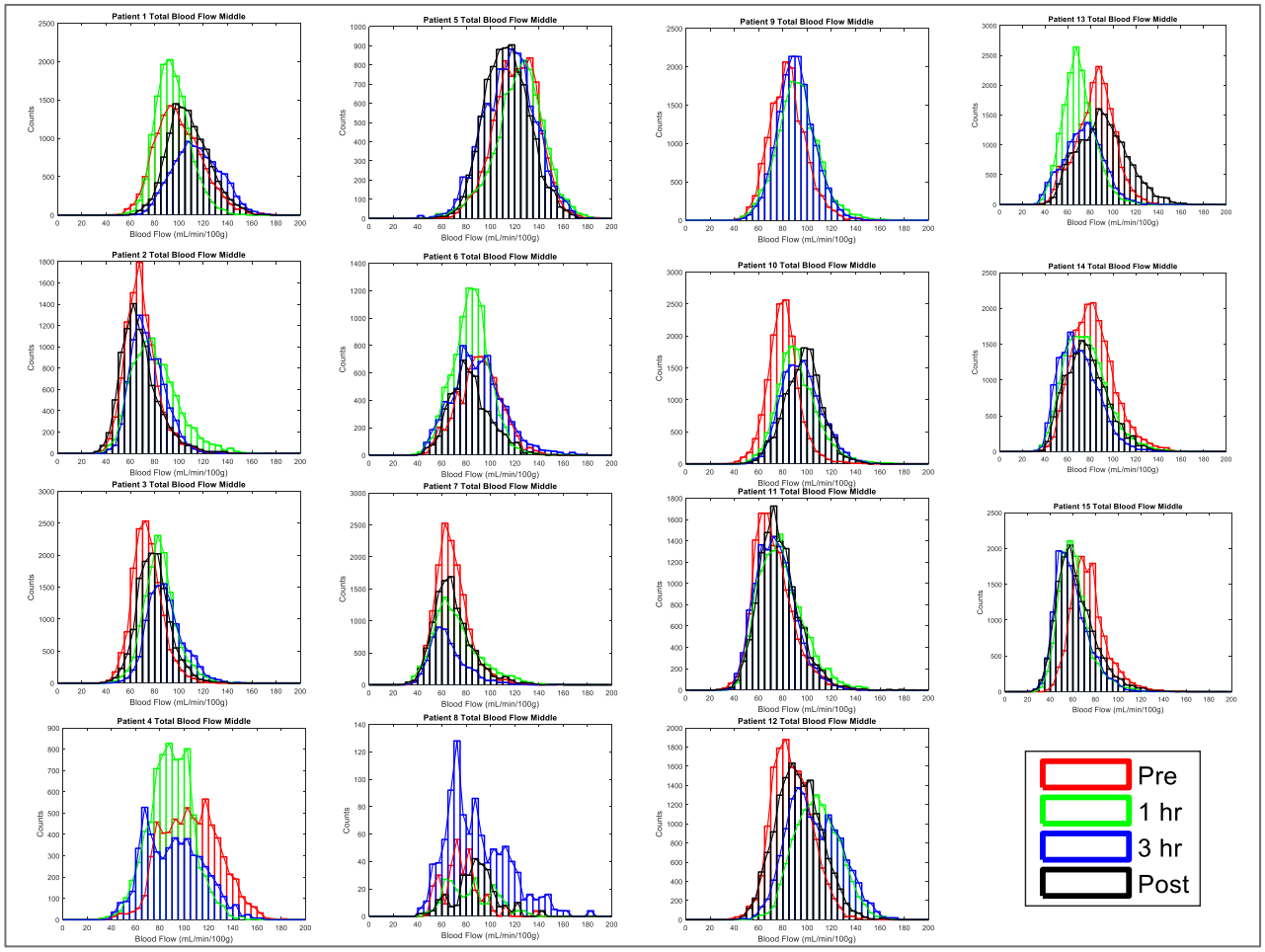
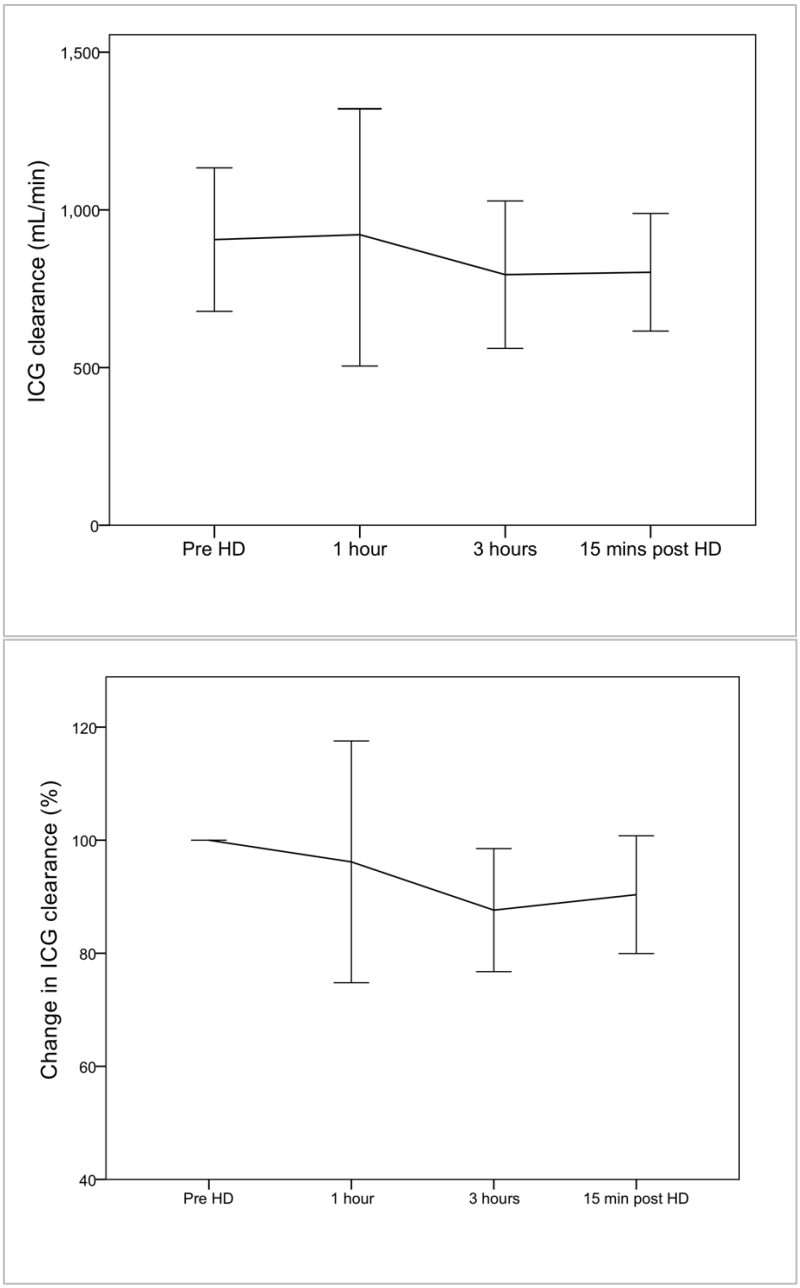


Figure 48: Pictorial example of heterogeneity of hepatic perfusion during dialysis. Each panel demonstrates an individual subject's liver blood flow histogram with the change in colour representing different timepoints.



Despite the overall preservation of total blood flow, indocyanine green (ICG) clearance, a marker of hepatic function, significantly decreased over the course of dialysis (falling from 916±420mL/min to 789±350mL/min, p=0.02 at three hours) (Figure 49). This fall in ICG clearance after three hours of dialysis was proportional to ultrafiltration (UF) volumes (r=0.69, p=0.02), but was not associated with session length, nadir blood pressure or minimum relative blood volume (RBV).

Figure 49: ICG clearance falls throughout dialysis and is significantly lower than baseline at three hours and post HD (n=15). The error bars in this figure represent 95% confidence intervals. Top panel indicates absolute change and bottom panel percentage change. p values 0.02 at 3 hours and p=0.03 post HD (paired t test) (n=15)



Comparative changes to arterial and portal derived liver perfusion were analyzed (Figure 50). Portal blood flow to hepatic tissue decreased early in dialysis from 62.8±12mL/min/100g to 57.9±14mL/min/100g and by three hours tended to remain lower at 58.3±13mL/min/100g. However, hepatic arterial flow tended to increase throughout dialysis and remained comparatively higher post-treatment (from 21.9±6.2ml/min/100g at baseline to 26.6±13mL/min/100g post-dialysis), although none of the observed changes were statistically significant.

Figure 50: Comparative origins of hepatic afferent blood flow throughout dialysis. Error bars represent 95% confidence intervals. Although there is a trend towards fall in portal flow and rise in HA flow, no statistically significant changes were observed.(n=15)

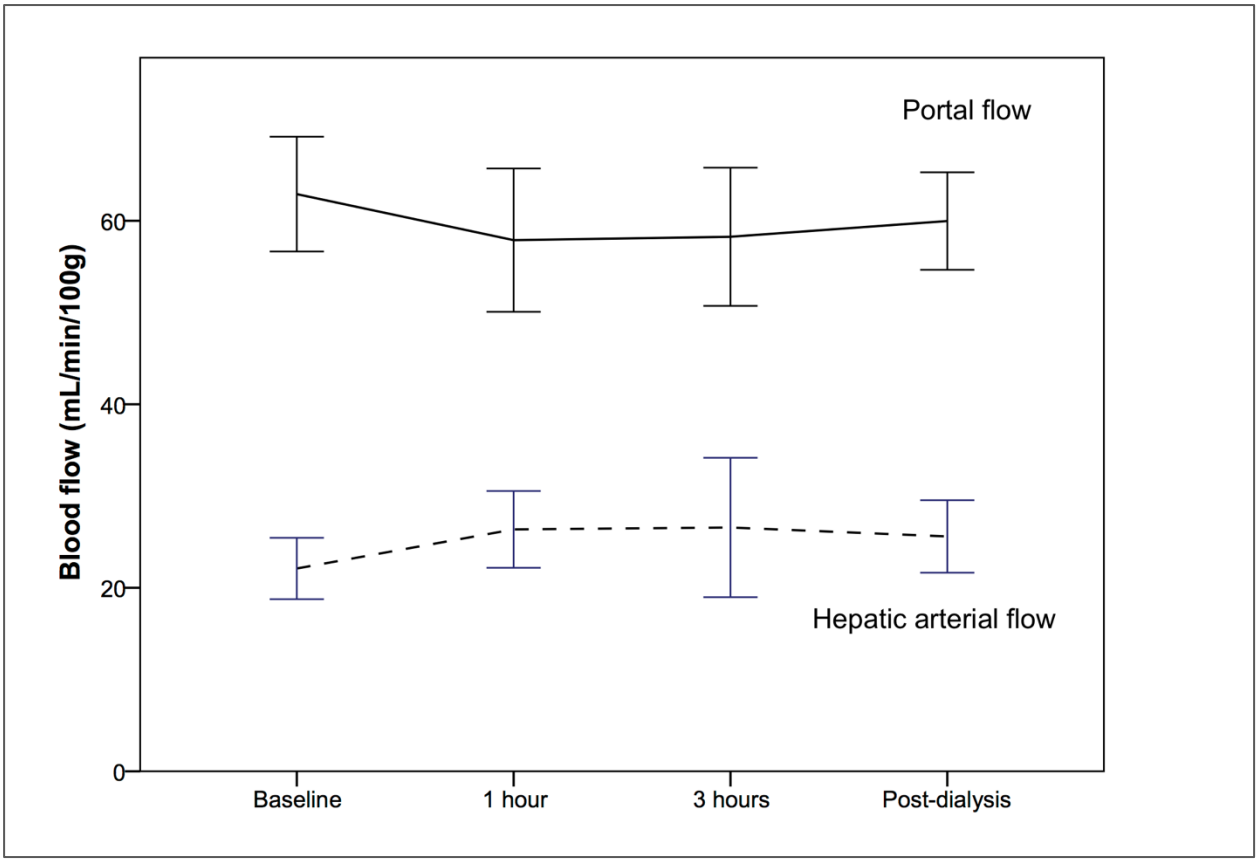
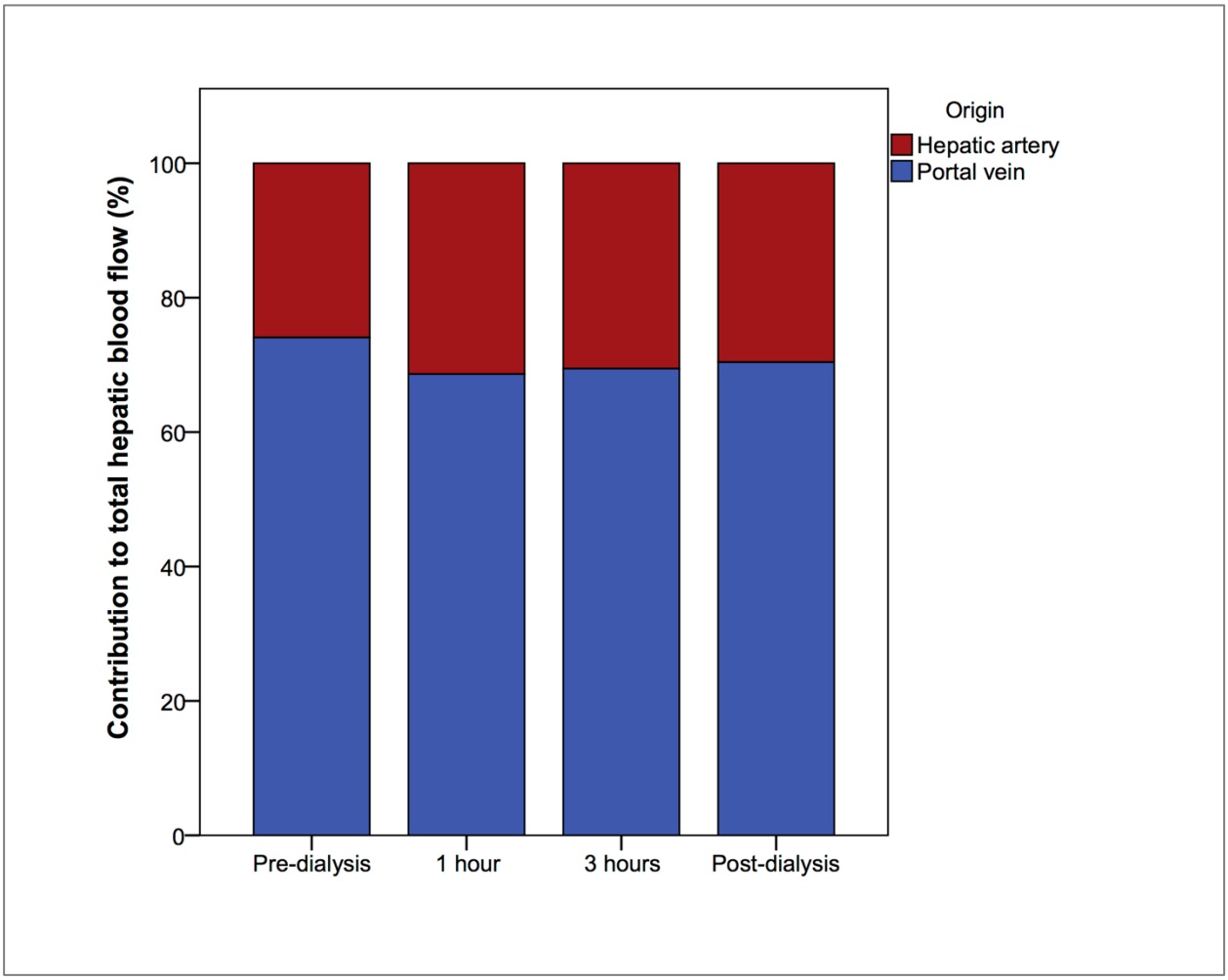


Figure 51: Schematic representation of the relative contributions to hepatic blood flow throughout dialysis. Blue bars represent PV contribution and red bars HA contribution to overall liver blood flow.



3.3 Mesenteric perfusion

Measurement of bowel perfusion throughout dialysis was possible for ten subjects (nine fasted, one fed). In fasted subjects, whilst hepatic perfusion was maintained throughout dialysis, intestinal perfusion decreased significantly (from 65.6±23.4mL/min/100g at baseline to 43.1±17.4mL/min/100g post dialysis, p=0.007).

