

Magnetic Resonance Assessment of Portal Hypertension and Liver Function

Thesis for a DPhil in Clinical Medicine

Christina Levick MA BM BCh MRCP

St John's College

September 2019

Supervisors

Prof. Eleanor Barnes BSc MBBS MRCP PhD

Prof. Stefan Neubauer MD FRCP FACC FMedSci

1 Contents

2 Acknowledgements 8

3 Declaration 9

4 Conflicts of Interest 10

5 Publications arising 10

6 Published abstracts 11

7 Abbreviations 12

8 Index of Tables 15

9 Index of Figures 16

10 Thesis abstract 18

1 Introduction 20

1.1 The pathophysiology of liver disease 20

1.1.1 Mechanisms of acute and chronic liver injury 20

1.1.2 Impairment of liver function caused by liver injury 22

1.1.3 The development and gold standard assessment of liver fibrosis in chronic liver disease 23

1.1.4 The development and gold standard assessment of portal hypertension in patients with cirrhosis 25

1.1.5 Decompensation of liver disease 28

1.1.5.1 Oesophageal varices 28

1.1.5.2 Ascites 29

1.1.5.3 Hepatic encephalopathy 30

1.1.5.4 Hepatocellular carcinoma 30

1.2 Liver disease severity scores and their prognostic significance 31

1.3 Review of existing non-invasive diagnostic methods for liver fibrosis, portal hypertension and liver function 32

1.3.1 Liver fibrosis markers 32

1.3.1.1 Blood-based markers of liver fibrosis 33

1.3.1.2 Elastographic markers of liver fibrosis 33

1.3.1.3 Imaging-based markers of liver fibrosis 34

1.3.2 Existing non-invasive assessments of portal hypertension 36

1.3.2.1 Liver-based markers of portal hypertension 36

1.3.2.2 Vascular flow-based markers of portal hypertension 36

1.3.2.3 Splenic markers of portal hypertension 37

1.3.3 Risk stratification of patients for varices needing treatment 39
1.3.4 Existing non-invasive assessments of liver function 40
1.3.4.1 Indocyanine green clearance for the assessment of liver function 40
1.3.4.2 MRI with gadoxetic acid for the assessment of liver function 41
1.4 A bit more about MRI physics 42
1.4.1 The principles of MRI and relaxation times 42
1.4.2 Factors that affect T1 43
1.4.3 Extracellular volume fraction measurement by MRI 44
1.5 General aims 45
2 General methods 46
2.1 Participants and recruitment 47
2.1.1 Patient groups 47
2.1.2 Recruitment 49
2.1.3 Eligibility criteria 50
2.2 Study assessments 52
2.2.1 Review of medical history 52
2.2.2 Blood tests 52
2.2.3 Anthropometric measurements 52
2.2.4 Transient elastography of the liver 53
2.2.5 Multiparametric MRI 53
2.2.5.1 T1 mapping 53
2.2.5.2 T2* and proton density fat fraction 55
2.2.5.3 MRI with gadoxetic acid administration 55
2.2.5.4 Volumetric interpolated breath hold examination 56
2.2.6 Varices, ascites and portal pressure results gathered from medical records 56
2.2.7 Patient follow up for clinical outcomes 57
2.3 MRI results analysis 57
2.3.1 LiverMultiScan 57
2.3.2 Spleen extracellular volume fraction 58
2.3.3 Change in liver T1 with gadoxetic acid administration 58
2.3.4 Organ size measurements 59
2.4 Statistical analysis 59
2.4.1 Target sample sizes 60
2.5 Validation of methods 61

2.5.1 Validation of the spleen ECV method 61
2.5.2 Reproducibility of non-invasive MR markers across study sites 64
2.5.3 Inter-observer reproducibility of MR analysis 65
3 Influence of the menstrual cycle on liver and spleen T1 68
3.1 Abstract 68
3.2 Introduction 69
3.3 Hypotheses 71
3.4 Aims 72
3.5 Methods 72
3.5.1 Participants and recruitment 72
3.5.2 Study procedures 73
3.5.3 Body composition estimation 73
3.5.4 Inferior vena cava area 73
3.5.5 Sample size calculation 74
3.5.6 Statistical methods 75
3.6 Results 75
3.6.1 Changes within the menstrual cycle 78
3.6.2 Correlations with liver cT1 81
3.6.3 Associations of spleen MRI parameters 82
3.7 Discussion 85
3.7.1 Limitations 87
3.7.2 Conclusions 87
4 Assessment of portal hypertension and oesophageal varices 89
4.1 Abstract 89
4.2 Introduction 90
4.3 Hypotheses 93
4.4 Aims 93
4.5 Methods 94
4.5.1 Participants 94
4.5.2 Study assessments 95
4.5.3 Calculation of non-invasive markers of portal hypertension 95
4.5.4 Application of criteria to exclude VNTs 96
4.5.5 Statistical methods 96
4.6 Results 97

4.6.1 Participants 97
4.6.2 Success of non-invasive measurements 100
4.6.3 Non-invasive markers in subjects with and without portal hypertension 100
4.6.4 Diagnostic accuracy of non-invasive markers for the identification of portal hypertension in cirrhosis 103
4.6.5 Association of non-invasive markers with portal pressure measurements 104
4.6.6 Non-invasive assessment of dynamic changes in portal pressure 106
4.6.7 Non-invasive markers in compensated advanced chronic liver disease with and without significant varices 108
4.6.8 Diagnostic accuracy for the detection of varices needing treatment 110
4.6.9 Identification of patients to avoid screening OGDs for varices 112
4.7 Discussion 115
4.7.1 Limitations 118
4.7.2 Conclusion 120
5 MR assessment of liver function and the ability of combined liver fibrosis, portal hypertension and liver function assessments to differentiate clinical phenotypes of liver disease 121
5.1 Abstract 121
5.2 Introduction 122
5.3 Hypotheses 124
5.4 Aims 125
5.5 Methods 125
5.5.1 Participants 126
5.5.2 MRI assessments 126
5.5.3 Statistical methods 127
5.6 Results 128
5.6.1 Missing results for non-invasive measures 128
5.6.2 Participant baseline characteristics 131
5.6.3 %rT1 in participants with and without liver dysfunction 134
5.6.4 Diagnostic accuracy of %rT1 to identify liver dysfunction 135
5.6.5 Associations with %rT1 137
5.6.6 The influence of liver volume on %rT1's association with MELD 140
5.6.7 Assessment of the contribution of liver fibrosis, liver function, and portal hypertension to different clinical phenotypes of liver disease 141
5.7 Discussion 145

5.7.1 Limitations 147
5.7.2 Conclusions 149
6 Prediction of clinical outcomes in patients with cirrhosis 150
6.1 Abstract 150
6.2 Introduction 151
6.3 Hypothesis 154
6.4 Aims 154
6.5 Methods 154
6.5.1 Participants 155
6.5.2 Assessments and follow up 155
6.5.3 Statistical methods 156
6.6 Results 157
6.6.1 Participants 157
6.6.2 Clinical outcomes during the follow up period 159
6.6.3 The effect of MRI-determined portal hypertension and liver dysfunction on the primary endpoint-free survival 159
6.6.4 Cox regression analysis for predictors of the primary endpoint 162
6.7 Discussion 164
6.7.1 Limitations 168
6.7.2 Conclusions 169
7 General discussion 170
7.1 Summary of thesis findings 170
7.2 Technical aspects of MR sequences used 173
7.3 Potential applications of multiparametric MR with gadoxetic acid in clinical practice 176
7.4 Comparison of multiparametric MR with other non-invasive tests for the multifactorial assessment of liver disease 178
7.4.1 Multifactorial assessments using transient elastography of the liver and spleen 178
7.4.2 Multifactorial assessments using magnetic resonance elastography of the liver and spleen 179
7.5 Limitations 180
7.6 Consideration of translation of research findings into clinical care 182
7.7 Suggested further work 184
7.8 Conclusion 185
8 References 186
9 Appendix 200

9.1 Appendix A. Letter to the research ethics committee regarding the safety of gadoxetic acid in the MAPLE study 200

2 Acknowledgements

The work of this thesis could not have been achieved without the help and support of a wide range of people.

My parents, Rodney and Angie Levick, and friends, particularly Sushma Shankar, have given invaluable support throughout the preparation and completion of this work. They've provided a sounding board for my ideas, highs and lows and have always been on hand to keep me fed and watered during intense times of writing.

Michael Pavlides was my first contact for this thesis, having met him at a conference in 2014 when he was mid-DPhil himself. He has remained enthusiastic and encouraging of my involvement and progress within our research group. He has always been on hand to answer my questions however big or small and has been an excellent role model and friend for me throughout my DPhil and beyond.

The departments I worked in, the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) and the Translational Gastroenterology Unit, provided the facilities, administrative support and academic debate needed for my progress. Special thanks go to Jane Francis for teaching me how to operate the MRI scanner and the other radiographers for trouble shooting my MR issues, Arjun Jayawsal for providing cover for my out of hours study visits, the OCMR receptionists for welcoming in all my study patients; Hanan Lamlum for her help with research governance and Kathryn Lacey for keeping the whole department running smoothly. I'm ever thankful for the physics support and exchange of ideas from Stefan Piechnik, Elizabeth Tunnicliffe, Chris Rodgers, Ferenc Mozes and Liam Young.

The research could not be possible without the help of clinicians in Oxford (Jane Collier, Jeremy Cobbold, John Ryan, Tony Ellis), Southampton (David Breen, Kathryn Nash, Mark Wright, Janisha

Patel) and Birmingham (Gideon Hirschfield, Dhiraj Tripathi, Homoyon Mehrzad) who helped to recruit patients and of course the help of all the patients and volunteers who participated in the studies. David Breen and Gideon Hirschfield were particularly helpful as local principle investigators. At each of the study sites I received research nurse support from Jacqueline Martin (Oxford BRC); Mac Mupudzi, Clare, Andrew Guy, Kim Harrison, Sanchia Rodriguez, Winningtom Ruiz (Southampton Clinical Research Facility) and Emma Burke (Birmingham Clinical Research Facility). Southampton also provided radiology support from Chris Everitt, Chris Watson and Angela Darekar.

I am grateful to Perspectum Diagnostics Ltd, with whom the University has a collaboration to use their proprietary software LiverMultiScan for cT1 analysis. In particular, Andrea Dennis and Andy Mackay helped with the analysis of baseline cT1 and Matt Kelly helped manage the collaboration.

My supervisors Ellie Barnes, Stefan Neubauer and Matthew Robson have done an outstanding job guiding me in my research and academic development. They have always encouraged me with my research ideas and helped me overcome multiple hurdles along the way.

Finally I would like to acknowledge my doctoral research fellowship funding provided by the National Institute of Health Research (NIHR) and the support of the Clinical Research Network.

3 Declaration

I declare that the work presented in this thesis is my own and has not been submitted for any other degree in this or any other University of Institute of Learning.

The studies within this thesis were designed by me under the supervision of Ellie Barnes, Stefan Neubauer and Matthew Robson and help from Michael Pavlides. The idea for the women's

hormone study was conceived by Michael Pavlides and refined further by myself. I recruited all participants, with some help from research nurses, performed all study procedures including the MRI scans in Oxford, analysed all MRI data, with some help analysing pre-contrast transverse thoracic vertebra 11 level scans from Perspectum Diagnostics, and performed all statistical analyses and interpretations. MRI scans in Southampton were acquired by Southampton radiographers under my supervision.

4 Conflicts of Interest

I have no conflicts of interest.

My supervisors EB, SN and MR have shares in Perspectum Diagnostics Ltd, a spin out company from the University of Oxford.

5 Publications arising

Phosphodiester content measured in human liver by in vivo 31P magnetic resonance spectroscopy at 7T. Purvis LAB, Clarke WT, Valkovič L, Levick C, Pavlides M, Barnes E, Cobbold JF, Robson MD and Rodgers CT. Magnetic Resonance in Medicine. 2017; 78: 2095–2105

Mapping tissue water T1 in the liver using the MOLLI T1 method in the presence of fat, iron and B0 inhomogeneity. Mozes FE, Tunnicliffe EM, Moolla A, Marjot T, Levick CK, Pavlides M, Robson MD. NMR in Biomedicine. 2019 Feb;32(2):e4030.

6 Published abstracts

Spleen T1 and spleen diameter criteria can identify and exclude oesophageal varices accurately. Presented at the International Liver Conference 2019.

Spleen extracellular volume fraction and platelet count/spleen volume ratio are accurate non-invasive markers of portal hypertension. Presented at the International Liver Conference 2019.

Platelet and spleen volume criteria exclude more oesophageal varices needing treatment than Baveno VI criteria with 100% sensitivity. Presented at the International Liver Conference 2018.

Liver function assessment using magnetic resonance imaging with gadoxetic acid administration. Presented at the British Association of the Study of the Liver annual meeting 2017 and the International Liver Conference 2018.

Multiparametric magnetic resonance imaging can quantitatively assess fibroinflammatory response to direct acting antiviral treatment of hepatitis C. Presented at the International Liver Conference 2018.

Associations of magnetic resonance imaging assessment of liver fibrosis with age, sex and total body water content in healthy individuals. Presented at the British Association of the Study of the Liver annual meeting 2018.

Multi-Parametric Magnetic Resonance Imaging of the Spleen: An Accurate and Non-Invasive Biomarker of Portal Pressure. Presented at the International Liver Conference 2016.

7 Abbreviations

AIH: Autoimmune hepatitis

ArLD: Alcohol-related liver disease

ALT: Alanine aminotransferase

ALP: Alkaline phosphatase

APRI: Aspartate aminotransferase-to-platelet ratio

AST: Aspartate aminotransferase

AUROC: Area under the receiver operator curve

cACLD: Compensated advanced chronic liver disease

CP: Child Pugh score

CPA: Collagen proportionate area

cT1: T1 corrected for iron content

DILI: Drug-induced liver injury

ECG: Electrocardiogram

ECM: Extracellular matrix

ECV: Extracellular volume fraction

DWI: Diffusion weighted imaging

ELF: Enhanced liver fibrosis

FHVP: Free hepatic vein pressure

FIB-4: Fibrosis-4

FOV: Field of view

Gd-EOB-DTPA : Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid, also known as gadoxetate disodium, gadoxetic acid, Primovist and Eovist