Reduction in intestinal perfusion was associated with markers of systemic circulatory stress; including nadir blood pressure (r=0.77, p=0.03) and the maximal drop in systolic pressure (r=0.74, p=0.04). Although data were only available for one of the subjects who ate during dialysis, their gut perfusion was relatively preserved (a reduction of 3.35mL/min/100g) compared to those who remained fasted (median reduction 32.2mLmin/100g, IQR 63.0mL/min/100g).

Table 20: Mesenteric perfusion before, during and after dialysis. The top table shows the absolute bowel perfusion, whereas the bottom shows relative change. Subject 3 (shaded) ate between 1 and 3 hours of HD.

Subject	Pre HD GBF (ml/min/100g)	1hr GBF (ml/min/100g)	3hrs GBF (ml/min/100g)	Post HD GBF (ml/min/100g)
2	39.7	45.9	38.1	35.6
3	110.6	99.2	107.2	70.1
6	80.8	39.5	25.4	31.9
7	41.7	40.1	28.3	25.7
8	72.6	83.3	49.2	67.1
9	69.7	-	23.1	36.2
11	49.6	84.3	-	27.6
12	95.6	137.8	106.4	-
13	-	-	64.2	102.5
15	60.2	45.5	46.3	50.3

Subject		% change 1hr GBF	% change 3hrs GBF	% change post HD GBF
2		15.7	-3.9	-10.4
3		-10.3	-3.0	-36.6
6		-51.1	-68.6	-60.5
7		-3.9	-32.1	-38.3
8		14.7	-32.2	-7.5
9		-	-66.9	-48.1
11		70.1	-	-44.3
12		44.2	11.4	-
13		-	-	-
15		-24.4	-23.1	-16.4

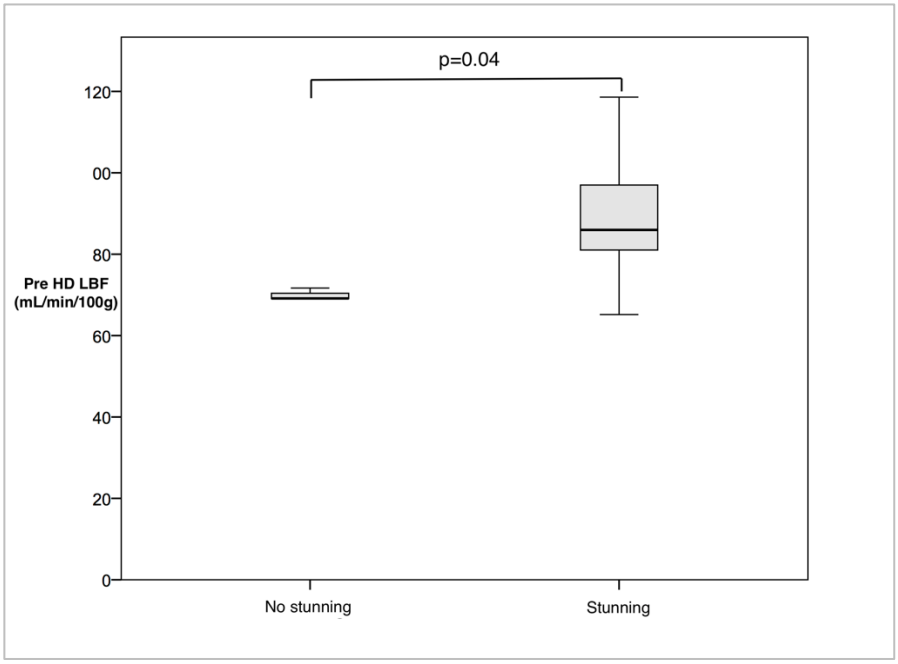
GBF = gut blood flow; HD = haemodialysis

3.4 Relationship of hepato-splanchnic changes to dialysis-related organ injury

Overall hepatic blood flow was maintained despite perfusion-related injury in other organ systems. Myocardial stunning (defined as a reduction of segmental longitudinal strain by more than 20% in two or more left ventricular segments) was present in 12 subjects, with the median number of segments affected four (range 2-7). There was no significant difference in global LV longitudinal strain ($p=0.55$) or ejection fraction ($p=0.43$) before and during HD.

As expected, severity of stunning was associated with the volume of UF ($r=0.65$, $p=0.008$) and showed a trend towards association with lower nadir blood pressure during dialysis ($r=0.46$, $p=0.08$). However, the presence of myocardial stunning was also associated with a higher total liver and portal venous blood flow throughout dialysis (Figure 52 and Figure 53). ICG clearance fell to a greater degree after 3 hours of dialysis in those who experienced myocardial stunning than those who did not ($p=0.026$) (Figure 54).

Figure 52: Boxplots to demonstrate the significant difference in pre-dialysis hepatic perfusion and portal blood flow in those who subsequently developed myocardial stunning during HD. The top panel illustrates the difference in hepatic perfusion whilst the bottom illustrates portal venous perfusion.



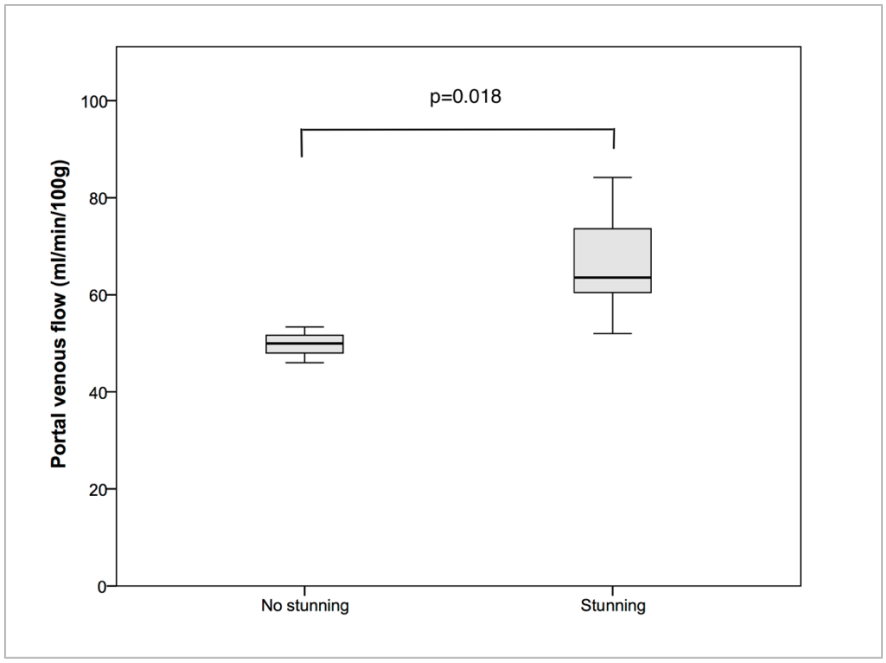
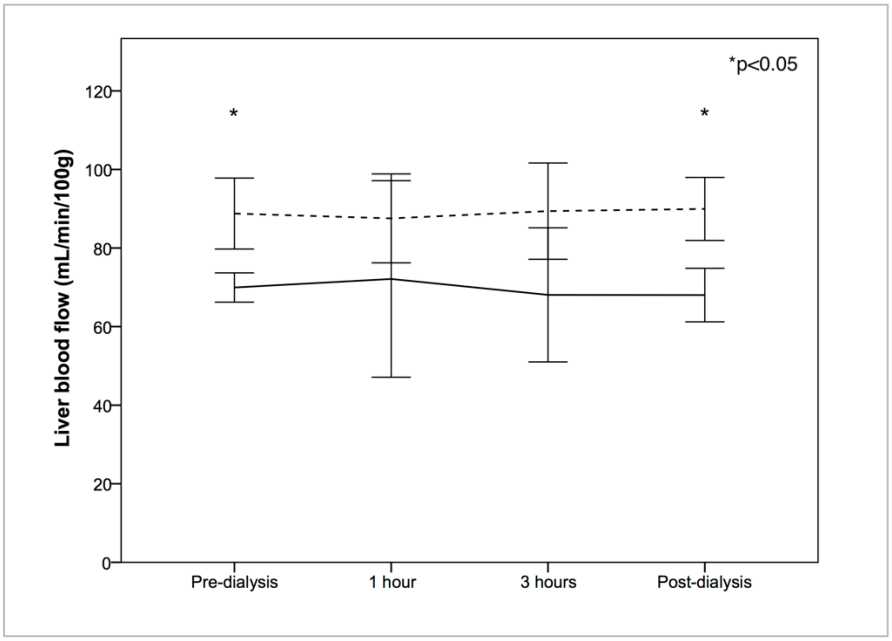


Figure 53: Serial blood flow comparisons in those with stunning and those without. The top panel illustrates the lower total liver blood flow before and after dialysis for those who had myocardial stunning. The bottom panel shows significantly lower portal venous perfusion for those without stunning. For each panel, the dotted line represents those with myocardial stunning whereas a solid line represents those without myocardial stunning. Error bars represent 95% confidence intervals.



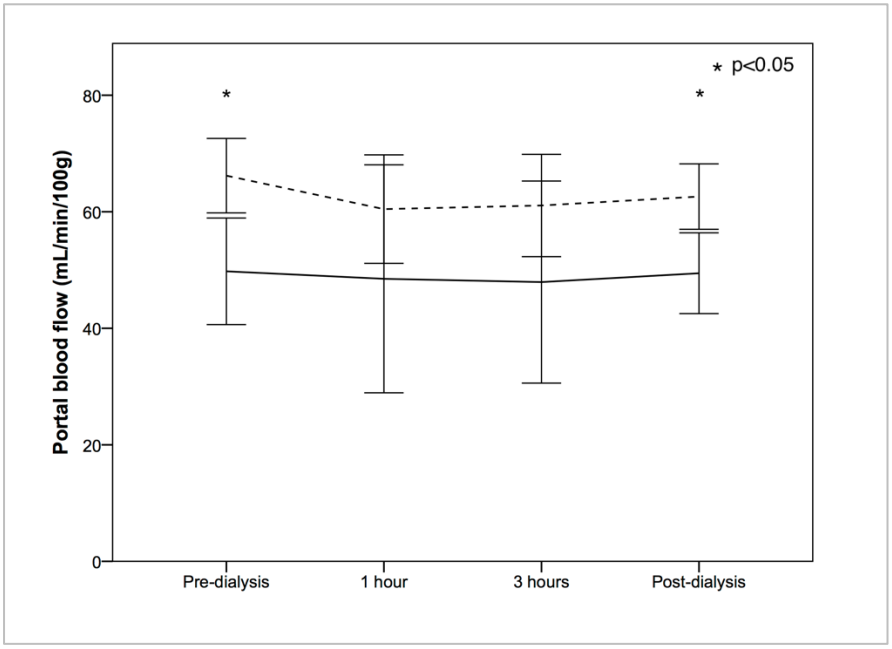
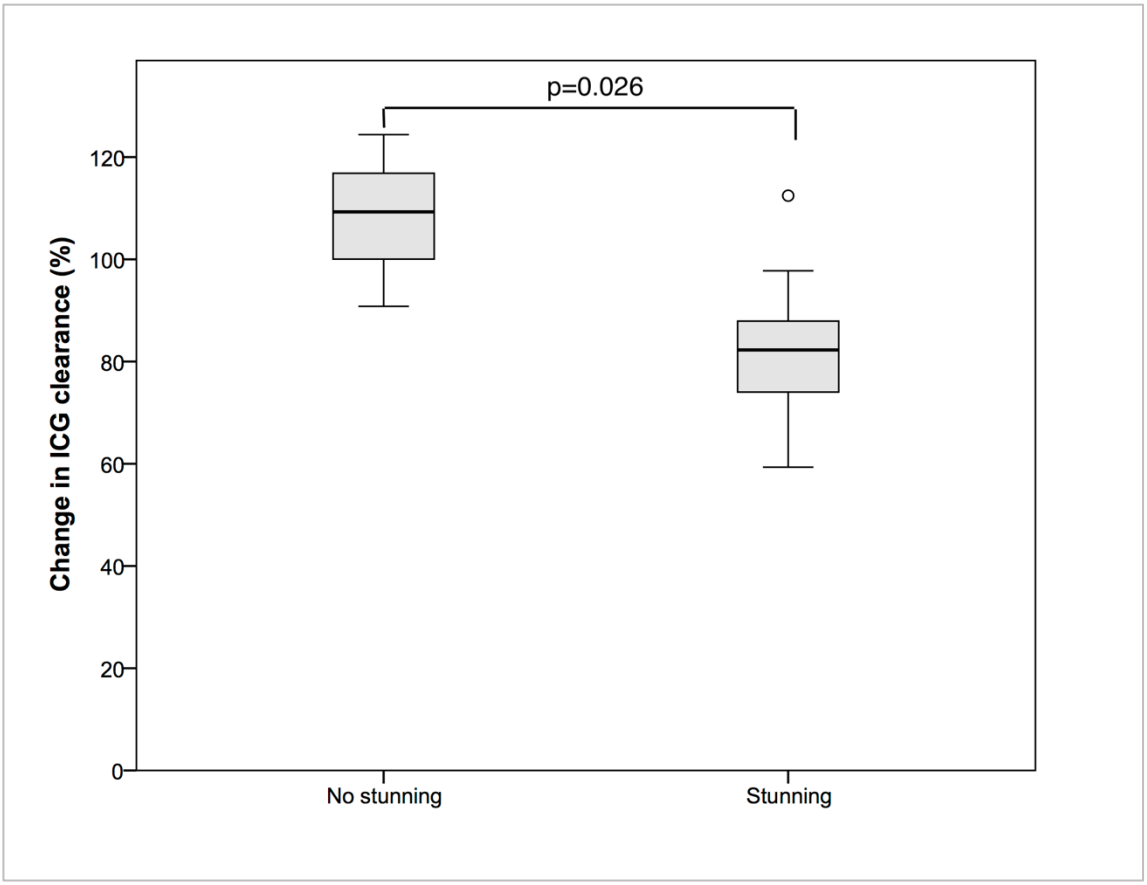


Figure 54: Boxplot demonstrating the relatively preserved ICG clearance in those without stunning compared to those who did stun.



3.5 Changes in uraemic toxin concentration

Pre-dialysis plasma concentrations of selected protein-bound uraemic toxins were comparable with those in previously published studies [88] (Table 21). Solute levels at this single session measurement were not significantly associated with age, BMI, dialysis vintage, symptom severity, pre-dialysis intestinal, total hepatic or portal venous flow. There were no significant differences in toxin levels between subjects who had myocardial stunning during dialysis and those who did not.

There was an association between the reduction ratio of indoxyl sulphate and the observed changes in hepatic blood flow during dialysis (Figure 55). However, there was no significant association between toxin clearance and changes in ICG clearance with dialysis (see Table 21).

Figure 55: Greater perturbation of hepatic perfusion was associated with lower reduction of indoxyl sulphate (Δ=fed patients). Left panel represents percentage change mean transit time, right panel represents percentage change in hepatic blood volume after 3 hours of HD.