HBV: Hepatitis B virus

HCC: Hepatocellular carcinoma

HCT: Haematocrit

HCV: Hepatitis C virus

HR: Hazard ratio

HSC: Hepatic stellate cells

HVPG: Hepatic vein pressure gradient

ICG: Indo-cyanine green

INR: International normalised ratio

IQR: Inter-quartile range

IVC: Inferior vena cava

LIF: Liver inflammation and fibrosis

LFTs: Liver function tests

LMS: LiverMultiScan^{TM}

LS: liver stiffness

MELD: Model of end stage liver disease

MRE: Magnetic resonance elastography

MRI: Magnetic resonance imaging

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

PBC: Primary biliary cholangitis

PDFF: Proton density fat fraction

PSC: Primary sclerosing cholangitis

PT: Prothrombin time

%rT1: Percentage reduction in liver T1 with gadoxetic acid administration

ROI: Region of interest

SD: Standard deviation

shMOLLI: Shortened modified look locker inversion

SSFP: Single shot steady state free precession

T2DM: Type 2 diabetes mellitus

TE: Transient elastography

TE: Echo time

TIMP-1: Tissue inhibitor of metalloproteinase 1

TIPSS: Transjugular intrahepatic porto-systemic shunt

TR: Repetition time

US: Ultrasound scan

VIBE: Volumetric interpolated breath hold examination

VNT: Varices needing treatment

WHVP: Wedged hepatic vein pressure

8 Index of Tables

Table 1. Description of histological liver fibrosis staging for the Ishak and METAVIR systems. 24
Table 2. Factorial design of patient groups by liver function, cirrhosis and portal hypertension status. 48
Table 3. F test for the equilibrium of spleen ECV for each participant group. 63
Table 4. Reproducibility of MR measures using healthy subjects (n = 5) repeat scanned in Oxford and Southampton 64
Table 5. Inter-observer reproducibility of MR markers analysis between 2 observers (n = 11) 66
Table 6. Baseline characteristics 77
Table 7. Change in parameters in different phases of the menstrual cycle 79
Table 8. Comparison of markers over time at the first and last study assessments in the same menstrual phase 80
Table 9. Correlation of liver cT1 with other factors. 81
Table 10. Correlation of spleen T1 and cT1 with other factors 83
Table 11. Baseline characteristics by participant groups used in the analysis of portal hypertension 99
Table 12. Comparison of subject groups with and without portal hypertension 101
Table 13. Diagnostic accuracy of non-invasive markers for the identification of portal hypertension in patients with cirrhosis 103
Table 14. Baseline characteristics of cACLD subjects with and without varices needing treatment (VNTs) 108
Table 15. Comparison of non-invasive markers in compensated advanced chronic liver disease (cACLD) patients with and without varices needing treatment (VNTs) 109
Table 16. Diagnostic accuracy of biomarkers in compensated advanced chronic liver disease patients for the identification of varices needing treatment. 111
Table 17. Ability of non-invasive criteria to avoid OGD screening for varices needing treatment (VNTs) in compensated advanced chronic liver disease. VNT prevalence was 14% 114
Table 18. Reported results from this and other studies for the application of Baveno criteria to exclude VNTs. 120
Table 19. Baseline characteristics of participant groups 132
Table 20. Associations of %rT1 with other liver-related measures (n = 169) 139
Table 21. Multiple linear regression for the prediction of %rT1 140
Table 22. Multivariable linear regression models using %rT1 and liver volume for the estimation of MELD as a gold standard for liver function 141
Table 23. MR markers in different phenotypes of liver disease 143
Table 24. Baseline characteristic of the patients followed up for clinical outcomes 158
Table 25. Univariate Cox regression analysis for the primary endpoint 162
Table 26. Multivariable Cox regression analysis model for the primary endpoint 164
Table 27. Results summary for MR markers of portal hypertension. 172
Table 28. Suggested MRI sequence for the multifactorial assessment of liver disease. 177

9 Index of Figures

Figure 1. Stages involved in the progression of chronic liver disease. 22
Figure 2. Anatomy of the portal venous system and measurement of wedged hepatic venous pressure. 26
Figure 3. Typical example of a transverse slice at the upper level of T11 through the liver and spleen. 54
Figure 4. Examples of 3-point slice T1 maps incorporating the left ventricular blood pool, spleen and liver at baseline (A) and post contrast (B). 55
Figure 5. Bland Altman plot showing the reproducibility of spleen ECV measured using gadoxetic acid versus gadobutrol as the contrast agent in 5 healthy volunteers. 62
Figure 6. Spleen ECV over time in study participant groups. 63
Figure 7. Bland Altman plot of spleen ECV (A) and %rT1 (B) repeated in Oxford and Southampton in 5 healthy subjects 65
Figure 8. Bland Altman plots for the inter-observer agreement of spleen ECV (A), %rT1 (B), spleen volume (C) and liver volume (D) (n = 11). 67
Figure 9. Hormone levels throughout the menstrual cycle. 70
Figure 10. Theoretical changes in extracellular fluid distribution during the menstrual cycle. 71
Figure 11. Example of inferior vena cava (orange) and aorta (yellow) area analysis performed using ClearCanvas DICOM viewer. 74
Figure 12. Flow diagram of study participation. 76
Figure 13. Liver cT1 throughout the menstrual cycle. 78
Figure 14. Correlation of liver cT1 with body water content as estimated by bioelectrical impedance (r = 0.544, p = 0.001). 82
Figure 15. Spleen cT1 correlations with (A) ferritin (r = -0.478, p = 0.004), (B) haemoglobin (r = -0.56, p = 0.001), (C) spleen T2* (r = 0.44, p = 0.008) and (D) progesterone (r = 0.37, p = 0.027). 84
Figure 16. Flow diagram of participants in the portal hypertension study. 98
Figure 17. Box and whisker plot of spleen ECV in healthy subjects (n = 20), cirrhotic subjects without portal hypertension (n = 49) and cirrhotic subjects with portal hypertension (n = 73). 102
Figure 18. Receiver operator curve for the identification of portal hypertension in subjects with cirrhosis using spleen ECV. 104
Figure 19. Associations of candidate portal pressure markers with portal pressure measurements in 7 subjects 105
Figure 20. Portal pressure gradient in patients pre and post TIPSS measured during the procedure (n = 4). 106
Figure 21. Scatter plots of the percentage change in non-invasive markers versus the percentage change in portal pressure gradient (PPG) following TIPSS (n = 4). 107
Figure 22. Comparison of spleen ECV in compensated advanced chronic liver disease with and without varices needing treatment (VNTs). 110
Figure 23. Receiver operator curves of spleen ECV (A), platelet/spleen volume ratio (B), spleen T1 x spleen diameter (C) and FIB-4 (D) for the diagnosis of varices needing treatment in compensated advanced chronic liver disease (n = 91). 112

Figure 24. Flow diagram of recruitment for the analysis of liver function and differentiation of liver disease phenotype using MR parameters. 130
Figure 25. The percentage reduction in liver T1 with gadoxetic acid (%rT1) in healthy volunteers (n = 24) and patients with acute liver injury (n = 7), cirrhosis with normal liver function (n = 66) and cirrhosis with liver dysfunction (n = 72). 134
Figure 26. Receiver operator curve of %rT1 in all participants with (n = 79) and without liver dysfunction (n = 90). 136
Figure 27. Receiver operator curve of %rT1 in patients with cirrhosis with (n = 72) and without (n = 66) liver dysfunction. 136
Figure 28. Associations of %rT1 with measures of liver function (n = 169). 138
Figure 29. Liver cT1 (A), spleen ECV (B) and percentage reduction in liver T1 with gadoxetic acid administration (%rT1) (C) by liver disease phenotype. 144
Figure 30. Kaplan Meier curves for patients with cirrhosis with spleen ECV-defined portal hypertension (spleen ECV >0.36; n = 57) and no portal hypertension (spleen ECV <0.36; n = 44) surviving free from the primary composite endpoint. 160
Figure 31. Kaplan Meier curves for patients with cirrhosis with platelet count/spleen volume ratio <0.28 (portal hypertension; n = 57) and platelet count/spleen volume ratio >0.28 (no portal hypertension; n = 50) surviving free from the primary composite endpoint. 161
Figure 32. Kaplan Meier curves for patients with cirrhosis with and without liver dysfunction defined by %rT1 >55.05% (n = 53) and %rT1 <55.05% (n = 56) surviving free from the primary endpoint. 162

10 Thesis abstract

Magnetic Resonance Assessment of Portal Hypertension and Liver Function

Christina Levick, St John's College

Thesis for a DPhil in Clinical Medicine

Trinity term 2019

In liver disease, measurements of portal hypertension and liver function can predict clinical outcomes and help guide management, but are either invasive or lack accuracy. This thesis explores magnetic resonance imaging (MRI) techniques with gadoxetic acid administration to assess portal hypertension and liver function non-invasively. This adds to the existing capabilities of multiparametric MRI to assess liver fibrosis, fat and iron.

Spleen extracellular volume fraction (ECV) and platelet count to spleen volume ratio identified portal hypertension accurately (both 0.79 area under the receiver operator curve (AUROC)). A combination of spleen T1 and spleen diameter identified oesophageal varices needing treatment (VNTs) in patients with compensated advanced chronic liver disease (cACLD) with the highest accuracy (AUROC 0.87) of all markers tested and could save 66% of screening endoscopies for VNTs without missing any VNTs.

Liver T1 reduction with gadoxetic acid (%rT1) correlated well with serum-based liver function markers and clinical scores and identified patients with impaired liver function with AUROC 0.86.

MRI-based markers of portal hypertension (spleen ECV; platelet count/spleen diameter ratio; spleen T1 x spleen diameter) and %rT1 were predictive of liver-related clinical outcomes in

patients with cirrhosis, who are at high risk of complications, but did not add to the predictive ability of age, albumin, sodium and APRI.

In conclusion, MRI provides accurate markers of portal hypertension and liver function. Of particular importance, spleen T1 and spleen diameter criteria could be used to avoid more screening endoscopies in cACLD than any other VNT exclusion criteria reported to date. Although prognostic in the high risk cohort studied here, the MRI markers of portal hypertension and liver function do not surpass the prognostic value of simpler measures.

1 Introduction

There is a rising tide of liver disease prevalence and mortality in the United Kingdom, unmatched by any other disease [1]. Liver disease particularly affects people of working age and is the 5th most common cause of death. In the 40-49 years age bracket, liver disease is the commonest cause of death in England, mostly due to alcohol [2]. Of the multifaceted approach required to tackle this problem, this thesis will contribute by focussing on the non-invasive identification and risk stratification of liver disease and its complications, so that patients can be managed better in the future. In particular I will investigate spleen extracellular volume fraction (ECV) as a measure of portal hypertension and the percentage reduction in liver T1 with gadoxetic acid administration (%rT1) as a measure of liver function, which could both be incorporated into gadoxetic acid-enhanced magnetic resonance imaging (MRI), used clinically for liver lesion assessment. In this section I will review the pathophysiology of liver disease, need for new diagnostic tests, existing diagnostic methods and aims of this thesis.

1.1 The pathophysiology of liver disease

1.1.1 Mechanisms of acute and chronic liver injury

There are many factors that can damage the liver acutely or chronically. Acute liver injury is caused commonly by acute viral hepatitis (e.g. hepatitis A virus; hepatitis E virus; Epstein Barr virus), drugs (e.g. paracetamol overdose) and toxins (e.g. alcoholic hepatitis), and occasionally by causes more commonly associated with chronic liver disease (e.g. hepatitis C virus, autoimmune

hepatitis, Wilson's disease). The insulting factor itself or the host response to the factor causes inflammation, apoptosis and/or necrosis within the liver, which leads to a rise in liver transaminases in the blood. If the liver injury is severe, acute liver failure may ensue, associated with high mortality. Fortunately, most acute liver injuries are less severe and so resolve spontaneously upon cessation of the causative drug, treatment of the cause or clearance of the virus by the host immune system. The liver has an amazing capacity to regenerate, so is usually able to return to its normal function without long-term sequelae.

Chronic liver disease from long term exposure to insult is caused commonly by alcohol, non-alcoholic fatty liver disease (NAFLD), viral hepatitis (hepatitis B and C viruses), metabolic disorders (e.g. haemochromatosis, Wilson's disease) and autoimmune diseases (e.g. primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH)). The prolonged injury in chronic liver disease can have long term effects, including the development of liver fibrosis and hepatocellular carcinoma (figure 1).

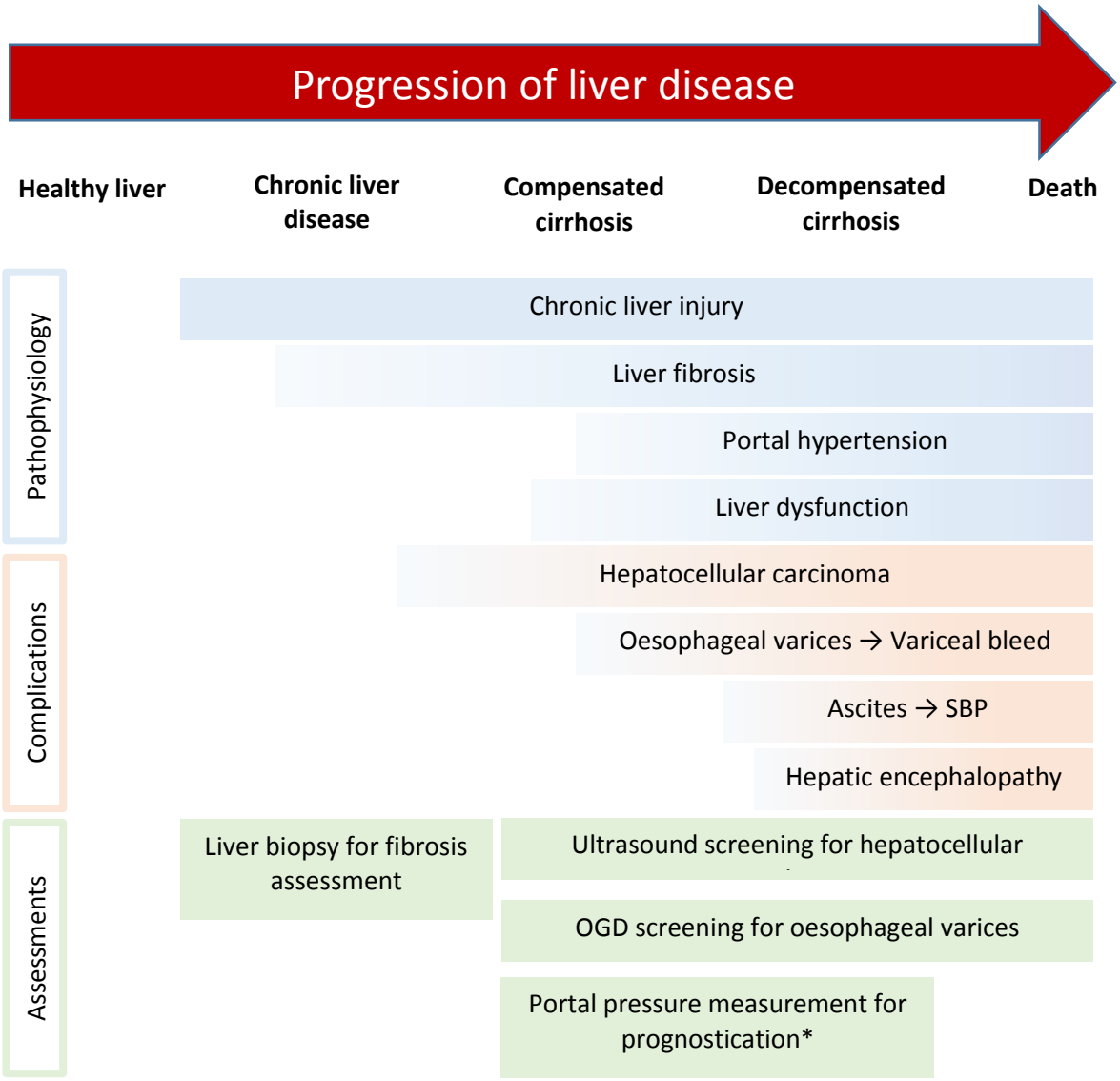


Figure 1. Stages involved in the progression of chronic liver disease.

Abbreviations: SBP, spontaneous bacterial peritonitis; OGD, oesophagogastrodoudenoscopy.

*Not part of routine care in most centres

1.1.2 Impairment of liver function caused by liver injury

In both acute and chronic liver disease, the ability of the liver to perform its normal functions can be affected. The liver has a vast array of metabolic roles that can be broadly divided into synthetic, homeostatic and excretory functions. It is no wonder therefore that liver function does

not have a well characterised definition in hepatology. The term 'liver function' will be used throughout this thesis to refer to the liver's ability to perform its metabolic roles.