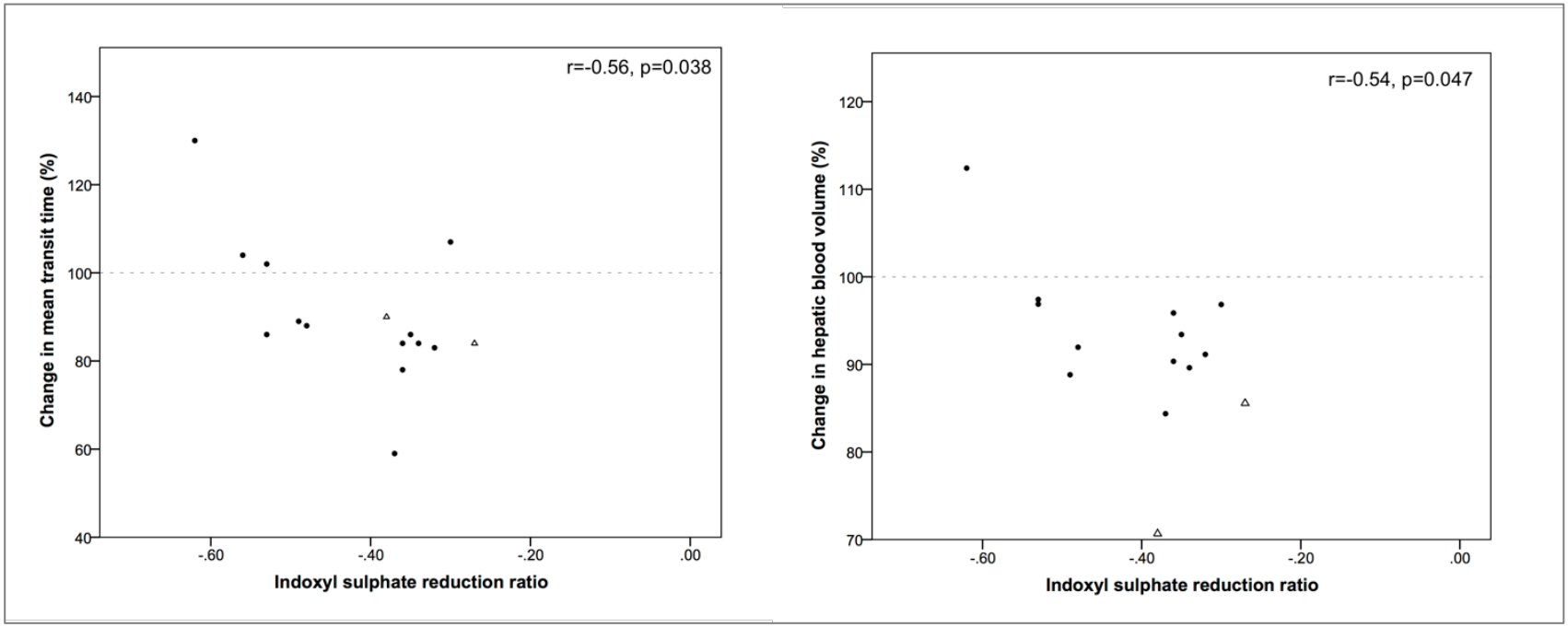


Table 21: Measured uraemic toxins concentrations before and after dialysis

Uraemic Toxin	Protein binding (%) [165]	Pre-dialysis concentration (mmol/l)	Post- dialysis concentration (mmol/l)	% reduction (range)	Correlation with change in hepatic blood volume at 3 hrs (r)	P value	Correlation with change in ICG clearance (r)	P value
Indoxyl Sulphate	97.7±0.2	119±45	69.3±33	37.2 (27-62)	**0.54**	**0.047**	0.06	0.83
p Cresyl Sulphate	95.1±0.6	219±160	144±120	30.0 (21-52)	**0.54**	**0.048**	0.04	0.89
Phenoxyl Sulphate	90.7±0.9	45.5±28	16.1±13	67.7 (50-89)	0.33	0.25	0.03	0.91
Hippuric acid	48.3±2.5	317±200	108±81	67.6 (48-80)	0.15	0.61	-0.46	0.09
p Cresyl Gluconate	32.3±2.6	8.57±6.4	2.31±1.8	72.3 (54-96)	0.09	0.75	-0.03	0.91
Urea	-	19.5±4.4	5.86±1.5	69.5 (62-82)	0.34	0.23	0.01	0.97

3.6 Symptoms and subjective experience

Levels of depression and anxiety were low in this cohort (HADS-D 3±4, HADS-A 4±3), with no subjects scoring abnormally (>10) in either domain. For appetite assessment, median SNAQ score was 15 out of 20 (range 13-18). Three patients had abnormally low appetite scores (<14). Appetite levels were not associated with measured albumin. Median 5D itch score was 10 (range 0-26) and was associated with higher anxiety scores ($r=0.72$, $p=0.003$) but not with other symptoms, nor with baseline uraemic toxin levels.

3.7 The effects of eating

This study was not intended nor powered to assess the effects of eating upon liver perfusion during HD. The subjects who ate during dialysis were included in the analysis of effects of HD on liver blood flow. However, all statistical analyses were repeated excluding these two subjects after the time of eating. Total liver blood flow for the fasted group was maintained throughout dialysis, yet both MTT and HBV fell significantly post HD ($p=0.022$ and $p=0.014$ respectively). However, there was no longer a statistically significant fall in ICG clearance at 3 hours ($p=0.051$) or post HD ($p=0.167$). The relationships between myocardial stunning and ICG clearance, myocardial stunning and LBF and myocardial stunning and portal

blood flow all remained statistically significant ($p<0.05$). The reduction in indoxyl sulphate remained significantly smaller in those with a greater fall in MTT at 3 hours ($p=0.04$) but not with HBV ($p=0.22$).

4 Discussion

This is study demonstrates that there are significant changes in splanchnic and hepatic perfusion during HD. This is associated with a fall in hepatic excretory function and is related to the degree of circulatory stress. Although HD-associated hypoperfusion in vulnerable circulations as a consequence of hemodynamic stress is well-recognized [8], this is the first time that this has been directly observed in the gut and liver.

A fall in mesenteric perfusion was seen in the majority of patients during dialysis. This was associated with severity of circulatory stress of dialysis, as evidenced by the relationship to intra-dialytic fall in blood pressure. Splanchnic hypoperfusion appears to be in part an adaptive mechanism for preservation of critical organ function during circulatory stress, being observed even in health during heavy exercise [166]. However, the intestinal epithelial barrier appears vulnerable to even brief periods of hypoperfusion in both health and disease, leading to the translocation of normally confined intra-luminal toxins, which stimulate the systemic inflammatory response. The exact mechanism for translocation of many of the identified uraemic toxins from the intestine into the portal circulation is yet to be described [87]. However, acute ischemia affects the totality of

intestinal barrier function, through disruption of both paracellular and transcellular mechanisms, as well as the mucosal portion [167].

A complex circulatory response to dialysis was observed within the liver, perhaps unsurprising given the sophistication of hepatic vascular regulation. The hepatic circulation is partially dependent upon changes within the mesenteric vascular bed, yet appears to be relatively protected from injury under conditions of circulatory compromise, unlike the gut. This is thought to be as a consequence of the hepatic arterial buffer response (HABR) which allows arterial dilatation when portal (and by inference, mesenteric) blood flow drops [62]. Here, we have shown that portal blood flow tends to fall during dialysis as intestinal perfusion falls and blood is diverted away from the splanchnic reservoir. Concurrently, hepatic arterial flow within the liver increases throughout dialysis, demonstrating an intact buffer response. This is consistent with other studies which show that the HABR remains intact in all but the most profound circulatory shock [168].

Despite the preservation of total hepatic blood flow, we observed that hepatic blood volume fell early in dialysis, in conjunction with a decrease in MTT. This may reflect the physiological response to a fall

in circulating volume, where an expulsion of hepatic pooled blood occurs in order to support the systemic circulation. The significance of dynamic hepatic MTT has yet to be well-established in health or disease, given the relatively recent advent of dynamic perfusion imaging in the liver. However, in this study, hepatic MTT was longer than reported values in healthy subjects in another study [169]. Whether this represents microcirculatory dysfunction within the liver remains to be established. However, a fall in MTT may decrease the availability of oxygen and substrate for extraction.

Furthermore, a change in regional distribution of perfusion was observed. Whilst portal-venous perfusion did not change across the imaged areas of the liver, the distribution of arterial-derived flow altered significantly. This is a novel observation but one which is biologically plausible given that the liver cannot regulate portal inflow.

We observed a significant fall in ICG clearance during and immediately after dialysis. In the normally functioning liver, ICG is completely and efficiently extracted from the sinusoids and secreted by hepatocytes into the bile canaliculi, therefore making it an effective measure of hepatic blood flow [149]. It is not clear why ICG clearance falls when hepatic perfusion is maintained. However, there

are several possible explanations for why this may occur. Firstly, we have demonstrated that although overall liver blood flow is preserved, there is regional variation in distribution of blood flow, with some areas experiencing a considerable change in perfusion coupled with a fall in the mean transit time of blood though the liver, potentially resulting in the reduced availability of ICG for extraction. Secondly, the demonstrated disconnect between blood flow and clearance could imply acute hepatocyte dysfunction, possibly as a direct result of toxin exposure from the increasingly toxin-laden portal blood from the stressed mesenteric circulation.

It was beyond the scope of this pilot study to conclusively establish the effect of circulatory stress findings upon uraemic toxin translocation and metabolism. However, in these anuric subjects, there appears to be a relationship between changes in the hepatosplanchnic circulation during dialysis and dynamic uraemic toxin levels, which deserves further attention and characterization in future studies, under direct experimental manipulation of the conditions within a single dialysis session. We found that disruption to hepatic perfusion during dialysis was associated with poorer clearance of indoxyl sulphate (IS), a potent toxin associated with cardiovascular

morbidity and mortality in HD patients, despite preservation of overall blood flow.

Perturbation of the hepatosplanchnic circulation may be compounded by the cardiac dysfunction that is observed during HD. Myocardial stunning with cumulative cardiac dysfunction occurs as a consequence of recurrent hypoperfusion to the myocardium and is common in HD patients [21]. Here we observed that stunning was present in the majority of patients during dialysis (for the most part without overt evidence of hemodynamic instability) yet appeared to be related to the degree of perturbation of hepato-splanchnic perfusion, despite a lack of change of global LV function. Recurrent cardiac injury has been shown to lead to cumulative cardiac dysfunction in dialysis patients [26], thereby potentially triggering a vicious cycle of continued multi-organ injury and acting as a driver of uraemic toxin accumulation, by both potentiation of splanchnic injury, as well as limiting tolerability of dialysis and thus, toxin removal.

Whilst our understanding of the potential harms of acute circulatory stress in HD may extend to increased uraemic toxin generation and exposure, simple interventions may be applied to lessen these effects. Addressing the other factors contributing to systemic exposure such

as dysbiosis and reduced clearance of protein-bound toxins have thus far proven difficult to reliably achieve long-term [137]. However, cooling dialysate, an inexpensive and widely applicable intervention, has been shown to be effective in reducing both cardiac and cerebral injury and promote stability on dialysis [22,29]. Future studies are needed to examine whether such treatments are similarly effective in protecting the hepato-splanchnic circulation.

Limitations

The symptom burden of this group of subjects was not as high as might be expected of the HD population, likely as a consequence of self-selective participation. Here symptom scores correlated poorly with multiple parameters, including those well-known to be associated with increased symptom burden, reflecting the limitations of currently available tools to capture patient experience. There were two further limitations to our ability to wholly represent the HD population in this pilot study. Firstly, we studied a group of largely fasted patients to remove the hemodynamic impact of eating as a confounder. However, patients exhibit differing preferences to eating during dialysis and the impact of this potentially important factor in the evolution of intestinal hemodynamic injury and toxin translocation has yet to be established. Secondly, owing to the methodology employed, we were restricted to

recruiting those who already had minimal residual renal function, hence may have pre-selected those who are more susceptible to dialysis-associated circulatory stress. However, studying anuric subjects negated any effects of residual renal function upon uraemic toxin clearance, allowing a more robust assessment of the relative reduction of gut-derived toxins. Nonetheless, future studies ought to ideally include incident dialysis patients or those with relatively preserved renal function as far as available methodology permits.

Despite an intention to study fasted patients, this study included two subjects who ate during HD. Interestingly, this seemed to have little effect on the changes during HD that were observed. However, it is impossible to be confident with such small subject numbers whether the changes which were observed with their exclusion were due to statistical error rather than true significance. As previously mentioned, the effects of eating during HD are an important subject for further study.

This is the first study to report intestinal perfusion measurement using dynamic contrast-enhanced CT. Assessment of a motile and heterogeneously-perfused organ is challenging, and this methodology requires validation in future studies. Comparison of absolute intestinal

perfusion was not possible due to the varying anatomical location of identifiable bowel selected and therefore relative changes in perfusion have reported. Quantification of SMA flow is less challenging, however, it fails to provide data on microcirculatory dysfunction and end-organ perfusion within the splanchnic circulation.

In this pilot study, we measured total uraemic toxin concentrations in serum, but not dialysate clearance. This data is essential in future studies to determine the contribution of intra-dialytic metabolism of gut-derived toxins to systemic toxin burden.

The number of subjects in this study precluded the application of multivariate analyses to determine independent associations, where correlation was demonstrated. Furthermore, this pilot study may not have been sufficiently powered to avoid Type 2 error. With the data obtained here, larger studies can be designed to rigorously test the hypotheses generated from this work.

5 Conclusion

Both splanchnic and hepatic perfusion are acutely affected by HD. Conventional HD may drive uraemic toxin exposure (to a variable extent between patients), potentially perpetuating the chronic inflammatory state characteristic of end-stage renal disease. This may be a key factor underlying the disconnect between uraemic symptom experience and dialysis adequacy. Furthermore, unlike the complex task of addressing dysbiosis and toxin generation within the gut, it may be more readily amenable to intervention through the application of dialysis-based therapies to reduce circulatory stress.

Chapter 5: Overall Discussion

1 Summary of Results

The aim of this project was to examine the effects of the circulatory stress of end-stage chronic kidney disease and HD upon the liver. It has been demonstrated that significant hepatic congestion is uncommon in dialysis patients and compliance is inaccurate as a surrogate marker for fluid status. Liver stiffness significantly alters with HD, although not consistently decreasing as expected with fluid removal. The liver appears to become more stiff in those with a tendency to develop intra-dialytic hypotension. Total hepatic blood flow does not significantly change throughout dialysis, although hepatic blood volume and mean transit time fall significantly. There appears to be a disconnect between hepatic blood flow and ICG extraction throughout dialysis, suggesting impaired hepatic function.

2 The liver and circulatory stress: the effects of fluid overload

Data from this project show that liver stiffness is not profoundly raised in the majority of dialysis patients, supporting findings in smaller studies [102,103] and suggesting an absence of significant liver congestion. Moreover, there is a lack of clear relationship between hydration status and measurements of hepatic compliance, even in the few patients with significantly elevated LS or in those with evidence of substantial fluid excess, effectively appearing to erase the utility of transient elastography to detect those with significant fluid overload pre-dialysis. This might be due to the impact of unidentified confounding factors such as fibrosis or steatosis, which were impractical to measure in this work. However, the presence of confounding factors elevating liver stiffness does not account for the subjects with considerable fluid overload, detectable both clinically and through bioimpedance, who had normal liver stiffness. This is particularly unexpected given recent animal studies which show that acute increases in central venous pressure and arterial pressure are independently capable of elevating liver stiffness [64,111].

In the CT-based study, there was also a lack of association between pre-dialysis measurements of hepatic haemodynamics and pre-dialysis volume status. Neither hepatic blood volume nor the transit time of blood across the hepatic vascular bed were associated with representative measures of volume status pre-dialysis: IDWG, bioimpedance-derived total body water, extracellular water or haematocrit.

From these studies, it is evident that profound hepatic congestion is not present in HD patients. However, it appears that neither is blood sequestered in the liver pre-dialysis, as would be expected with evidence of hypervolaemia. It is beyond the scope of this work to identify whether this represents a failure of a protective mechanism against cardiovascular stress. However, hepatic vasoregulation relies upon local endothelial and systemic autonomic activity, both of which have been shown to be dysfunctional in the HD population [11,14]. Further study is needed to establish whether the hepatic response to volumetric stress impacts upon the evolution of LVH and cardiac dysfunction in the dialysis population.

3 The liver and circulatory stress: the acute effects of HD

This work has shown that the HABR is intact in HD patients undergoing HD with ultrafiltration. PV flow decreases early in dialysis, presumably as a result of splanchnic vasoconstriction, with a concurrent rise in HA flow observed. Consequently, total liver blood flow is maintained throughout dialysis. However, hepatic blood volume falls early, suggesting that physiological expulsion of pooled blood to support the cardiovascular system also occurs.

In recognising that overall liver blood flow is not significantly affected by the stress of HD, it must be noted that this occurred in the context of myocardial stunning as well as a significant decrease in perfusion to the splanchnic vascular bed. This observation is consistent with haemodynamic changes observed in other low flow states, where clinically-detectable hepatic ischaemia is uncommon. Regulation of hepatic perfusion, although influenced by systemic haemodynamics, is also locally regulated through the HABR, allowing maintenance of hepatic perfusion in the face of falling perfusion elsewhere.

Nevertheless, it was also observed that higher liver blood flow was associated with a more severe cardiac dysfunction during dialysis. Concurrent measurements of hepatic blood flow and cardiac function have rarely been assessed in experimental or observational studies of other low-flow states, therefore interpreting this novel finding is challenging. However, the finding may be supported by data from the elastography and MRI based studies which showed that many HD patients exhibit a rise in liver stiffness and water content which tended to be associated with haemodynamic instability during dialysis. It is uncertain whether these reflects the hepatic response to cardiovascular stress or a pathological association, with the liver receiving a disproportionately large proportion of a falling cardiac output.