Impaired liver function (liver dysfunction) can result from direct toxic effects on the liver, liver cell death (reducing functional hepatic cell mass) and/or impaired liver perfusion. The liver's synthetic function is judged commonly in clinical practice by albumin and prothrombin time/international normalised ratio (INR) blood tests, and its excretory function is judged by serum bilirubin level. Worsening liver dysfunction can cause hypoalbuminaemia, coagulopathy, jaundice and hepatic encephalopathy amongst other effects.

1.1.3 The development and gold standard assessment of liver fibrosis in chronic liver disease

Regardless of the cause, chronic liver disease is associated with activation of hepatic stellate cells (HSC). These proliferate and turn into myofibroblasts, which lay down collagen and other extracellular matrix (ECM) products that form scar tissue (fibrosis). ECM degradation is inhibited by the production of tissue inhibitor of metalloproteinase 1 (TIMP-1) by activated HSCs, whilst both ECM components and TIMP-1 help maintain HSC activation, thus promoting further fibrosis. Chronic liver disease can therefore result in the accumulation of liver fibrosis and eventual cirrhosis (end stage fibrosis) [3].

The gold standard assessment of liver fibrosis is by histological staging from liver biopsies. The most commonly used staging systems are based on fibrosis morphology, two examples of which are described in table 1. Cirrhosis, the highest stage, describes marked bands of fibrosis linking portal tracts to create distinct nodules of hepatocytes. The risk of developing complications of liver disease increases with fibrosis stage, with cirrhosis posing the highest risk [4]. There is

variance in the definition of lower stages of fibrosis however, with most staging systems scoring fibrosis F0 –F4 [5-7] and the Ishak system staging fibrosis F0 – F6 [8] (table 1).

Table 1. Description of histological liver fibrosis staging for the Ishak and METAVIR systems.

Fibrosis staging system	Description
Ishak [8]	F0 – No fibrosis F1 – Fibrous expansion of some portal areas ± short fibrous septa F2 – Fibrous expansion of most portal areas ± short fibrous septa F3 – Fibrous expansion of most portal areas with occasional portal to portal bridging F4 – Fibrous expansion of portal areas with marked portal to portal or portal to central bridging F5 – Marked bridging with occasional nodules F6 – Cirrhosis
METAVIR [5]	F0 – No fibrosis F1 – Portal fibrosis without septa F2 – Portal fibrosis with few septa (portal to portal) F3 – Numerous septa (portal to central) without cirrhosis F4 – Cirrhosis

More recently fibrosis has been quantified using the collagen proportionate area (CPA) [9]. This is derived using picrosirius red stain of a histological slide for collagen, which is then digitalised and the area of collagen (red stain) calculated as a percentage of the liver tissue area. Although traditional fibrosis stages are predictive of clinical outcomes, they divide patients into coarse categories and reach a ceiling at cirrhosis. The CPA is a continuous measure and has been found to predict clinical outcomes with greater accuracy than fibrosis stages [10]. Fibrosis staging remains the mainstay of histological fibrosis assessment in clinical practice however.

Despite the prognostic benefits of fibrosis staging histologically, it is widely regarded that liver biopsy is not a true gold standard assessment due to its small sampling volume and inter-observer

variability [11]. Liver biopsy is also an invasive and costly procedure with a 0.7% risk of major complications [12].

1.1.4 The development and gold standard assessment of portal hypertension in patients with cirrhosis

Once cirrhotic, patients are at increased risk of developing complications of liver disease. An important pathological step is the development of portal hypertension. Portal hypertension describes increased pressure in the portal vein, which supplies venous blood from the gut and spleen to the liver (figure 2). Portal hypertension is defined as a portal pressure ≥5 mmHg.

Portal hypertension is caused primarily by increasing hepatic resistance through the mechanical action of fibrosis and altered liver architecture. As liver disease progresses, dynamic mechanisms also contribute to raise portal pressure. These include further increased hepatic resistance through increased hepatic sinusoidal tone, and increased portal flow from splanchnic vasodilatation, mesenteric angiogenesis and a hyperdynamic systemic circulation [13]. Dilated veins (varices) and portosystemic venous shunts can develop as a result. Due to the portal vein's connection to the spleen, portal hypertension leads to splenic venous congestion and hypersplenism, often detected in patients as splenomegaly and thrombocytopaenia. Splenic fibrosis may also occur [14].

It is portal hypertension that causes the majority of complications of liver disease (bleeding from oesophageal varices, ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, hepatopulmonary syndrome and hepatic encephalopathy) and subsequent mortality. The development of any of these complications is termed decompensation of liver disease. These liver disease complications will be described in more detail later. A portal pressure ≥10 mmHg

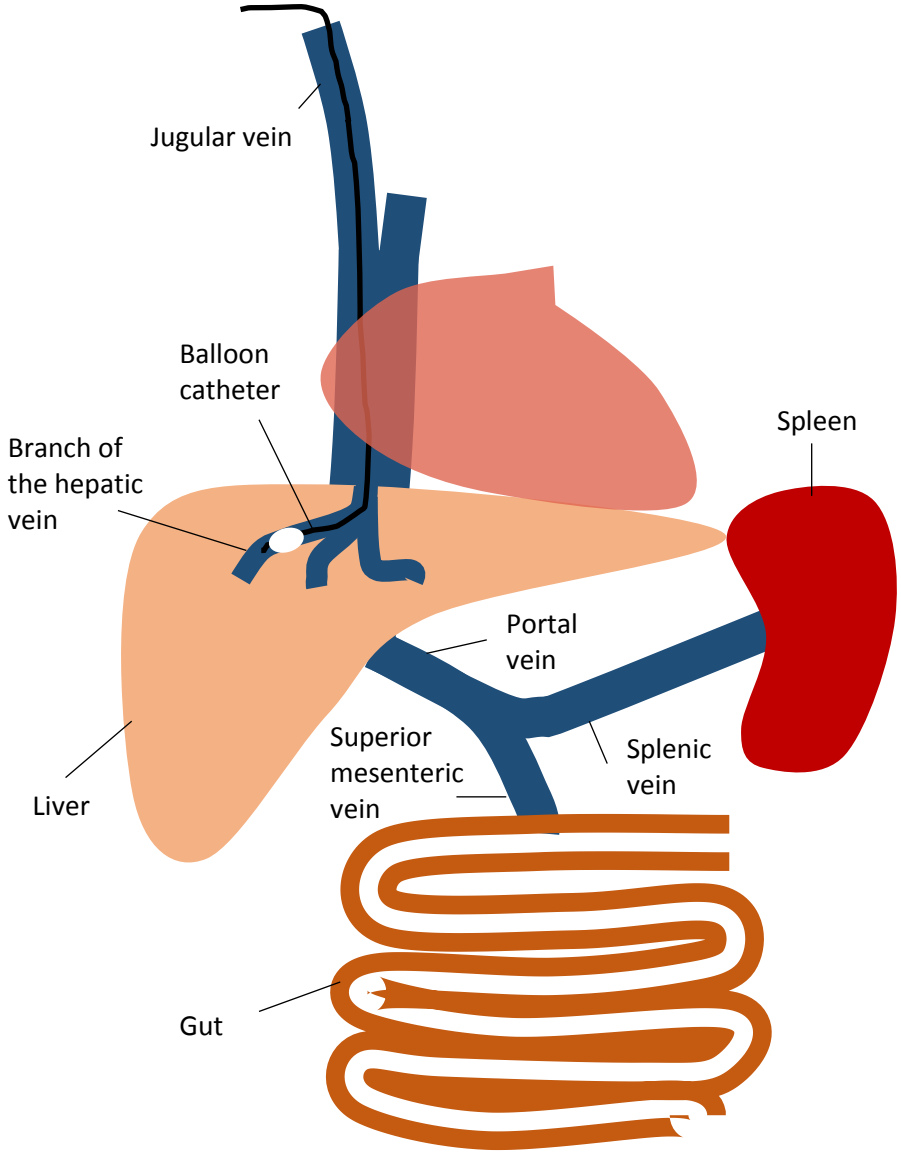


Figure 2. Anatomy of the portal venous system and measurement of wedged hepatic venous pressure.

A catheter is passed from the jugular vein down into a branch of the hepatic vein and wedged by inflation of a balloon near the catheter tip. The wedged hepatic venous pressure is measured using a pressure transducer.

(clinically significant portal hypertension, CSPH) confers an increased risk of decompensation events [15]. Portal pressure measurements can also predict the development of hepatocellular

carcinoma (HCC) [16] in compensated cirrhosis. The accurate identification of portal hypertension could help risk stratify patients and guide their management.

Direct portal pressure can only be measured by puncture through the liver or spleen into the portal vein - a procedure too invasive for diagnostic purposes. Surrogate markers of portal pressure are therefore used, with the hepatic venous pressure gradient (HVPG) being the gold standard. HVPG is the difference between the free hepatic venous pressure (FHVP) and wedged hepatic vein pressure (WHVP). HVPG has excellent correlation ($R^2 = 0.90$) and good agreement by Bland-Altman analysis with directly measured portal pressure gradient (PPG, difference between the portal vein and right atrium pressures) [17]. HVPG is a Food and Drug Agency approved surrogate outcome for clinical trials [18].

HVPG is measured by passing a catheter via the right jugular vein down to the hepatic vein, where FHVP is measured. WHVP is then measured after either wedging the catheter tip in a branch of the hepatic vein (straight catheter method) or occluding the hepatic vein with the balloon (balloon catheter method) (figure 2). All measurements are made in triplicate with a pressure transducer. The balloon catheter method produces less variable and more accurate results than the straight tip method, likely because it captures a larger liver circulation volume and is therefore better representative of the total portal pressure [19]. HVPG measurement with a balloon tip catheter is therefore the optimal method [20] and its HVPG tracings can be interpreted with high reproducibility ($r = 0.98$, $p = 0.001$; intra-class correlation coefficient 0.991, $p = 0.001$) [21]. Despite the prognostic value that can be gained from HVPG measurement, its invasiveness, cost and expertise required mean it is performed rarely in clinical practice [22].

1.1.5 Decompensation of liver disease

Decompensated liver disease describes the failure of the liver to perform its usual functions. Most mortality and morbidity in liver disease results from portal hypertension, liver dysfunction and hepatocellular carcinoma (figure 1). I will now discuss the common complications of liver disease in turn. These include oesophageal varices, ascites, hepatic encephalopathy and hepatocellular carcinoma.

1.1.5.1 Oesophageal varices

The effect of rising portal pressure is the venous congestion of organs connected with the portal venous system, expanding veno-venous connections called shunts and subsequent dilation of their connected collateral veins (creation of varices). Varices can develop in multiple sites including the stomach, rectum, spleen, and umbilicus, but the varices with the most clinical relevance occur in the oesophagus.

Oesophageal varices are present in 24% to 80% of all patients with cirrhosis [23], with the incidence increasing over time and with worsening liver decompensation [24]. Varices are a consequence of portal hypertension and have a risk of bleeding in proportion to the variceal wall tension (a function of internal pressure and diameter). Oesophageal varices have an increased risk of bleeding with portal pressures >12 mmHg [25] and if they bleed, they have a high risk of treatment failure with portal pressure >20 mmHg [26]. Overall, variceal bleeding is associated with a 15% 30 day mortality [27].

Patients with cirrhosis are screened for varices routinely using oesophagogastroduodenoscopy (OGD) to identify those at high risk of bleeding. Prophylactic treatment with β-blockers or variceal

band ligation (VBL) is offered when the varices are grade II, III or I with red sign. These criteria define varices needing treatment (VNTs). The frequency of screening depends on the clinical status of the patient and is typically every 2-3 years in compensated cirrhosis and every 1-2 years in decompensated cirrhosis [24, 28]. As OGD is an invasive and uncomfortable procedure, strategies to risk stratify patients for oesophageal varices have been developed and are discussed in section 1.3.3.

1.1.5.2 Ascites

Ascites describes the accumulation of free fluid within the abdomen - a consequence of portal hypertension. Ascites may be detected on ultrasound examination of patients with cirrhosis attending for hepatocellular carcinoma (HCC) screening or if large enough, may be detected on clinical examination as “shifting dullness” i.e. the movement of fluid within the abdomen. Gross ascites may cause abdominal discomfort, breathlessness and impaired nutrition. It may also become infected spontaneously (spontaneous bacterial peritonitis: SBP), which is associated with a high mortality [29].

Patients are managed with diuretics primarily, ascitic drains if diuretic refractory and with a transjugular intrahepatic portosystemic shunt (TIPSS) if requiring frequent drains [29]. The TIPSS connects the portal vein to the hepatic vein through the liver, thus lowering portal pressure immediately and reducing the accumulation of ascites. TIPSS is also a second line treatment of variceal bleeding. Although TIPSS is highly effective at reducing portal hypertension, 22% of patients will develop hepatic encephalopathy (described next) as a consequence of the shunt [30] by preventing adequate removal of toxins and waste products from the blood, which can then affect brain function.

1.1.5.3 Hepatic encephalopathy

Hepatic encephalopathy is an impairment in brain function that ranges from subclinical neurological/psychiatric impairment to coma. It is caused by the accumulation of ammonia affecting the brain, which the liver has failed to clear from the blood due to the presence of portosystemic shunts, impaired liver function and/or increased ammonia absorption. Hepatic encephalopathy is associated with high mortality, high usage of healthcare facilities and a significant impairment to patients' quality of life [31].

1.1.5.4 Hepatocellular carcinoma

Cirrhosis is associated with an increased risk of hepatocellular carcinoma (HCC). The risk of HCC increases with portal pressure [16] and severity of liver decompensation, as well as being determined by aetiology, age and sex [32]. In the UK, patients with cirrhosis are screened for HCC every 6 months using ultrasound examination, so that lesions can be detected at an early stage and treated with curative intent [28]. If a lesion is found, further assessment with triple phase computer tomography (CT) and/or MRI with contrast can characterise the lesion [33]. Biopsy is performed rarely since imaging is often diagnostic and biopsy carries a risk of tumour seeding.

Gadoxetic acid is an MRI contrast agent developed specifically for liver lesion assessment. It has hepatocyte uptake via organic anion transporting polypeptides OATP1B1 and B3, followed by excretion unchanged into bile via multidrug resistance-associated proteins MRP2. OATP are lost in HCC and so HCCs are seen to enhance less in the hepatobiliary phase than normal liver tissue [34]. Gadoxetic acid enhanced MRI has higher diagnostic accuracy for HCCs than CT [35, 36] and if used for HCC screening, may have a lower false positive rate than ultrasound scans [37].

1.2 Liver disease severity scores and their prognostic significance

A number of scores have been developed to help predict the development of complications and death in liver disease. One of the best-performing scores is the Child Pugh score (CP) that was developed originally to predict outcomes of surgical treatment of bleeding oesophageal varices [38]. CP incorporates blood measures of bilirubin, albumin and prothrombin time and clinical measures of ascites and hepatic encephalopathy and so utilises markers of liver function as well as portal hypertension. Each factor is assigned a score 1-3, with the total CP ranging 5-15. CP is highly predictive of mortality in cirrhosis [39] and was used to help allocate patients for life-saving liver transplantation. CP is easy to apply in clinical practice, but is criticised for the subjectivity of ascites and hepatic encephalopathy scores and for the coarse categorisation of otherwise continuous liver function markers. Such categorisation loses prognostic information and creates an unnatural ceiling to the possible disease severity.