Despite the gross maintenance of LBF, there was disruption to regional blood flow. Physiologically, liver blood flow tends to be homogeneous [170], however, redistribution of hepatic blood has been shown to occur in low-flow states [171,172]. It remains unclear whether this is part of physiological compensation or a maladaptive response to altered oxygen demand. Microcirculatory dysfunction, even in the absence of gross hepatic ischaemia, may provoke cytokine release, alter hepatic metabolism and impair function [173].

Indeed, we observed a reduction in ICG clearance from the circulation. Reduction in ICG clearance has been observed in a previous study of HD patients [77], however this is the first time that blood flow and clearance have been measured simultaneously, allowing the identification of the disproportionate fall in ICG clearance despite maintenance of total hepatic blood flow. Interestingly, the fall in ICG clearance was proportional to the degree of myocardial stunning. The pathophysiology underlying this association is unclear but it links a well-described sequela of dialysis-associated stress with functional disruption in the liver. It was beyond the scope of this project to ascertain if the fall in ICG clearance was reflective of more extensive hepatic dysfunction. Nevertheless, there was a relationship observed between protein-bound uremic toxin clearance and dialysis-associated changes in hepatic haemodynamics which requires further exploration.

The hypothesis that the relative contributions of portal venous and hepatic arterial supply would significantly change during HD was not supported by this work.

In summary, HD disrupts hepatic perfusion, although overall blood flow is maintained and physiological mechanisms to protect both the

liver and the cardiovascular system from stress appear intact. During dialysis, hepatic dysfunction is observed, which appears to be related to extra-hepatic organ injury. The significance of a rise in dynamic liver stiffness remains uncertain.

4 Clinical Significance

With observational data from these studies, it would be premature to draw firm conclusions about the full clinical significance of the hepatic role in the response to dialysis. Nevertheless, there are several direct clinical implications from what has been found.

Firstly, it is evident that conventional methods to assess hydration or stability during dialysis fail to reflect the complexity of regional haemodynamic response to circulatory stress in HD patients. Identification of those most at risk of dialysis-associated organ dysfunction requires a more comprehensive understanding of regional haemodynamics and organ cross-talk in cardiovascular stress.

Secondly, the consequences of altered hepatic perfusion and function during dialysis need to be considered. The hepatic immune response provides the final functional barrier against the potentially toxic products of intra-luminal bacterial fermentation [139]. Impairment of this response as a consequence of regional hypoperfusion provides another mechanism to explain the increased bacterial translocation in HD patients, which is strongly linked with mortality. Although attention has been largely directed at modifying the intestinal milieu to reduce endotoxaemia, methods to reduce circulatory stress upon

the hepato-splanchnic system may provide another therapeutic target to reduce morbidity and mortality.

The liver also plays a role in the metabolism of many gut-derived uraemic toxins. If temporary hepatic dysfunction occurs as a consequence of altered hepatic perfusion, the release of gut-derived toxins, or indeed urea, into the systemic circulation may be affected by the stress of dialysis. This has implications for modelling the kinetics of toxin clearance. Conventional methods to measure dialysis adequacy are largely based upon the assumption that the advent of toxins into the circulation is constant.

Finally, it has been demonstrated that hepatic perfusion is relatively well-preserved at a time where cardiac, renal and splanchnic blood flow appears to be significantly compromised. Whilst the relative rarity of hepatic ischaemia in low-flow states has been recognised, it appears that, in maintaining constant afferent blood flow, the liver may consume a greater proportion of cardiac output, potentially disproportionately reducing other regional blood flow. It remains to be established whether this contributes to the pathophysiology of specific extra-hepatic organ injury in other low-flow states.

5 Future Work

A study is currently underway to assess the effect of implementing strategies to alleviate circulatory stress during HD. Subjects will undergo assessment of hepato-splanchnic perfusion using dynamic CT scanning during a normal HD session and during a session where their dialysate is cooled – a technique which has been shown to reduce other dialysis-associated organ injury [22,29].

A project is also being conducted using imaging data from the CT study to examine the relationship between markers of endothelial dysfunction, systemic levels of endotoxin and dynamic changes in hepatic perfusion during HD. Detection of circulating endothelial microparticles using nanoscale flow cytometry will be used to quantify endothelial dysfunction.

A study is also being prepared to determine the impact of food ingestion during dialysis upon cardiovascular stability and the splanchnic response to HD. Eating during dialysis remains a controversial subject with some studies demonstrating a link to intra-dialytic hypotension [174], whilst others have contested this view [175]. Given the variability we have observed in hepato-splanchnic response, a trial of feeding and fasting with a cross-over design

appears to be the most appropriate methodology for testing the hypothesis that food ingestion alleviates the fall in intestinal perfusion.

6 Conclusion

Physiological mechanisms to preserve overall hepatic perfusion remain intact during HD, despite evidence of concurrent critical organ injury. However, hepatic haemodynamics appear to be both affected by and to affect the circulatory stress of HD. The liver's multi-dimensional functions, which include cardiovascular protection, host defence and toxin metabolism, render it of hitherto under-appreciated importance in the study of dialysis-associated circulatory stress and in morbidity in end-stage renal failure.

Appendices

1. Patient Information Leaflet for Chapter 2
2. Patient Information Leaflet for Chapter 3
3. Patient Information Leaflet for Chapter 4
4. Questionnaires used in Chapter 4
    - 4.1. HADS
    - 4.2. SNAQ
    - 4.3. SF-36
    - 4.4. 5D-Itch

LETTER OF INFORMATION AND CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Study Title

Bedside ultrasound-based assessment of liver compliance to measure fluid status in hemodialysis patients

Name of Principal Investigator (Study Doctor)

Dr Shih-Han Susan Huang

Associate Professor
Kidney Clinical Research Unit
London Health Sciences Centre
Victoria Hospital
800 Commissioners Road East
London, ON, Canada, N6A 5W9

Co-Investigator

Dr Claire Grant
Research Fellow
Kidney Clinical Research Unit

Dr Mark Levstik
Hepatologist
University Hospital

Dr Chris McIntyre
Professor of Nephrology
Kidney Clinical Research Unit

Contact Information

Phone: 519 685-8500 Ext 55975

Conflict of Interest

None

Introduction

We would like to invite you to take part in our research study. We are asking you as you are a patient having regular hemodialysis. Before you decide, we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. You are free to talk to others about the study if you wish. Please feel free to ask us if there is anything that is not clear.

Background/Purpose

Patients on hemodialysis have higher rates of death from heart disease or heart failure than the normal population. We know from previous research that damage is done to the heart from having too much or too little fluid removed during dialysis and gaining too much fluid between dialysis sessions. Therefore, assessing how much fluid to remove is a critical importance to maintaining health. We are unsure whether the liver suffers from fluid imbalances during dialysis and whether measuring fluid content in the liver could help us to tell how much fluid to remove during dialysis. We are planning to conduct a research study to help to answer these two questions.

Study Design

This is a non-invasive study where you will be asked to have a couple of painless tests before and after your normal dialysis for one session, in your normal unit. We will not ask you to take any additional or experimental medication for this study.

Procedures

If you are given information about the study and are considering taking part, one of our research team will contact you in person to meet you to discuss the study.

You will have an opportunity during this initial meeting to discuss any questions or worries you may have. After this, you will be asked to sign a form to say you consent to be part of the study. Once you have agreed to take part in the study, we arrange the date for you to be involved in the study. This visit will be the only time you will be required to spend as part of the study. We expect the entire study will add on approximately 20 minutes before and after dialysis.

Before and after dialysis, the following tests will take place (in any order):

- ***Physical examination –*** a doctor will listen to your lungs and heart as well as examining your ankles for swelling.

- ***Blood test*** – this will be no different to a normal blood test taken on dialysis. We will take 20mls in total. This involves no additional needles as the same access will be used for your dialysis.

- ***Bioimpedance test*** – this is a way to measure the amount of fluid in your body using small amounts of electrical current. You will have four stickers placed on your wrist and foot which are hooked up to the analyzer for approximately 2 minutes. The test is completely painless and has no side effects or risks attached.

- ***Fibroscan® test*** – this is a device used to measure how stiff your liver tissue is. This is measured by placing a probe over the right side of your ribcage and taking 10 measurements using an ultrasound. It is also painless and has no side effects. In order to measure the same part of liver before and after dialysis, we will mark the spot measured with a washable marker.

Voluntary Participation

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights. If you decided to not take part, this would not affect your future care in any way.

Withdrawal from Study

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and this information may still be used in the project analysis.

Risks/disadvantages of participation

None of the above procedures carry any specific risks or long-term side effects.

We anticipate that being in the study will require you to stay approximately an extra 40 minutes at one of your visits for dialysis. .

There will be no delay to your routine healthcare if you participate in the study. The treatment of your disease will continue as normal throughout the time that you spend in the study.

Benefits

This study will not benefit you directly but the information we get from this study may help us understand what causes some of the complications of dialysis and therefore, in the future, to look at ways of helping prevent these from happening.

Alternatives to Being in the Study

If you do not wish to participate in the study, your care will continue as normal and not be affected in any way. If you wish to, you can indicate to your usual doctor that you want to be informed of any future studies that you might be eligible to take part in.

Confidentiality

We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorized persons only who are part of the research team. Representatives of Western University's Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research. All will have a duty of confidentiality to you, as a research participant, and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidentially**, stored in a secure and locked office, and on a password protected database. Any information recorded from your health records which is used in the research will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognized from it.

Your personal data (telephone number/email) will be kept for 6 months after the end of the study. All other data (research data) will be kept securely for 15 years. After this time, your data will be disposed of securely. During this time, all precautions will be taken by all those involved to maintain your confidentiality,

only members of the research team will have access to your personal data. Representatives of Lawson Quality Assurance (QA) Education Program may look at study data for QA purposes, however this data will be anonymized and you will not be identifiable from this. All data will be stored and protected in accordance with the Personal Information Protection and Electronic Documents Act, 2000.

Costs

This study will not cost you any money. All travel expenses or parking will be covered (see below).

Compensation

You will not be paid to participate in the study. Travel expenses will be offered for any visits incurred as a result of participation.

Questions about the Study

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the beginning of this information sheet. If you have any questions about your rights as a research participant or the conduct of the study, you may contact Dr. David Hill, Scientific Director, Lawson Health Research Institute (519) 667-6649.

Consent Form

Evaluation of the Relationship between Liver Stiffness and Fluid Balance in Chronic Hemodialysis Patients

Name of researchers: Dr S Huang, Dr M Levstik, Dr C Grant, Dr C McIntyre

Please initial in the box

1. I confirm that I have read and understand the information sheet dated 20-JUL-2015 (version 2) for the above study and I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

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

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities, where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4. I agree to taking of blood samples as part of this study. ☐

5. I agree to participate in this study ☐

_____	_____	_____
Name of patient	Signature	Date *(DD-MMM-YYYY)*
_____	_____	_____
Name of person taking consent (if different from researcher)	Signature	Date *(DD-MMM-YYYY)*
_____	_____	_____
Researcher	Signature	Date *(DD-MMM-YYYY)*

Consent form (continued)

☐ The person signing below acted as a translator for the participant during the consent process and attests that the study as set out in this form was accurately translated and has had any questions answered.

_____	_____	_____
Print name of translator	Signature	Date *(DD-MMM-YYYY)*

_____
Language

On completion: 1 copy for patient; 1 copy for researcher site file

LETTER OF INFORMATION AND CONSENT

Study Title:	A New MRI Technique for Total Body Water Measurement
Principal Investigator:	Dr. Susan Huang, MD, FRCPC Nephrology London Health Sciences Centre (519) 685-8066
Co-Investigator:	Dr. Charles McKenzie, PhD Medical Biophysics Western University (519) 661-2111 ext. 85686
	Dr. Robert Lindsay, MB.Chb, FRCP(E), FRCP(Glasg), FACP Nephrology London Health Sciences Centre 519-685-8349
FRCPC,	
	Dr. Guido Filler, MD, PhD, FRCPC Paediatrics Children's Hospital (519) 685-8377 ext. 58377
Contact Information:	Anuradha Sawant, PhD Candidate Physiotherapy London Health Sciences Centre (519) 685-8500 ext. 33503
	Claire Grant Research Fellow London Health Sciences Centre (519) 685-8500 ext. 55975
Name of Sponsor:	Western University Division of Nephrology Academic Fund

In this Consent document, "you" always refers to the study participant.

This consent form is only part of the process of informed consent. It should give you a basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, please ask. Please take the time to read this carefully and to understand any accompanying information. You will receive a copy of this form.

Introduction
You are being invited to participate in this research study about using scans from a magnetic resonance imaging (MRI) machine to measure the total amount of water in the body of patients with chronic kidney disease (CKD) undergoing dialysis treatment because you are a dialysis patient at University Hospital.

Background/Purpose
Many dialysis patients have high blood pressure and a buildup of water in their body. We are looking for a way to accurately measure the total amount of water in the bodies of dialysis patients so that we can better manage the complications that arise when there is too much water.

The purpose of the study is to: (i) see if a new way of measuring the total amount of water in the bodies of dialysis patients using an MRI machine is accurate (ii) to compare this to other methods that we currently use to measure body water, and (iii) to assess the effect of a buildup of water in the body on the liver using an ultrasound machine for the liver (the Fibroscan). At present, doctors still use blood pressure and measure swelling in the body to estimate the normal weight of patients with CKD, to use this weight as a target when ordering dialysis treatments. This way of measuring body water is often inaccurate. If we can accurately measure how much extra water a patient is retaining, we will be able to set a more accurate target weight and we will be able to better manage the complications with the heart and liver that happen because of this extra water.

You are being asked to participate because you have been diagnosed with end-stage renal disease (a CKD) by your doctor and are currently undergoing dialysis treatments at University Hospital.

The usual treatment for your condition is to have 3 hemodialysis sessions per week every other day. You have your blood taken and tested at least monthly (before the dialysis session) and you most likely receive specific medications such as erythropoietin (for red blood cell production), Replavite (contains vitamins), phosphate binders (to limit phosphate in your blood), and iron (a supplement). If you choose to participate in this study, you will receive all of the care you would normally receive along with other tests that are beyond the standard of care. For example, your blood will be tested both before and after the dialysis treatment, and Cystatin C (a blood test that measures how well your kidneys are working) will be measured. Other extra testing includes bioimpedance measurement (a different machine that measures how much water is in your body), inferior vena cava (an important vein in your heart) diameter using an ultrasound (another way to measure the water in your body), the MRI scan, and a Fibroscan (ultrasound) of your liver.

Up to 20 dialysis patients from University Hospital will participate in this pilot study at University Hospital, and participation will only involve measurements before and after one dialysis session. It is expected that you will be in the study for several hours over the course of one day.

Study Design

This is a cross-sectional study, meaning that the study is only carried out at one point in time. All participants will receive the same treatment that they would receive if they were not participating in the study, but with extra testing.

You should not have an MRI scan (unless supervised by a doctor) if: You have any history of head or eye injury involving metal fragments; if you have some type of implanted electrical device (such as a cardiac pacemaker); if you have severe heart disease (or are prone to an irregular heart rhythm); if you have conductive implants or devices such as skin patches, body piercing or tattoos that have metallic inks (there is a risk that the implant, device or tattoo/piercing may heat up and burn the tissue around it).

Procedures

If you choose to participate in the study, we will put your some of your basic demographic information into a database (date of birth and age, gender, ethnicity, etc). We will also record any treatments you may receive for your condition, such as the drugs you are given and how long you take them, and the results of our tests.

- After providing informed consent by signing this form, you will have an MRI scan 60 minutes before one of your hemodialysis treatments at University Hospital. An MRI involves the use of magnetic waves to take pictures of the inside of your body. You will have to lie still in the machine but will be able to speak to someone at all times. The MRI scan will take place at the Robarts Research Institute (beside the hospital). The scan lasts about 30 minutes and is beyond your usual treatment.

A description of the MRI procedure: The MRI machine uses a strong magnet and radio waves to make images of the body interior. You will be asked to lie on a long narrow couch for about half an hour while the machine gathers data. During this time you will be exposed to magnetic fields and radio waves. You will not feel either. You will, however, hear repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones that you will be required to wear to minimize the sound and protect your hearing. The space within the large magnet in which you lie is somewhat confined, although we have taken many steps to relieve the "claustrophobic" feeling. There are no known significant risks with this procedure at this time because the radio waves and magnetic fields, at the strengths used, are thought to be without harm. The exception is if you have a cardiac pacemaker, or a metallic clip in your body (e.g., an aneurysm clip in your brain), have severe heart disease, body piercings, tattoos containing metallic ink or slow release pharmaceutical skin patches.

There is a possibility that you will experience a localized twitching sensation due to the magnetic field changes during the scan. This is no unexpected and should not be painful. However, you can stop the exam at any time. The magnetism and radio waves do not cause harmful effects at the levels used in the MRI machine. However, because the MR scanner uses a very strong magnet that will attract metal, all metallic objects must be removed from your person before you approach the scanner. In addition, watches and credit cards should also be removed as these could be damaged. (These items will be

watched for you).

- After the MRI scan, 2 other tests that also measure how much water is in your body will be done: bioimpedance (uses an electric current that you cannot feel) and an ultrasound to measure the diameter of an important vein in your heart. These are also extra tests outside of the care you would normally receive.
- You will then be moved to the University Hospital dialysis unit. You will have your weight measured and will undergo a physical exam to see if you are dehydrated or over-hydrated. You will also have your blood drawn for testing. You will then have your routine four-hour hemodialysis treatment that is a part of your usual care. During your treatment, we will use an ultrasound (Fibroscan) to test your liver – this is outside of the care you would usually receive. Once the hemodialysis session is done, your weight will be measured.
- You will then be transferred back to Robarts, and 30-60 minutes after your hemodialysis session, we will perform the 3 tests again (MRI scan, ultrasound, and bioimpedance). We will also collect about 15 ml of blood for testing. Another ultrasound (Fibroscan) will also be performed immediately after these tests. These tests are all outside of the care you would normally receive.
- We will record anything you drink or any urination, and we will note down your laboratory and dialysis results.

Your participation in this study only takes place over the course of 1 day.

Voluntary Participation
Your participation in this study is voluntary. You may decide not to be in this study, or to be in the study now and then change your mind later. You may leave the study at any time without affecting your care. We will give you new information that is learned during the study that might affect your decision to stay in the study.

Withdrawal from Study
Your participation in this research is voluntary. You may withdraw from this study at any time. Your withdrawal does not have to be in writing and you do not have to provide a reason. If you choose to withdraw, your decision will not affect your medical care.

If you decide to withdraw from the study, you have the right to request withdrawal of information collected about you and of the extra blood collected from you. Let your study doctor know.

Risks
The risks associated with ultrasounds (Fibroscan and inferior vena cava measurement) are minimal. You may experience some discomfort and/or pressure during the procedures. Bioimpedance measurement also poses minimal risk.

Part of your participation in this study will involve a research test with Magnetic Resonance Imaging (MRI) system, a common medical diagnostic tool that uses a strong magnetic field, a low frequency magnetic field, and a radio frequency field. No X-rays

are used. As with any technology there is a risk of death or injury. For MRI the risk of death is less than 1 in 10 million and the risk of injury is less than 1 in 100,000. These risks do not arise from the MRI process itself, but from a failure to disclose or detect MRI incompatible objects in or around the body of the subject or the scanner room. It is therefore very important that you answer all the questions honestly and fully on the MRI screening questionnaire. Almost all the deaths and injuries related to MRI scans have occurred because the MRI operator did not know that surgically implanted metal hardware (such as a cardiac pacemaker) was present inside the subject during the MRI scan. Other remote risks involve temporary hearing loss from the loud noise inside the magnet. This can be avoided with ear headphone protection that also allows continuous communication between the subject and staff during the scan. For comparison, the risk of death in an MRI is similar to travelling 10 miles by car, while the risk of injury during an MRI is much less than the risks associated with normal daily activities for 1 hour.

You may not be allowed to continue in this research study if you are unable to have a MRI scan because, for example, you have some MRI incompatible metal in your body, you may be pregnant or attempting to become pregnant, or you may have a drug patch on your skin that contains a metal foil. Should you require a medically necessary MRI scan in the future, the final decision as to whether you can be scanned will be made by a qualified physician considering all the risks and benefits.

You may be asked to hold your breath no more than 6 times for periods of about 20-30 seconds during the scan but if this is too uncomfortable the scan will be stopped. Some patients may experience anxiety when receiving an MRI scan related to the limited space within the machine (claustrophobia).

Only the research staff and the study physician(s) will review your health information.

Benefits
You may not receive direct benefit from being in this study. However, your individual results from the study may help with adjusting your future target weight for hemodialysis treatments. These techniques could also be incorporated into your future dialysis assessments.

Information learned from this study may help lead to improved treatment of end-stage renal disease in the future.

Alternatives to Being in the Study
An alternative to the procedures described above is not to participate in the study and continue on just as you do now.

Confidentiality
Your personal information will be kept strictly confidential except as required or permitted by law. Your identity will not be disclosed in any published materials as a result of this study. Information about you is confidential and study results will only be presented in a group format to ensure your identity is protected at all times. Please note that your date of birth will

be collected within the data collection form and therefore collected and stored with the data. The patient-level data will be stored in a secure place at the London Health Sciences Centre (LHSC). It will be accessible to the study team at the LHSC by user id and passwords.

Representatives of the University of Western Ontario Health Sciences Research Ethics Board and from the Lawson Quality Assurance Education Program (QAEP) may look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines.

The study doctor will keep any personal health information about you in a secure and confidential location for 25 years. A list linking your study number with your name will be kept by the study doctor in a secure place, separate from your study file.

Costs
You will not have to pay to participate in this study. In the event that you are required to visit the clinic for study purposes only, you will be reimbursed up to $15 for your parking costs at the hospital. You will also be provided with lunch or breakfast, depending on when the scan takes place.

Compensation
You will not be paid to participate in this study.

Rights as a Participant
If you require treatment for any illness directly related to procedures required by the study, or if you suffer side effects from any of the procedures performed as part of the study, you should contact your study doctor as soon as possible. The necessary medical care will be provided to you at no additional cost to you.

You do not waive any legal rights by signing the consent form.

Questions about the Study
If you have any questions or desire further information about this study before or during participation, you can either contact Dr. Susan Huang at 519-685-8066, Anuradha Sawant at 519-685-8500, ext. 33503, or Marta Kobrzynski at 519-685-8500, ext. 52663.

If you have any questions about your rights as a research participant or the conduct of this study, you may contact The Office of Research Ethics (519) 661-3036, email: ethics@uwo.ca.

Consent

Study Title: A New MRI Technique for Total Body Water Measurement

Principal Investigator: Dr. Susan Huang, MD, FRCPC

☐ Your signature on this form indicates that the study has been explained to you and all your questions have been answered to your satisfaction. You agree to take part in the study. You know that you can leave the study any time.

You will be given a copy of consent document once it has been signed.

Print Name of Participant	Signature	Date *(DD-MMM-YYYY)*
Print Name of Person Obtaining Consent	Signature	Date *(DD-MMM-YYYY)*

MRI Research Questionnaire

To ensure your safety, this form MUST BE completed in the presence of a member of the study team.

Question	Yes	No
Have you ever had a previous MRI?		
Have you ever been a metal worker, grinder or welder?		
Have you ever had a metal foreign body in or around the eyes?		
Are you pregnant or breast-feeding?		
Are you claustrophobic?		
Are you connected to any supportive medical device? (pumps, catheters)		
Have you ever had any surgery?		
Do you have any of the following in place:		
Cardiac Pacemaker, Implantable Cardioverter Defibrillators, or Leads		
Heart Valve Prosthesis		
Aneurysm Clip(s)		
Intraventricular Shunt		
Orbital Implants		
Neurostimulator, Bone Growth Stimulator, Biostimulator		
Implanted Drug Infusion Device/Insulin Pump		
Inner Ear Implants - Cochlear, Stapes, Aids		
Joint Replacements/Prosthesis		
Coil, Filter or Stent (intravascular)		
Genital Prosthesis/Devices (Penile, Diaphragm, Intra Uterine Device, Pessary)		
Surgical Rods/Wires/Plates/Shrapnel/Bullets		
Vascular access port (Peripherally Inserted Central Catheter, Swan Ganz, Port-a-cath)		
Dentures, Braces		
Tattoos, Permanent Cosmetics		
Body Piercing, Body Jewellery		
Medication Patches		

If you answered yes to any of the above, please speak to the MR Technologist or the Principal Investigator.

LETTER OF INFORMATION & CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Study Title

Evaluation of the hemodynamic effects of hemodialysis and feeding on liver perfusion, endotoxemia and uremic toxin handling

Name of Principal Investigator (Study Doctor)

Professor Chris McIntyre
Director, Kidney Clinical Research Unit
London Health Sciences Centre
Victoria Hospital
800 Commissioners Road East
London, ON, Canada, N6A 5W9

Co-Investigators

Dr Claire Grant
Research Fellow
Kidney Clinical Research Unit
London Health Sciences Centre

Professor T.Y. Lee
Professor of Medical Physics &
Lawson Scientist
Lawson Health Research Institute

Dr Mark Levstik
Associate Professor of Hepatology
University Hospital, LHSC

Research Team: Jarrin Penny, RN, Hemodialysis Research Nurse, KCRU

Contact Information

Phone: 519 685-5800 Ext 55975 Email: claire.grant@lhsc.on.ca

Name of Sponsor

Lawson Health Research Institute

Conflict of Interest

None

Introduction

We would like to invite you to take part in our research study which is being done as part of a PhD student project. Before you decide, we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. You are free to talk to others about the study if you wish. Please feel free to ask us if there is anything that is not clear. You are being asked to participate in this study as you are having regular hemodialysis.

Background/Purpose

Having hemodialysis affects the blood supply to various organs in the body including the heart and the brain. With time, these effects build up and can affect the way these organs work to keep you in health. It is only just being recognized how many organs are affected during dialysis but one organ which has not been examined up until now is the liver. The liver works to help clean the blood, make proteins and turn all your food into energy, therefore its role is crucial to keep you well. We want to examine whether the blood supply to the liver is affected during dialysis treatment.

Study Design

We will not ask you to take any additional or experimental medication for this study. This study involves one study visit. We will ask you to come for one of your dialysis treatments to the Lawson Institute at St Joseph's Hospital for a detailed scans of the liver and your heart during dialysis (see below for further details).

Procedures

If you are given information about the study and are considering taking part, one of our research team will contact you at one of your usual dialysis sessions to discuss the study. You will have an opportunity during this initial meeting to discuss any questions or worries you may have. After this, you will be asked to sign a form to say you consent to be part of the study. Once you have agreed to take part in the study, we will arrange the dates for the visit (see below). This visit will be the only time you will be required to spend as part of the study.

The Study Visit – at the Radiology Department, St. Joseph's Health Care, London

This visit will take place on a day where you are due for dialysis and we expect it to take approximately 5-6 hours in total (including your usual dialysis time). Transport to the hospital can be arranged if desired. There will be an experienced dialysis nurse present with you for the whole visit. We would ask you to have the following at this visit (see below for more details of each test):

Before dialysis:	• Complete 4 questionnaires relating to your health and symptoms • Have a heart scan • Have ICG measurement • Have a liver scan • Have blood taken
During dialysis: 1 hour in 3 hours in	 • Have a liver scan • Have a liver scan; have a heart scan; have blood taken; have ICG measurement
After dialysis:	• Have a liver scan • Have ICG measurement • Have blood taken

How the day will run

This visit will involve having 4 detailed scans of your liver called CT scans, before, during and after dialysis as well as 2 ultrasound scans of your heart (called an echocardiogram) before and during dialysis. This type of scan is regularly done on hospital patients for non-research purposes.

Once you arrive for the visit, we would go over all the details of the morning or afternoon to make sure you were happy before we started. You would then have a small plastic tube (called an IV line) inserted into a vein in your arm to enable us to give you the dye needed to have the CT scan and for ICG clearance measurements (see below). You would then be taken to the CT scanner to have the first scans (see details below).

You would then be prepared for dialysis as per your usual routine (have your weight and blood pressure taken, have your needles inserted to your fistula if required) and begin your dialysis using a dialysis chair placed in the scanning room. At 1 and 3 hours into your treatment, we would ask you to transfer back onto the scan bed for another scan (without disconnecting from your dialysis treatment).

We want to see what happens to the liver's blood supply when people do not eat during dialysis. Therefore, we will ask you not to eat until after dialysis has finished but we will provide a meal after the session. The meal will consist of a cheese sandwich, two biscuits and a glass of apple juice (alternative arrangements can be made if dietary restrictions or allergies exist).

Once your dialysis treatment has finished, you have been safely disconnected from the machine and had your weight and blood pressure taken, you will be asked to have a fourth scan. After this, you will have finished the study. You would then return back to your usual routine at your next dialysis session.

The Study Tests

- Echocardiogram (heart scan)

  This involves having your heart assessed using ultrasound. This involves placing a small ultrasound probe on the chest with some clear, washable jelly to help see the heart on a screen. It takes about 10 minutes and is completely painless with no side-effects.

- Questionnaires

  We will ask you to complete 4 questionnaires relating to your health symptoms and quality of life (takes approximately 15 minutes). There are four questionnaires – two relating to you general health and mood, one which asks questions about specific symptoms such as itching and tiredness and one which asks about your appetite and food intake.

- Blood tests

  We will take a blood sample (approximately 15mls) before, during and after dialysis (three in total) for this visit. This can be done from your dialysis access and therefore no extra needles will be used. These tests are only to be used as part of the study.

- Indocyanine green (ICG) clearance

  This involves injection of a dye into your blood through the plastic tube (cannula) we will have placed for the CT scan (CT dye and this dye will be mixed as one injection). The dye is removed from the blood by the liver and therefore it acts as a way for us to tell how well the liver is working. We measure the rate of disappearance of the dye from your blood stream 15 minutes after its injection by placing a probe on your finger for a few seconds. This is painless. The dye only remains in your system for less than an hour and has no long-term side effects. A short-term side effect of the dye is that it colors your stool green; however this is harmless and only lasts a few days.

- The CT Scans

  A CT scanner is a ring-shaped scan machine as pictured below. You will be asked to sit on the scan bed which will then move in and out of the ring. While the machine is working, you may hear some clicks and noises but you will not feel anything. Each scan will last approximately 2 minutes. The cannula (small plastic tube) inserted into one of the veins in one arm will be used for the scan. A preliminary CT scan of your abdomen will be done to locate the liver for blood flow measurements. X-ray dye mixed with a green dye for liver function measurement (see above) will be injected into the catheter in your arm with an automatic injector at the rate of 3-4 mL per second. The total volume of the x-ray and green dye that will be injected is about 80 mL (for an average size person). After the dye is injected the liver is scanned 33 times at 2,3,5 and 15 second intervals for 120 seconds to monitor the flow of blood to the liver. Each time you have a scan, we will inject a dye into the plastic tube in a blood vessel in your hand. This is painless although you may experience a few side effects (see Section **Risks and Burdens**).



Voluntary Participation

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

Withdrawal from Study

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far may still be used in the project analysis. Your usual care would not be affected in any way if you were to withdraw from the study.

Risks and Burdens

Having the CT dye injected as part of the study has a few common side effects which are all temporary and harmless. These include a metallic taste in the mouth, feeling flushed or the sensation of emptying your bladder. There is also a smaller risk of having an allergic reaction to the dye. This happens in less than 3% of people who receive the contrast injection. If this occurs, we will stop the scanning and not give you the dye again. The chance of a serious allergic reaction is very small (less than 1 in 200 people) but we would have all the medical equipment and personnel to treat this if it were necessary. The ICG dye has also much more rarely been known to cause allergic reactions. We will monitor you for these and use the same precaution as outlined above for the CT dye.

The discomfort felt during the insertion of a cannula into a vein is the same as having an injection.

You may read that having CT dye carries a risk to kidney function. This is true but we have asked you to be part of the study because your kidneys do not function and therefore there is no risk at all for you to have the dye. It is completely removed by the dialysis process and will cause no long-term side effects.

Having these CT scan means that you are exposed to radiation. You will have four CT scans of the liver. The radiation dose you will receive is about the same as two routine CT liver scans or the equivalent of 200 chest X rays. The risk of developing fatal cancer anywhere in the body in about twenty years' time after the radiation dose received in this research study is less than 1 in 250.

There is a small chance that in having the scan, we detect a physical abnormality which we are not looking for. This would then need to be investigated further. Our team would contact the hospital team looking after you to inform them of the findings and responsibility for any further tests would then lie with your team. We would also contact you at the same time to let you know the abnormal finding and what to expect next. We may, in this instance, have to withdraw you from the study.