The model for end stage liver disease (MELD) replaced CP as a liver transplant allocation score due to its objectivity and superior prediction of short term mortality. MELD was first created to predict 3 month survival following TIPSS and later applied to predict survival as a tool for liver transplant allocation [40]. MELD comprises bilirubin, INR and creatinine into an integer score ranging 6-40. Although objective, some differences in laboratory processing of samples can cause variation in MELD scores [41]. None-the-less, MELD has been used widely since its creation. MELD is associated with death in decompensated liver disease (hazard ratio 1.18) [42], decompensation of compensated liver disease (hazard ratio 1.12) [42], postoperative mortality in patients with cirrhosis [43], and mortality following variceal bleeding [44].

Serum sodium level was recognised to have prognostic significance and so was later incorporated into MELD to create new scores, e.g. MELD-Na, with improved prediction of transplant waiting list mortality [45, 46]. MELD-Na has now superseded MELD for transplant allocation in America,

whilst the United Kingdom use a different formula of the same components called the United Kingdom end stage liver disease score (UKELD) [47].

The CP, MELD and MELD-Na scores are good at predicting survival, particularly at 6 months with pooled AUROCs 0.91, 0.83 and 0.90 respectively [48]. Other prognostic scores exist for acute or critical illness, but are beyond the remit of this thesis. Scores are lacking, however, in the prediction of decompensation events and longer timescale prognostication for liver disease.

1.3 Review of existing non-invasive diagnostic methods for liver fibrosis, portal hypertension and liver function

The gold standard methods for the diagnosis and grading of liver fibrosis, portal hypertension and oesophageal varices are all invasive tests, often tolerated poorly by patients. To reduce procedural risks and improve patient experience, new non-invasive assessment methods are needed. The optimal methods should be accurate, well-tolerated and cost-effective. Here, I will review the alternatives.

1.3.1 Liver fibrosis markers

The most promising markers of liver fibrosis can be divided into blood-based, elastographic and imaging-based biomarkers.

1.3.1.1 Blood-based markers of liver fibrosis

Blood-based biomarkers include AST/ALT ratio [49], AST to platelet ratio index (APRI) [50] and FIB-4 [51], which can be calculated from standard blood tests and the enhanced liver fibrosis (ELF) score that requires specific testing for liver ECM metabolites [52, 53]. They lack the accuracy to diagnose cirrhosis when used alone, but are useful to risk stratify patients to undergo further testing when cirrhosis is likely.

1.3.1.2 Elastographic markers of liver fibrosis

Non-invasive tests that measure liver stiffness may relate to the amount of liver fibrosis. These tests examine the viscoelastic qualities of the liver in response to a sheer wave. Examples include transient elastography (TE), acoustic radiation force impulse (ARFI, which includes point sheer wave elastography (pSWE) and 2D sheer wave elastography (2D SWE)), and magnetic resonance elastography (MRE).

TE is the most widely used method in the UK and can be performed at the bedside by operators with minimal training. A handheld probe is used to generate a 50 Hz mechanical wave through the liver and the speed of the resultant sheer wave is tracked using ultrasound in the probe tip. In a meta-analysis of mixed aetiologies, TE diagnosed cirrhosis with 89% sensitivity and 87% specificity [54] and has been recommended as a diagnostic tool for cirrhosis in guidelines [55]. The degree of liver stiffness is also associated with the development of decompensation events, HCC and death, although the size of the effect is variable across studies [56]. TE samples a larger volume of liver than standard liver biopsy, but this still only equates to approximately 3 cm^3. TE, however, becomes less accurate with increasing obesity [57] and raised liver transaminases or inflammation [58, 59], has different aetiology-specific diagnostic cut-offs and cannot be used in the presence of ascites. A larger probe size (XL vs M) was created to improve accuracy in obesity.

The failure of TE to obtain reliable results poses a significant problem and has been reported as 18.4% in a mixed cohort of liver disease [60] and 23% to 3% in NAFLD [61-63].

ARFI has similar diagnostic accuracy for advanced fibrosis and cirrhosis to TE and benefits from a 3-fold lower failure to obtain reliable measures [54]. It can be incorporated into standard ultrasound examination on compatible ultrasound machines and requires specific training in the technique to produce reproducible results [64].

1.3.1.3 Imaging-based markers of liver fibrosis

Imaging-based biomarkers can overcome the effects of obesity and ascites. Unlike blood-based biomarkers, imaging techniques allow visualisation of the liver and so could assess regional differences in liver fibrosis, particularly common in PSC [65], and screen for HCCs during the same scan. Of the imaging-based biomarkers of liver fibrosis, ultrasound scans (US) lack accuracy, but magnetic resonance imaging (MRI) holds great promise.

MRE has 91% sensitivity and 81% specificity for the diagnosis of cirrhosis with area under the receiver operator curve (AUROC) 0.92 and a 4.3% failure rate [66]. It performs superiorly to blood-based markers of fibrosis [67]. The cut offs for advanced fibrosis and cirrhosis were 4.11 kPa and 4.71 kPa, respectively in a meta-analysis of 12 retrospective studies. No influences on accuracy were found for aetiologies, obesity or sex, but the presence of moderate-severe liver inflammation had a significant influence on the diagnostic ability for cirrhosis [66]. MRE is the most common elastographic technique used in North America, but requires hardware additional to the MRI scanner. 2D-MRE is commercially approved, whilst 3D-MRE has been developed that shows superior diagnostic ability for advanced fibrosis in NAFLD (AUROC 0.921 vs 0.981, $p<0.05$) in a prospective study, but is technically more challenging to perform [68].

Diffusion weighted MRI may also be useful for fibrosis staging, but there is no established consensus in its acquisition parameters and it was shown in a meta-analysis to perform inferiorly to MRE [69].

A multiparametric MRI technique called the iron-corrected T1 was developed at our institution that can differentiate fibrosis from no fibrosis with AUROC 0.91 [70]. In MRI, the relaxation time T1 is an inherent property of tissues that largely reflects water content, which increases with fibrosis, congestion and inflammation. Iron lowers T1 [71] and because liver iron content varies between individuals, this can confound the relationship of T1 to fibrosis. A correction algorithm was created that returns the measured liver T1 to the value it would be were the liver iron content normal [72]. This is the iron-corrected T1 (cT1). In this algorithm, the liver iron content is estimated from the measured T2* relaxation time. Further explanation of MRI relaxation times is given in section 1.3.

Liver cT1 was standardised to the liver inflammation and fibrosis (LIF) score, which allows comparison between magnet strengths and scanner vendors. In NAFLD alone (n = 71), LIF had high diagnostic accuracy for the identification of cirrhosis (AUROC 0.85, 0.76 – 0.95 95% CI) [73]. In this cohort, although TE had 0.93 AUROC for cirrhosis, TE had a 41% failure rate versus 5% for MRI, making TE perform poorly on an intention to diagnose basis. LIF was also shown to predict clinical outcomes in a cohort of patients with mixed liver disease aetiologies [74].

Iron-corrected T1 has the benefit of not requiring additional equipment (unlike MRE), sampling a much larger area of the liver than TE or biopsy and being able to examine regional variation in liver fibrosis [75]. Liver cT1 has now been standardised across MRI scanner models and field strengths [76] (making LIF redundant) and has been shown to have excellent repeatability [77]. Liver fat measurement by proton density fat fraction (PDFF) can also be obtained within the same examination [70], giving a multifactorial, non-invasive assessment of liver histology.

1.3.2 Existing non-invasive assessments of portal hypertension

1.3.2.1 Liver-based markers of portal hypertension

Given the role of liver fibrosis in the pathophysiology of portal hypertension, liver fibrosis markers also reflect portal pressure quite well at the milder end of the spectrum. A meta-analysis performed in 2013 found liver TE to have 81% positive predictive value (PPV) for CSPH (HVPG >10 mmHg) with 11% negative predictive value (NPV), but <70% PPV for oesophageal varices detection and there was a range of diagnostic cut offs for both CSPH and varices [78]. The correlation of liver stiffness with HVPG deteriorates above 10 mmHg [79, 80], perhaps due to the increasing contribution of dynamic changes to higher portal pressure. ARFI liver stiffness has also been found to have good diagnostic accuracy for CSPH (AUROC 0.86) with a lower test failure rate than TE (1 vs 25%) [81].