There will be no delay to your routine healthcare if you participate in the study. The treatment of your disease will continue as normal throughout the time that you spend in the study.

Benefits

This study will not benefit you directly but the information we get from this study may help us understand what causes some of the complications of dialysis and therefore, in the future, to look at ways of helping prevent these from happening.

Alternatives to Being in the Study

If you do not wish to participate in the study, your care will continue as normal and not be affected in any way. If you wish to, you can indicate to your usual doctor that you want to be informed of any future studies that you might be eligible to take part in.

Confidentiality

All information about you collected as part of the study will be handled in confidence.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorized persons only (the research team listed at the top of this document). Representatives of Western University's Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research. All will have a duty of confidentiality to you, as a research participant, and we will do our best to meet this duty, however we cannot 100% guarantee that a breach will not occur. If this should happen, we would inform you of the occurrence as soon as possible and what information has been shared.

All information which is collected about you during the course of the research will be kept **strictly confidentially**, stored in a secure and locked office, and on a password protected database. Any information recorded from your health records which is used in the research will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognized from it.

Your personal data (address, telephone number) will be kept for 6 months after the end of the study. All other data (research data) will be kept securely for 15 years. After this time, your data will be disposed of securely. During this time,

all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data. Representatives of Lawson Quality Assurance (QA) Education Program may look at study data for QA purposes, however this data will be anonymized and you will not be identifiable from this. All data will be stored and protected in accordance with the Personal Information Protection and Electronic Documents Act, 2000.

Costs

This study will not cost you any money. All travel expenses or parking will be covered (see below). There will be a time burden of approximately two extra hours to participate in this study.

Compensation

You will not be paid to participate in the study. Travel expenses will be offered for any visits incurred as a result of participation. For the visit to St. Joseph's, a taxi will be arranged and paid for, if desired.

Questions about the Study

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the beginning of this information sheet. If you wish to discuss the study with someone outside the study team in confidence, you can do this by contacting Dr. David Hill, Scientific Director, Lawson Health Research Institute at (519) 667-6649.

Consent Form

Evaluation of the hemodynamic effects of hemodialysis and feeding on liver perfusion, endotoxemia and uremic toxin handling

Name of researchers: Dr. CW McIntyre, Dr Levstik, Dr C Grant, Dr TY Lee

Please initial in the box

1. I confirm that I have read and understand the information sheet dated 13-JUL-2015 (version 2) for the above study and I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

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

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities, where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4. I agree to have CT Scans as part of this study. ☐

5. I agree to taking of blood samples as part of this study. ☐

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

_____	_____	_____
Name of patient	Signature	Date
_____	_____	_____
Name of person taking consent (if different from researcher)	Signature	Date
_____	_____	_____
Researcher	Signature	Date

On completion: 1 copy for patient; 1 (original) for researcher site file; 1 copy to be kept in medical notes

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:

Total score: Depression (D) _____ Anxiety (A) _____

0-7 = Normal

8-10 = Borderline abnormal (borderline case)

11-21 = Abnormal (case)

Simplified nutritional appetite questionnaire (SNAQ)

Study Number: _____
Date: _____

Please complete the questionnaire by circling the correct answers for you :

1. My appetite is

a. very poor
b. poor
c. average
d. good
e. very good

2. When I eat

a. I feel full after eating only a few mouthfuls
b. I feel full after eating about a third of a meal
c. I feel full after eating over half a meal
d. I feel full after eating most of the meal
e. I hardly ever feel full

3. Food tastes

a. very bad
b. bad
c. average
d. good
e. very good

4. Normally I eat

a. less than one meal a day
b. one meal a day
c. two meals a day
d. three meals a day
e. more than three meals a day

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Thank you for completing this survey!

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
❑1	❑2	❑3	❑4	❑5

2. **Compared to one year ago**, how would you rate your health in general **now**?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
❑1	❑2	❑3	❑4	❑5

3. The following items are about activities you might do during a typical day. **Does your health now limit you** in these activities? If so how much?

	Yes limited a lot	Yes limited a little	No, not limited at all
Vigorous activities, such as lifting heavy objects, participating in strenuous sports	❑1	❑2	❑3
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	❑1	❑2	❑3
Lifting or carrying groceries	❑1	❑2	❑3
Climbing several flights of stairs	❑1	❑2	❑3
Climbing one flight of stairs	❑1	❑2	❑3
Bending, kneeling, or stooping	❑1	❑2	❑3
Walking more than a kilometer	❑1	❑2	❑3
Walking several hundred meters	❑1	❑2	❑3
Walking one hundred meters	❑1	❑2	❑3
Bathing or dressing yourself	❑1	❑2	❑3

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All the time	Most of the time	Some of the time	A little of the time	None of the time
Cut down the amount of time you spend on work or other activities	❑1	❑2	❑3	❑4	❑5
Accomplished less than you would have liked	❑1	❑2	❑3	❑4	❑5
Were limited in the kind of work or other activities	❑1	❑2	❑3	❑4	❑5
Had difficulty performing the work or other activities (for example, it took extra effort)	❑1	❑2	❑3	❑4	❑5

5. During the **past 4 weeks**, how much of the time have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	All the time	Most of the time	Some of the time	A little of the time	None of the time
Cut down the amount of time you spend on work or other activities?	❑1	❑2	❑3	❑4	❑5
Accomplished less than you would have liked	❑1	❑2	❑3	❑4	❑5
Did work or other activities less carefully than usual.	❑1	❑2	❑3	❑4	❑5

6. During the **past 4 weeks**, to what **extent** have your **physical health or emotional problems** interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all	Slightly	Moderately	Quite a lot	Extremely
❑1	❑2	❑3	❑4	❑5

7. How much **bodily** pain have you had during the **past 4 weeks?**

None	Very mild	Mild	Moderate	Severe	Very severe
❑1	❑2	❑3	❑4	❑5	❑6

8. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
❑1	❑2	❑3	❑4	❑5

9. These questions are about how you feel and how things have been with you **during the past 4 weeks.** For each question, please give the one answer that comes closest to the way you have been feeling.

   How much of the time during the **past 4 weeks…**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
Did you feel full of life?	❑1	❑2	❑3	❑4	❑5
Have you been very nervous?	❑1	❑2	❑3	❑4	❑5
Have you felt so down in the dumps that nothing could cheer you up?	❑1	❑2	❑3	❑4	❑5
Have you felt calm and peaceful?	❑1	❑2	❑3	❑4	❑5
Did you have a lot of energy?	❑1	❑2	❑3	❑4	❑5
Have you felt downhearted and blue?	❑1	❑2	❑3	❑4	❑5
Did you feel worn out?	❑1	❑2	❑3	❑4	❑5
Have you been a happy person?	❑1	❑2	❑3	❑4	❑5
Did you feel tired?	❑1	❑2	❑3	❑4	❑5

10. During the **past 4 weeks,** how much of the **time** have your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little bit of the time	None of the time
❑1	❑2	❑3	❑4	❑5

11. How **TRUE** or **FALSE** each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly False	Definitely False
I seem to get sick a little easier than other people	❑1	❑2	❑3	❑4	❑5
I am as healthy as anybody I know	❑1	❑2	❑3	❑4	❑5
I expect my health to get worse	❑1	❑2	❑3	❑4	❑5
My health is excellent	❑1	❑2	❑3	❑4	❑5

Thank you for completing these questions

5-D Pruritus Scale

1. **Duration**: During the last 2 weeks, how many hours a day have you been itching?

Less than 6hrs/day	6-12 hrs/day	12-18 hrs/day	18-23 hrs/day	All day
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. **Degree**: Please rate the intensity of your itching over the past 2 weeks

Not present	Mild	Moderate	Severe	Unbearable
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. **Direction**: Over the past 2 weeks has your itching gotten better or worse compared to the previous month?

Completely resolved	Much better, but still present	Little bit better, but still present	Unchanged	Getting worse
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. **Disability**: Rate the impact of your itching on the following activities over the last 2 weeks

	Never affects sleep	Occasionally delays falling asleep	Frequently delays falling asleep	Delays falling asleep and occasionally wakes me up at night	Delays falling asleep and frequently wakes me up at night
Sleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

	N/A	Never affects this activity	Rarely affects this activity	Occasionally affects this activity	Frequently affects this activity	Always affects this activity
Leisure/Social	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Housework/ Errands	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Work/School	☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. **Distribution:** Mark whether itching has been present in the following parts of your body over the last 2 weeks. If a body part is not listed, choose the one that is closest anatomically.

	Present		Present
Head/Scalp	☐	Soles	☐
Face	☐	Palms	☐
Chest	☐	Tops of Hands/Fingers	☐
Abdomen	☐	Forearms	☐
Back	☐	Upper Arms	☐
Buttocks	☐	Points of Contact w/ Clothing	
Thighs	☐	(e.g waistband, undergarment)	☐
Lower legs	☐	Groin	☐
Tops of Feet/Toes	☐		

References

1. KDIGO. KDIGO Clinical Practice Guideline. Chapter 1: Definition and classification of CKD. Kidney Int Suppl. 2013;3(1):19–62.
2. National Institutes of Health: National Kidney and Urologic Diseases. Treatment Methods for Kidney Failure: Hemodialysis. 2006.
3. MacNeill SJ, Ford D, Evans K, Medcalf JF. UK Renal Replacement Therapy Adult Prevalence in 2016: National and Centre-specific Analyses. Nephron. 2018;139(1):47–74.
4. Canadian Institute for Health Information. Canadian Organ Replacement Register Annual Report. Treatment of End-Stage Organ Failure in Canada, 200 to 2009. 2011.
5. Ontario Renal Network System Statistics 2016/2017. 2018.
6. Parfrey PS. Cardiac disease in dialysis patients : diagnosis , burden of disease , prognosis , risk factors and management. Nephrol Dial Transplant. 2000;15(Suppl 5):58–68.
7. Cheung AK, Sarnak MJ, Yan G, Dwyer JT, Heyka RJ, Rocco M V, Teehan BP, Levey AS. Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. Kidney Int. 2000;58(1):353–62.
8. McIntyre CW. Recurrent circulatory stress: the dark side of

dialysis. Semin Dial. 2010;23(5):449–51.

9. Ito S, Yoshida M. Protein-bound Uremic toxins: New culprits of cardiovascular events in chronic kidney disease patients. Toxins (Basel). 2014;6(2):665–78.
10. Shroff R, Long DA, Shanahan C. Mechanistic insights into vascular calcification in CKD. J Am Soc Nephrol. 2013;24(2):179–89.
11. Briet M, Burns KD. Chronic kidney disease and vascular remodelling: molecular mechanisms and clinical implications. Clin Sci. 2012;123(7):399–416.
12. Sigrist M, Bungay P, Taal MW, McIntyre CW. Vascular calcification and cardiovascular function in chronic kidney disease. Nephrol Dial Transplant. 2006;21(3):707–14.
13. Sigrist MK, Taal MW, Bungay P, McIntyre CW. Progressive vascular calcification over 2 years is associated with arterial stiffening and increased mortality in patients with stages 4 and 5 chronic kidney disease. Clin J Am Soc Nephrol. 2007;2(6):1241–8.
14. Chesterton LJ, Sigrist MK, Bennett T, Taal MW, McIntyre CW. Reduced baroreflex sensitivity is associated with increased vascular calcification and arterial stiffness. Nephrol Dial Transplant. 2005;20(6):1140–7.

15. Stewart G, Mark PB, Johnston N, Foster JE, Cowan M, Rodger RSC, Dargie HJ, Jardine AG. Determinants of hypertension and left ventricular function in end stage renal failure: a pilot study using cardiovascular magnetic resonance imaging. Clin Physiol Funct Imaging. 2004;24(6):387–93.
16. Fagugli RM, Pasini P, Quintaliani G, Pasticci F, Ciao G, Cicconi B, Ricciardi D, Santirosi PV, Buoncristiani E, Timio F, Valente F, Buoncristiani U. Association between extracellular water, left ventricular mass and hypertension in haemodialysis patients. Nephrol Dial Transplant. 2003;18(11):2332–8.
17. Zoccali C, Benedetto F a, Mallamaci F, Tripepi G, Giacone G, Cataliotti A, Seminara G, Stancanelli B, Malatino LS. Prognostic value of echocardiographic indicators of left ventricular systolic function in asymptomatic dialysis patients. J Am Soc Nephrol. 2004;15(4):1029–37.
18. Sands JJ, Usvyat LA, Sullivan T, Segal JH, Zabetakis P, Kotanko P, Maddux FW, Diaz-Buxo JA. Intradialytic hypotension: Frequency, sources of variation and correlation with clinical outcome. Hemodial Int. 2014;18(2):415–22.
19. Flythe JE, Xue H, Lynch KE, Curhan GC, Brunelli SM. Association of Mortality Risk with Various Definitions of Intradialytic Hypotension. J Am Soc Nephrol. 2015;26(3):724–34.

20. Reeves PB, Mc Causland FR. Mechanisms, Clinical Implications, and Treatment of Intradialytic Hypotension. Clin J Am Soc Nephrol. 2018 Aug 7;13(8):1297–303.
21. McIntyre CW, Burton JO, Selby NM, Leccisotti L, Korsheed S, Baker CSR, Camici PG. Hemodialysis-induced cardiac dysfunction is associated with an acute reduction in global and segmental myocardial blood flow. Clin J Am Soc Nephrol. 2008 Jan;3(1):19–26.
22. Eldehni MT, Odudu A, McIntyre CW. Randomized clinical trial of dialysate cooling and effects on brain white matter. J Am Soc Nephrol. 2015;26(4):957–65.
23. Buchanan C, Mohammed A, Cox E, Kohler K, Canaud B, Taal MW, Selby NM, Francis S, McIntyre CW. Intradialytic Cardiac Magnetic Resonance Imaging to Assess Cardiovascular Responses in a Short-Term Trial of Hemodiafiltration and Hemodialysis. J Am Soc Nephrol. 2017;28(4):1269–77.
24. Selby NM, Lambie SH, Camici PG, Baker CS, McIntyre CW. Occurrence of regional left ventricular dysfunction in patients undergoing standard and biofeedback dialysis. Am J Kidney Dis. 2006;47(5):830–41.
25. Burton JO, Jefferies HJ, Selby NM, McIntyre CW. Hemodialysis-induced cardiac injury: determinants and associated outcomes.

Clin J Am Soc Nephrol. 2009;4(5):914–20.

26. Burton JO, Jefferies HJ, Selby NM, McIntyre CW. Hemodialysis-induced repetitive myocardial injury results in global and segmental reduction in systolic cardiac function. Clin J Am Soc Nephrol. 2009;4(12):1925–31.
27. Hothi DK, Rees L, Marek J, Burton J, McIntyre CW. Pediatric myocardial stunning underscores the cardiac toxicity of conventional hemodialysis treatments. Clin J Am Soc Nephrol. 2009;4(4):790–7.
28. Jefferies HJ, Virk B, Schiller B, Moran J, Mcintyre CW. Frequent hemodialysis schedules are associated with reduced levels of dialysis-induced cardiac injury (myocardial stunning). Clin J Am Soc Nephrol. 2011;6(6):1326–32.
29. Odudu A, Eldehni MT, McCann GP, McIntyre CW. Randomized controlled trial of individualized dialysate cooling for cardiac protection in hemodialysis patients. Clin J Am Soc Nephrol. 2015;10(8):1408–17.
30. Polinder-Bos HA, Garcia DV, Kuipers J, Elting JWJ, Aries MJH, Krijnen WP, Groen H, Willemsen ATM, van Laar PJ, Strijkert F, Luurtsema G, Slart RHJA, Westerhuis R, Gansevoort RT, Gaillard CAJM, Franssen CFM. Hemodialysis Induces an Acute Decline in Cerebral Blood Flow in Elderly Patients. J Am Soc Nephrol.

2018;29(4):1317–25.

31. Kjaergaard KD, Jensen JD, Peters CD, Jespersen B. Preserving residual renal function in dialysis patients: an update on evidence to assist clinical decision making. NDT Plus. 2011;4(4):225–30.
32. van der Wal WM, Noordzij M, Dekker FW, Boeschoten EW, Krediet RT, Korevaar JC, Geskus RB. Full loss of residual renal function causes higher mortality in dialysis patients; findings from a marginal structural model. Nephrol Dial Transplant. 2011;26(9):2978–83.
33. Vilar E, Wellsted D, Chandna SM, Greenwood RN, Farrington K. Residual renal function improves outcome in incremental haemodialysis despite reduced dialysis dose. Nephrol Dial Transplant. 2009;24(8):2502–10.
34. Vilar E, Farrington K. Emerging importance of residual renal function in end-stage renal failure. Vol. 24, Seminars in dialysis. 2011. p. 487–94.
35. Shafi T, Jaar BG, Plantinga LC, Fink NE, Sadler JH, Parekh RS, Powe NR, Coresh J. Association of residual urine output with mortality, quality of life, and inflammation in incident hemodialysis patients: The Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (CHOICE) study. Am J

Kidney Dis. 2010;56(2):348–58.

36. Mathew AT, Fishbane S, Obi Y, Kalantar-Zadeh K. Preservation of residual kidney function in hemodialysis patients: reviving an old concept. Kidney Int. 2016;90(2):262–71.
37. Ng TG, Johnson DW, Hawley CM. Is it time to revisit residual renal function in haemodialysis? Nephrology. 2007;12(3):209–17.
38. Obi Y, Streja E, Rhee CM, Ravel V, Amin AN, Cupisti A, Chen J, Mathew AT, Kovesdy CP, Mehrotra R, Kalantar-Zadeh K. Incremental Hemodialysis, Residual Kidney Function, and Mortality Risk in Incident Dialysis Patients: A Cohort Study. Am J Kidney Dis. 2015;1–10.
39. Davies SJ, Brown EA, Frandsen NE, Rodrigues AS, Rodriguez-Carmona A, Vychytil A, Macnamara E, Ekstrand A, Tranaeus A, Filho JCD. Longitudinal membrane function in functionally anuric patients treated with APD: data from EAPOS on the effects of glucose and icodextrin prescription. Kidney Int. 2005;67(4):1609–15.
40. Van Stone JC. The effect of dialyzer membrane and etiology of kidney disease on the preservation of residual renal function in chronic hemodialysis patients. ASAIO J. 1995;41(3):M713-6.
41. Menon MK, Naimark DM, Bargman JM, Vas SI, Oreopoulos DG.

Long-term blood pressure control in a cohort of peritoneal dialysis patients and its association with residual renal function. Nephrol Dial Transplant. 2001;16(11):2207–13.

42. Moist LM, Port FK, Orzol SM, Young EW, Ostbye T, Wolfe R a, Hulbert-Shearon T, Jones C a, Bloembergen WE. Predictors of loss of residual renal function among new dialysis patients. J Am Soc Nephrol. 2000;11(3):556–64.
43. Jansen M, Hart A, Korevaar JC, Dekker FW, Boeschoten EW, Krediet RT. Predictors of the rate of decline of residual renal function in incident dialysis patients. Kidney Int. 2002;62(3):1046–53.
44. Lysaght MJ, Vonesh EF, Gotch F, Ibels L, Keen M, Lindholm B, Nolph KD, Pollock CA, Prowant B, Farrell PC. The influence of dialysis treatment modality on the decline of remaining renal function. ASAIO Trans. 1991;37(4):598–604.
45. Rowell LB, Blackmon JR, Kenny M a, Escourrou P. Splanchnic vasomotor and metabolic adjustments to hypoxia and exercise in humans. Am J Physiol. 1984;247(2 Pt 2):H251–8.
46. Jakob SM. Splanchnic Blood Flow in Low-Flow States. Anesth Analg. 2003;96(34):1129–38.
47. Jakob SM. Clinical review : Splanchnic ischaemia. Crit Care. 2002;6(4):306–12.

48. Ruokonen E, Takala J, Kari A, Saxen H, Mertsola J, Hansen EJ. Regional blood flow and oxygen transport in septic shock. Crit Care Med. 1993;21(9):1296–303.
49. Swank GM, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and permeability changes. World J Surg. 1996;20(4):411–7.
50. Naaber P, Smidt I, Tamme K, Liigant A, Tapfer H, Mikelsaar M, Talvik R. Translocation of indigenous microflora in an experimental model of sepsis. J Med Microbiol. 2000;49(5):431–9.
51. Yu P, Martin CM. Increased gut permeability and bacterial translocation in Pseudomonas pneumonia-induced sepsis. Crit Care Med. 2000;28(7):2573–7.
52. McClave S a, Chang W-K. Feeding the hypotensive patient: does enteral feeding precipitate or protect against ischemic bowel? Nutr Clin Pract. 2003;18(4):279–84.
53. Berger MM, Chioléro RL. Enteral nutrition and cardiovascular failure: from myths to clinical practice. J Parenter Enter Nutr. 2009;33(6):702–9.
54. Wells DL. Provision of Enteral Nutrition During Vasopressor Therapy for Hemodynamic Instability: An Evidence-Based Review. Nutr Clin Pract. 2012;27(4):521–6.

55. Gaddy MC, Max MH, Schwab CW, Kauder D. Small bowel ischemia: a consequence of feeding jejunostomy? South Med J. 1986;79(2):180–2.
56. Zetti G, Tagliabue F, Barabino M, Fontana S, Ceppi M, Samori G. Small bowel necrosis associated with postoperative enteral feeding. Chir Ital. 2002;54(4):555–8.
57. Crispe IN. Liver Antigen-Presenting Cells. J Hepatol. 2011;54(2):357–65.
58. Elvevold K, Smedsrød B, Martinez I. The liver sinusoidal endothelial cell: a cell type of controversial and confusing identity. Am J Physiol Gastrointest Liver Physiol. 2008;294(2):G391-400.
59. Connolly MK, Bedrosian AS, Malhotra A, Henning JR, Ibrahim J, Vera V, Cieza-Rubio NE, Hassan BU, Pachter HL, Cohen S, Frey AB, Miller G. In hepatic fibrosis, liver sinusoidal endothelial cells acquire enhanced immunogenicity. J Immunol. 2010;185(4):2200–8.
60. Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver Int. 2006;26(10):1175–86.
61. Kietzmann T. Metabolic zonation of the liver: The oxygen gradient revisited. Redox Biol. 2017;11:622–30.
62. Eipel C. Regulation of hepatic blood flow: The hepatic arterial

buffer response revisited. World J Gastroenterol. 2010;16(48):6046.

63. Bosch J, Abraldes JG, Fernández M, García-Pagán JC. Hepatic endothelial dysfunction and abnormal angiogenesis: new targets in the treatment of portal hypertension. J Hepatol. 2010;53(3):558–67.
64. Millonig G, Friedrich S, Adolf S, Fonouni H, Golriz M, Mehrabi A, Stiefel P, Pöschl G, Büchler MW, Seitz HK, Mueller S. Liver stiffness is directly influenced by central venous pressure. J Hepatol. 2010;52(2):206–10.
65. Lautt W. Hepatic Circulation: Physiology and Pharmacology. San Rafael, CA: Morgan & Claypool Life Sciences; 2009.
66. Henrion J. Hypoxic hepatitis. Liver Int. 2012;32(7):1039–52.
67. Henrion J, Descamps O, Luwaert R, Schapira M, Parfonry A, Heller F. Hypoxic hepatitis in patients with cardiac failure: incidence in a coronary care unit and measurement of hepatic blood flow. J Hepatol. 1994;21(5):696–703.
68. Spapen H. Liver perfusion in sepsis, septic shock, and multiorgan failure. Anat Rec. 2008;291(6):714–20.
69. Strnad P, Tacke F, Koch A, Trautwein C. Liver - guardian, modifier and target of sepsis. Nat Rev Gastroenterol Hepatol. 2017;14(1):55–66.

70. Van Leeuwen P, Boermeester M, Houdijk A, Ferwerda C, Cuesta M, Meyer S, Wesdorp R. Clinical significance of translocation. Gut. 1994;35(Supplement 1):S28–34.
71. Guerville M, Boudry G. Gastrointestinal and hepatic mechanisms limiting entry and dissemination of lipopolysaccharide into the systemic circulation. Am J Physiol - Gastrointest Liver Physiol. 2016;311(1):G1–15.
72. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int. 1999;55(2):648–58.
73. Gonçalves S, Pecoits-Filho R, Perreto S, Barberato S, Stinghen A, Lima E, Fuerbringer R, Sauthier S, Riella M, Gonc S. Associations between renal function , volume status and endotoxaemia in chronic kidney disease patients. Nephrol Dial Transplant. 2006;21(10):2788–94.
74. Sabatino A, Regolisti G, Brusasco I, Cabassi A, Morabito S, Fiaccadori E. Alterations of intestinal barrier and microbiota in chronic kidney disease. Nephrol Dial Transplant. 2015;30(6):924–33.
75. Yu AW, Nawab ZM, Barnes WE, Lai KN, Ing TS, Daugirdas JT. Splanchnic erythrocyte content decreases during hemodialysis: a new compensatory mechanism for hypovolemia. Kidney Int.

1997;51(6):1986–90.

76. Schneditz D, Haditsch B, Jantscher A, Ribitsch W, Krisper P. Absolute blood volume and hepato-splanchnic blood flow measured by indocyanine green kinetics during hemodialysis. ASAIO J. 2014;60(4):452–8.

77. Ribitsch W, Schneditz D, Franssen CFM, Schilcher G, Stadlbauer V, Horina JH, Rosenkranz AR. Increased hepato-splanchnic vasoconstriction in diabetics during regular hemodialysis. PLoS One. 2015;10(12):1–14.

78. Jakob SM, Ruokonen E, Vuolteenaho O, Lampainen E, Takala J. Splanchnic perfusion during hemodialysis: evidence for marginal tissue perfusion. Crit Care Med. 2001;29(7):1393–8.

79. McIntyre CW, Harrison LE, Eldehni MT, Jefferies HJ, Szeto C-C, John SG, Sigrist MK, Burton JO, Hothi D, Korsheed S, Owen PJ, Lai K-B, Li PKT. Circulating endotoxemia: a novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. Clin J Am Soc Nephrol. 2011;6(1):133–41.

80. Jefferies HJ, Crowley LE, Harrison LEA, Szeto C-C, Li PKT, Schiller B, Moran J, McIntyre CW. Circulating endotoxaemia and frequent haemodialysis schedules. Nephron Clin Pract. 2014;128(1–2):141–6.

81. Cannistrà M, Ruggiero M, Zullo A, Gallelli G, Serafini S, Maria M,

Naso A, Grande R, Serra R, Nardo B. Hepatic ischemia reperfusion injury: A systematic review of literature and the role of current drugs and biomarkers. Int J Surg. 2016;33:S57–70.

82. Henrion J, Schapira M, Luwaert R, Colin L, Delannoy A, Heller FR. Hypoxic hepatitis: clinical and hemodynamic study in 142 consecutive cases. Medicine (Baltimore). 2003;82(6):392–406.
83. Owen PJ, Priestman WS, Sigrist MK, Lambie SH, John SG, Chesterton LJ, Mcintyre CW. Myocardial contractile function and intradialytic hypotension. Hemodial Int. 2009;13(3):293–300.
84. Nolan JP. The role of intestinal endotoxin in liver injury: a long and evolving history. Hepatology. 2010;52(5):1829–35.
85. La Mura V, Pasarin M, Rodriguez-Vilarrupla A, Garcia-Pagan JC, Bosch J, Abraldes JG. Liver sinusoidal endothelial dysfunction after LPS administration: A role for inducible-nitric oxide synthase. J Hepatol. 2014;61(6):1321–7.
86. Moradi H, Sica DA, Kalantar-Zadeh K. Cardiovascular burden associated with uremic toxins in patients with chronic kidney disease. Am J Nephrol. 2013;38(2):136–48.
87. Evenepoel P, Meijers BKI, Bammens BRM, Verbeke K. Uremic toxins originating from colonic microbial metabolism. Kidney Int Suppl. 2009;76(114):S12–9.
88. Duranton F, Cohen G, De Smet R, Rodriguez M, Jankowski J,

Vanholder R, Argiles a. Normal and Pathologic Concentrations of Uremic Toxins. J Am Soc Nephrol. 2012;23(7):1258–70.

89. Pahl M V., Vaziri ND. The Chronic Kidney Disease-Colonic Axis. Semin Dial. 2015;28(5):459–63.
90. Wu M-J, Chang C-S, Cheng C-H, Chen C-H, Lee W-C, Hsu Y-H, Shu K-H, Tang M-J. Colonic transit time in long-term dialysis patients. Am J Kidney Dis. 2004;44(2):322–7.
91. Bossola M, Luciani G, Rosa F, Tazza L. Appetite and gastrointestinal symptoms in chronic hemodialysis patients. J Ren Nutr. 2011;21(6):448–54.
92. Vaziri ND, Yuan J, Nazertehrani S, Ni Z, Liu S. Chronic kidney disease causes disruption of gastric and small intestinal epithelial tight junction. Am J Nephrol. 2013;38(2):99–103.
93. Lin CJ, Liou TC, Pan CF, Wu PC, Sun FJ, Liu HL, Chen HH, Wu CJ. The role of liver in determining serum colon-derived uremic solutes. PLoS One. 2015;10(8):1–12.
94. Ochi A, Mori K, Nakatani S, Emoto M, Morioka T, Motoyama K, Fukumoto S, Imanishi Y, Shoji T, Ishimura E, Inaba M. Indoxyl sulfate suppresses hepatic fetuin-A expression via the aryl hydrocarbon receptor in HepG2 cells. Nephrol Dial Transplant. 2015;30(10):1683–92.
95. Tsujimoto M, Nagano Y, Hosoda S, Shiraishi A, Miyoshi A,

Hiraoka S, Furukubo T, Izumi S, Yamakawa T, Minegaki T, Nishiguchi K. Effects of decreased vitamin D and accumulated uremic toxin on human CYP3A4 activity in patients with end-stage renal disease. Toxins (Basel). 2013;5(8):1475–85.

96. Tsujimoto M, Higuchi K, Shima D, Yokota H, Furukubo T, Izumi S, Yamakawa T, Otagiri M, Hirata S, Takara K, Nishiguchi K. Inhibitory effects of uraemic toxins 3-indoxyl sulfate and p-cresol on losartan metabolism in vitro. J Pharm Pharmacol. 2010;62(1):133–8.
97. Barnes KJ, Rowland A, Polasek TM, Miners JO. Inhibition of human drug-metabolising cytochrome P450 and UDP-glucuronosyltransferase enzyme activities in vitro by uremic toxins. Eur J Clin Pharmacol. 2014;70(9):1097–106.
98. Abreo K, Sella M, Gautreaux S, De Smet R, Vogeleere P, Ringoir S, Vanholder R. p-Cresol, a Uremic Compound, Enhances the Uptake of Aluminium in Hepatocytes. J Am Soc Nephrol. 1997;8(6):935–42.
99. Stewart EE, Chen X, Hadway J, Lee T-Y. Hepatic perfusion in a tumor model using DCE-CT: an accuracy and precision study. Phys Med Biol. 2008;53(16):4249–67.
100. Hopper I, Kemp W, Porapakkham P, Sata Y, Condon E, Skiba M, Farber L, Porapakkham P, Williams TJ, Menahem S, Roberts S,

Krum H. Impact of heart failure and changes to volume status on liver stiffness: non-invasive assessment using transient elastography. Eur J Heart Fail. 2012;14(6):621–7.

101. Taniguchi T, Sakata Y, Ohtani T, Mizote I, Takeda Y, Asano Y, Masuda M, Minamiguchi H, Kanzaki M, Ichibori Y, Nishi H, Toda K, Sawa Y, Komuro I. Usefulness of transient elastography for noninvasive and reliable estimation of right-sided filling pressure in heart failure. Am J Cardiol. 2014;113(3):552–8.
102. Khunpakdee N, Jayanama K, Kaewdoung P, Promson K, Rattanasiri S, Warodomwichit D, Kantachuvesiri S, Sobhonslidsuk A. Transient Elastography in End-Stage Renal Disease Patients on Hemodialysis: The Effect of Net Fluid Withdrawal. Blood Purif. 2015;40(3):256–9.
103. Kellner P, Anadol E, Huneburg R, Hundt F, Bos D, Klein B, Woitas RP, Spengler U, Sauerbruch T, Trebicka J. The effect of hemodialysis on liver stiffness measurement: a single-center series. Eur J Gastroenterol Hepatol. 2013;25(3):368–72.
104. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: A new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol. 2003;29(12):1705–13.

105. Friedrich-Rust M, Ong M-F, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology. 2008;134(4):960–74.
106. Moissl UM, Wabel P, Chamney PW, Bosaeus I, Levin NW, Bosy-Westphal A, Korth O, Müller MJ, Ellegård L, Malmros V, Kaitwatcharachai C, Kuhlmann MK, Zhu F, Fuller NJ. Body fluid volume determination via body composition spectroscopy in health and disease. Physiol Meas. 2006;27(9):921–33.
107. Chamney PW, Wabel P, Moissl UM, Müller MJ, Bosy-Westphal A, Korth O, Fuller NJ. A whole-body model to distinguish excess fluid from the hydration of major body tissues. Am J Clin Nutr. 2007;85(1):80–9.
108. Chamney PW, Krämer M, Rode C, Kleinekofort W, Wizemann V. A new technique for establishing dry weight in hemodialysis patients via whole body bioimpedance. Kidney Int. 2002;61(6):2250–8.
109. Faul F, Erdfelder E, Buchner A, Lang A. Statistical power analyses using G * Power 3.1: Tests for correlation and regression analyses. Behav Res Methods. 2009;41(4):1149–60.
110. Liu C-H, Liang C-C, Huang K-W, Liu C-J, Chen S-I, Lin J-W, Hung P-H, Tsai H-B, Lai M-Y, Chen P-J, Chen J-H, Chen D-S, Kao J-H.

Transient elastography to assess hepatic fibrosis in hemodialysis chronic hepatitis C patients. Clin J Am Soc Nephrol. 2011;6(5):1057–65.

111. Piecha F, Peccerella T, Bruckner T, Seitz H-K, Rausch V, Mueller S. Arterial pressure suffices to increase liver stiffness. Am J Physiol - Gastrointest Liver Physiol. 2016;311(5):G945–53.
112. Thalhammer C, Segerer S, Augustoni M, Jacomella V, Clemens RK, Wüthrich RP, Amann-Vesti BR, Husmann M. Acute effects of haemodialysis on central venous and arterial pressure characteristics. Nephrology. 2015;20(2):91–5.
113. Kooman JP, Gladziwa U, Bocker G, van Bortel LM, van Hooff JP, Leunissen KM. Role of the venous system in hemodynamics during ultrafiltration and bicarbonate dialysis. Kidney Int. 1992;42(3):718–26.
114. Turgut O, Yilmaz A, Yalta K, Karadas F, Yilmaz MB. Gamma-glutamyltransferase is a promising biomarker for cardiovascular risk. Med Hypotheses. 2006;67(5):1060–4.
115. Bradley RD, Fitzpatrick AL, Jacobs Jr DR, Lee D, Jenny NS, Wake DH. Associations between γ-glutamyltransferase (GGT) and Biomarkers of Atherosclerosis: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2014;233(2):387–93.
116. Ilker M, Faruk Y, Mehmet T, Mutlu K, Alper S. Serum gamma-

glutamyltransferase levels are inversely related to endothelial function in chronic kidney disease. Int Urol Nephrol. 2013;45(4):1071–8.

117. Park WY, Koh ES, Kim S, Kim YO, Jin DC. Serum Gamma-Glutamyltransferase Levels Predict Clinical Outcomes in Hemodialysis Patients. PLoS One. 2015;10(9):1–13.
118. Chan C, McIntyre C, Smith D, Spanel P, Davies SJ. Combining near-subject absolute and relative measures of longitudinal hydration in hemodialysis. Clin J Am Soc Nephrol. 2009;4(11):1791–8.
119. Jaffrin MY, Morel H. Body fluid volumes measurements by impedance : A review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods. Med Eng Phys. 2008;30:1257–69.
120. Hsiao S-H, Huang W-C, Sy C-L, Lin S-K, Lee TY, Liu C-P. Doppler tissue imaging and color M-mode flow propagation velocity: are they really preload independent? J Am Soc Echocardiogr. 2005;18(12):1277–84.
121. Kjærgaard M, Thiele M, Jansen C, Strassburg C, Trebicka J, Krag A. High risk of misinterpreting liver and spleen stiffness using 2D shear-wave and transient elastography after a moderate or high calorie meal. PLoS One. 2017;12(4):1–15.

122. Ozkahya M, Toz H, Unsal A, Ozerkan F, Asci G, Gurgun C, Akcicek F, Mees EJ. Treatment of hypertension in dialysis patients by ultrafiltration: role of cardiac dilatation and time factor. Am J kidney Dis. 1999;34(2):218–21.
123. Charra B. Control of blood pressure in long slow hemodialysis. Blood Purif. 1994;12(4–5):252–8.
124. Zoccali C, Benedetto FA, Mallamaci F, Tripepi G, Giacone G, Stancanelli B, Cataliotti A, Malatino LS. Left ventricular mass monitoring in the follow-up of dialysis patients: Prognostic value of left ventricular hypertrophy progression. Kidney Int. 2004;65(4):1492–8.
125. Krane V, Heinrich F, Meesmann M, Olschewski M, Lilienthal J, Angermann C, Störk S, Bauersachs J, Wanner C, Frantz S. Electrocardiography and outcome in patients with diabetes mellitus on maintenance hemodialysis. Clin J Am Soc Nephrol. 2009;4(2):394–400.
126. Niebauer J, Volk HD, Kemp M, Dominguez M, Schumann RR, Rauchhaus M, Poole-Wilson P a, Coats a J, Anker SD. Endotoxin and immune activation in chronic heart failure: a prospective cohort study. Lancet. 1999;353(9167):1838–42.
127. Charalambous BM, Stephens RCM, Feavers IM, Montgomery HE. Role of bacterial endotoxin in chronic heart failure: the gut of

the matter. Shock. 2007;28(1):15–23.

128. Mandelbaum A, Link A, Wambach G, Ritz E. Vena cava ultrasonography for the assessment of hydration status in kidney insufficiency. Dtsch Med Wochenschr. 1993;118(37):1309–15.
129. Moissl U, Wabel P, Chamney P, Bosaeus I, Levin N, Bosy-Westphal A, Korth O, Müller M, Ellegård L, Malmros V, Kaitwatcharachai C, Kuhlmann M, Zhu F, J Fuller N. Body fluid volume determination via body composition spectroscopy in health and disease. Vol. 27, Physiological measurement. 2006. 921-933 p.
130. Ciozda W, Kedan I, Kehl DW, Zimmer R, Khandwalla R, Kimchi A. The efficacy of sonographic measurement of inferior vena cava diameter as an estimate of central venous pressure. Cardiovasc Ultrasound. 2016;14(1).
131. Cheriex EC, Leunissen KM, Janssen JH, Mooy JM, van Hooff JP. Echography of the inferior vena cava is a simple and reliable tool for estimation of "dry weight" in haemodialysis patients. Nephrol Dial Transplant. 1989;4(6):563–8.
132. Soliman AS, Wiens CN, Wade TP, McKenzie CA. Fat quantification using an interleaved bipolar acquisition. Magn Reson Med. 2016;75(5):2000–8.

133. Yu H, Reeder SB, Shimakawa A, McKenzie CA, Brittain JH. Robust multipoint water-fat separation using fat likelihood analysis. Magn Reson Med. 2012;67(4):1065–76.
134. Joy TR, McKenzie CA, Tirona RG, Summers K, Seney S, Chakrabarti S, Malhotra N, Beaton MD. Sitagliptin in patients with non-alcoholic steatohepatitis: A randomized, placebo-controlled trial. World J Gastroenterol. 2017;23(1):141–50.
135. Cui Y. Fast Absolute Quantification of In Vivo Water and Fat Content with Magnetic Resonance Imaging. The University of Western Ontario; 2012.
136. Glorieux G, Tattersall J. Uraemic toxins and new methods to control their accumulation: Game changers for the concept of dialysis adequacy. Clin Kidney J. 2015;8(4):353–62.
137. Vanholder RC, Eloot S, Glorieux GLRL. Future avenues to decrease uremic toxin concentration. Am J Kidney Dis. 2016;67(4):664–76.
138. Vaziri ND, Zhao Y-Y, Pahl M V. Altered intestinal microbial flora and impaired epithelial barrier structure and function in CKD: the nature, mechanisms, consequences and potential treatment. Nephrol Dial Transplant. 2016;31(5):737–46.
139. Balmer ML, Slack E, de Gottardi A, Lawson MAE, Hapfelmeier S, Miele L, Grieco A, Van Vlierberghe H, Fahrner R, Patuto N,

Bernsmeier C, Ronchi F, Wyss M, Stroka D, Dickgreber N, Heim MH, McCoy KD, Macpherson AJ. The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. Sci Transl Med. 2014;6(237):237–66.

140. Terao N, Shen DD. Reduced extraction of I-propranolol by perfused rat liver in the presence of uremic blood. J Pharmacol Exp Ther. 1985;233(2):277–84.
141. Klein JD, Timmer RT, Rouillard P, Bailey JL, Sands JM. UT-A Urea Transporter Protein Expressed in Liver : Upregulation by Uremia. J Am Soc Nephrol. 1999;10(10):2076–83.
142. Vilela P, Rowley HA. Brain ischemia: CT and MRI techniques in acute ischemic stroke. Eur J Radiol. 2017;96(5):162–72.
143. Seitun S, De Lorenzi C, Cademartiri F, Buscaglia A, Travaglio N, Balbi M, Bezante GP. CT Myocardial Perfusion Imaging: A New Frontier in Cardiac Imaging. Biomed Res Int. 2018;Oct:1–21.
144. Jensen NKG, Lock M, Fisher B, Kozak R, Chen X, Chen J, Wong E, Lee TY. Prediction and Reduction of Motion Artifacts in Free-Breathing Dynamic Contrast Enhanced CT Perfusion Imaging of Primary and Metastatic Intrahepatic Tumors. Acad Radiol. 2013;20(4):414–22.
145. Kim SH, Kamaya A, Willmann JK. CT Perfusion of the Liver in Oncology. Radiology. 2014;272(2):322–44.

146. Petralia G, Bonello L, Viotti S, Preda L, D'Andrea G, Bellomi M. CT perfusion in oncology: How to do it. Cancer Imaging. 2010;10(1):8–19.
147. Hsieh CB, Chen CJ, Chen TW, Yu JC, Shen KL, Chang TM, Liu YC. Accuracy of indocyanine green pulse spectrophotometry clearance test for liver function prediction in transplanted patients. World J Gastroenterol. 2004;10(16):2394–6.
148. Sugimoto H, Okochi O, Hirota M, Kanazumi N, Nomoto S, Inoue S, Takeda S, Nakao A. Early detection of liver failure after hepatectomy by indocyanine green elimination rate measured by pulse dye-densitometry. J Hepatobiliary Pancreat Surg. 2006;13(6):543–8.
149. Caesar J, Shaldon S, Chiandussi L, Guevara L, Sherlock, S. The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. Clin Sci. 1961;21(2):43–57.
150. Vos JJ, Wietasch JKG, Absalom AR, Hendriks HGD, Scheeren TWL. Green light for liver function monitoring using indocyanine green? An overview of current clinical applications. Anaesthesia. 2014;69(12):1364–76.
151. Reekers M, Simon MJG, Boer F, Mooren RAG, Van Kleef JW, Dahan A, Vuyk J. Pulse dye densitometry and indocyanine green

plasma disappearance in ASA physical status I-II patients. Anesth Analg. 2010;110(2):466–72.

152. Grainger SL, N KPW, Brown IMH, Marigold JH, Thompson RPH. Clearance and non-invasive determination of the hepatic extraction of indocyanine green in baboons and man. Clin Sci. 1983;64:207–12.
153. Huang S-HS, Crowley LE, Jefferies HJ, Eldehni MT, Odudu A, McIntyre CW. The impact of hemodialysis on segmental and global longitudinal myocardial strain. Can J Cardiol. 2014;30(11):1422–8.
154. Blessberger H, Binder T. Two dimensional speckle tracking echocardiography: Basic principles. Heart. 2010;96(9):716–22.
155. Marwick TH, Leano RL, Brown J, Sun JP, Hoffmann R, Lysyansky P, Becker M, Thomas JD. Myocardial Strain Measurement With 2-Dimensional Speckle-Tracking Echocardiography. Definition of Normal Range. JACC Cardiovasc Imaging. 2009;2(1):80–4.
156. Vanholder R, De Smet R, Glorieux G, Argiles A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn P, Deppisch R, Descamps-Latscha B, Henle T, Jorres A, Lemke H, Massy Z, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidel W. Review on uremic toxins : Classification , concentration , and interindividual variability. Kidney Int.

2003;63:1934–43.

157. Vanholder R, Schepers E, Pletinck A, Nagler E V., Glorieux G. The Uremic Toxicity of Indoxyl Sulfate and p-Cresyl Sulfate: A Systematic Review. J Am Soc Nephrol. 2014;25(9):1897–907.
158. Vanholder R, Boelaert J, Glorieux G, Eloot S. New Methods and Technologies for Measuring Uremic Toxins and Quantifying Dialysis Adequacy. Semin Dial. 2015;28(2):114–24.
159. Feigenbaum J, Neuberg CA. Simplified Method for the Preparation of Aromatic Sulfuric Acid Esters. J Am Chem Soc. 1941;63(12):3529–30.
160. Murtagh FEM, Addington-Hall J, Higginson IJ. The Prevalence of Symptoms in End-Stage Renal Disease: A Systematic Review. Adv Chronic Kidney Dis. 2007;14(1):82–99.
161. Brazier JE, Harper R, Jones NM, O'Cathain A, Thomas KJ, Usherwood T, Westlake L. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. BMJ. 1992;305(7):160–4.
162. Yosipovitch G, Zucker I, Boner G, Gafter U, Shapira Y, David M. A questionnaire for the assessment of pruritus: Validation in uremic patients. Acta Derm Venereol. 2001;81:108–11.
163. Loosman WL, Siegert CEH, Korzec A, Honig A. Validity of the Hospital Anxiety and Depression Scale and the Beck Depression

Inventory for use in end-stage renal disease patients. Br J Clin Psychol. 2010;49(4):507–16.

164. Wilson M-MG, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, Diebold MR, Morley JE. Appetite assessment : simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. Am J Clin Nutr. 2005;82(5):1074–81.
165. Yamamoto S, Kazama JJ, Wakamatsu T, Takahashi Y, Kaneko Y, Goto S, Narita I. Removal of uremic toxins by renal replacement therapies: a review of current progress and future perspectives. Ren Replace Ther. 2016;2(1):43.
166. van Wijck K, Lenaerts K, van Loon LJC, Peters WHM, Buurman WA, Dejong CHC. Exercise-Induced splanchnic hypoperfusion results in gut dysfunction in healthy men. PLoS One. 2011;6(7):1–9.
167. Grootjans J, Lenaerts K, Buurman WA, Dejong CHC, Derikx JPM. Life and death at the mucosal-luminal interface: New perspectives on human intestinal ischemia-reperfusion. World J Gastroenterol. 2016;22(9):2760.
168. Jakob SM, Tenhunen J, Laitenen S, Heino A, Alhava E, Takala J. Effects of systemic arterial hypoperfusion on splanchnic hemodynamics and hepatic arterial buffer response in pigs. Am J

Physiol Gastrointest Liver Physiol. 2001;280(5):819–27.

169. Chen M, Zeng Q, Huo J, Yin X, Li B, Liu J. Assessment of the hepatic microvascular changes in liver cirrhosis by perfusion computed tomography. World J Gastroenterol. 2009;15(28):3532–7.
170. Greenway BC V, Oshiro G. Intrahepatic distribution of portal and hepatic arterial blood flows in anaesethetized cats and dogs and the effects of portal occlusion, raised venous pressure and histamine. J Physiol. 1972;227(2):473–85.
171. Slater G, Vladeck BC, Bassin R, Shoemaker WC. Sequential changes in hepatic blood flows during hemorrhagic shock. Am J Phsyiology. 1972;223(6):1428–32.
172. Hiltebrand LB, Krejci V, Banic A, Erni D, Wheatley AM, Sigurdsson GH. Dynamic study of the distribution of microcirculatory blood flow in multiple splanchnic organs in septic shock. Crit Care Med. 2000;28(9):3233–41.
173. Vollmar B, Glasz J, Leiderer R, Post S, Menger MD. Hepatic Microcirculatory Perfusion Failure Is a Determinant of Liver Dysfunction in Warm Ischemia-Reperfusion. Am J Pathol. 1994;145(6):1421–31.
174. Sivalingam M, Banerjee A, Nevett G, Farrington K. Haemodynamic effects of food intake during haemodialysis.

Blood Purif. 2008;26(2):157–62.

175. Kistler BM, Fitschen PJ, Ikizler TA, Wilund KR. Rethinking the Restriction on Nutrition During Hemodialysis Treatment. J Ren Nutr. 2015;25(2):81–7.