Quantification of liver surface nodularity (LSN) by post-processing of CT images has shown excellent correlation with HVPG (r = 0.75) and AUROC 0.88 for the identification of CSPH in cirrhotic patients without clinically apparent portal hypertension [82]. LSN, however, could not be acquired in 7% of patients and involves high dose radiation, which increases cancer risk and makes it unsuitable for a screening or monitoring tool.

In the same study cohort, simple blood-based liver fibrosis scores also had reasonable diagnostic accuracy for CSPH (AUROCs 0.79 for APRI and 0.77 for FIB-4) [82]. In other studies, APRI had AUROC 0.72-0.76 for identifying HVPG >12 mmHg [83] [84] and 0.84 for HVPG ≥10 mmHg [81].

1.3.2.2 Vascular flow-based markers of portal hypertension

Perhaps surprisingly, assessments of portal venous flow lack the accuracy for clinical assessment of portal hypertension [80, 85]. Superior mesenteric artery, splenic artery and azygous vein flow

measured by MRI have better correlation with HVPG than portal vein flow [80, 85]. Azygous vein flow could identify patients with HVPG ≥16 mmHg with AUROC 0.96 (95% CI 0.91 – 1.00) [85]. A model combining liver T1 and splenic artery velocity has also been found to correlate well with HVPG (r = 0.90) [80]. Unfortunately, measures of flow can be technically challenging, time consuming and have poor repeatability (coefficients of variation 7.3 – 18.6%) [86]. The vascular anatomy can be highly variable in patients with portal hypertension and so translation of these techniques into clinical practice is challenging.

1.3.2.3 Splenic markers of portal hypertension

Many of the most promising markers of portal hypertension relate to the spleen [80, 87-93]. This is because the spleen is connected to the portal vein directly by the splenic vein, transmitting the portal pressure effects from all the pathological contributors of portal hypertension (hepatic resistance and portal venous flow). Portal hypertension causes splenic venous congestion and fibrosis [14, 94] [95], which increase the spleen's water content and stiffness. Splenomegaly and increased platelet consumption, which decreases platelet count, may occur. Platelet count and scores involving platelet count have shown promise in the non-invasive assessment of portal pressure and complications of portal hypertension [96, 97].

Giannini et al. proposed a platelet count/spleen diameter ratio ≤909 n/mm^3 for the identification of oesophageal varices of any size in a mixed cohort of patients with cirrhosis [98]. A meta-analysis of 20 studies (n = 3063) found platelet count/spleen diameter ratio ≤909 had 92% sensitivity and 87% specificity for varices of any size with a hierarchical summary receiver operating curve of 0.95 [90]. When only patients with compensated cirrhosis are included, however, diagnostic accuracy reduced to a summary AUROC of 0.85 with 87% sensitivity and 71% specificity [99].

For the diagnosis of CSPH, a liver stiffness and platelet count/spleen diameter ratio score (LSPS) performed superiorly (AUROC 0.88) to liver stiffness (AUROC 0.82) or platelet count/spleen diameter ratio (AUROC 0.77) alone [97]. LSPS also performs superiorly to liver stiffness for the diagnosis of varices (AUROC 0.851 vs 0.817) [93].

Spleen stiffness can be measured by elastographic methods similar to liver stiffness. Across all methods, spleen stiffness has AUROC 0.899 with 90% sensitivity and 73% specificity for oesophageal varices of any size, but lacks specificity for varices needing treatment (AUROC 0.807, 87% sensitivity, 52% specificity) [93]. TE spleen stiffness correlates well with HVPG (R^2 0.78) in compensated HCV cirrhosis, with AUROC 0.769 for the identification of CSPH [100]. Spleen stiffness outperforms liver stiffness for the diagnosis of oesophageal varices [93] and risk assessment for variceal bleeding [101] and prediction of liver decompensation [102]. The combination of liver and spleen stiffness together improves correlation with portal pressure to R^2 0.85 [100] and accuracy of varices identification [89] and variceal bleeding risk [101]. Unfortunately, the ability to obtain useable measurements is worse for spleen stiffness than liver stiffness, with 16% failed/unreliable spleen stiffness measurements vs 2% unreliable liver stiffness measurements in a slim Asian cohort with chronic HBV [103].

TE was designed for the liver and has an upper limit of 75kPa on the standard machines, which would limit the range of portal pressures detectable. Modification of TE parameters for spleen stiffness measurement specifically, can identify grade 2-3 oesophageal varices with AUROC 0.82 in patients with compensated HCV cirrhosis [92] and a new TE model was released recently for spleen stiffness measurement.

MRE spleen stiffness measures have correlated with HVPG [88, 104, 105] and identified severe portal hypertension (HVPG ≥12 mmHg) with AUROC 0.81, oesophageal varices with AUROC 0.83 [106] and varices needing treatment with AUROC 0.93 [88]. Spleen MRE has also correlated with

the change in HVPG following TIPSS (R^2 = 0.659), indicating it may be a useful modality for monitoring treatment response [104].

Iron-corrected T1 has also been applied to the spleen and found to have r = 0.66 correlation with HVPG and 0.91 accuracy for the diagnosis of portal hypertension (HVPG ≥5 mmHg) and CSPH (HVPG ≥10 mmHg) [107]. These results support the hypothesis that splenic water content increases with portal pressure. Splenic cT1 reflects the total volume of fluid within the tissue, whereas the pathological features of portal hypertension are predominantly extracellular. The extracellular volume fraction (ECV), which is the fraction of extracellular (interstitial and intravascular) to total fluid content of tissues, may therefore be more accurate.

ECV can be measured using established MRI techniques with a freely diffusible contrast agent and is a technique used often in cardiac MRI [108, 109]. Application of ECV to the spleen may provide an accurate marker of portal pressure and so has been employed in this thesis. More details of the ECV method are described in section 1.4 and the methods section.

1.3.3 Risk stratification of patients for varices needing treatment

The identification of oesophageal varices is important so that β-blockers or variceal band ligation can be employed to reduce the risk of variceal bleeding. One strategy to reduce OGD screening for varices needing treatment (VNTs) is to identify patients at low risk of VNTs and reserve OGD for those at high risk where endoscopic treatment can be delivered. The Baveno VI consensus was published during the course of this thesis, which recommends that patients with compensated advanced chronic liver disease (cACLD, which includes compensated cirrhosis) do not need OGD screening for varices if they have platelets >150x10^9/l and liver stiffness <20 kPa [96]. These criteria were validated in numerous studies. Although Baveno VI criteria can avoid 10-34% of all

screening OGDs performed in cACLD [110, 111], the prevalence of VNTs in cACLD is just 5-17% [110, 112], so there remains a large proportion of unnecessary OGDs performed. Newer criteria have been suggested to avoid more OGDs, but often miss too many VNTs [111, 113, 114]. Scope remains to improve on these criteria.

1.3.4 Existing non-invasive assessments of liver function

Liver function is assessed most commonly in clinical practice using blood tests including bilirubin, prothrombin time (or international normalised ratio) and albumin. These blood tests can be affected by other disease processes and medications, however, and so lack specificity to liver disease. The blood results can be incorporated into composite scores such as the MELD [40] and Child Pugh [38] scores, which have prognostic value as described in section 1.2.

Beyond these tests, the best tests of liver function are dynamic tests, which require the liver to exhibit its function in response to a challenge. A number of non-invasive quantitative liver function tests have been shown to predict clinical outcomes in a hepatitis C cohort [115], but many involve complex administration and sampling protocols that are difficult to perform in clinical practice. Perhaps the most established dynamic liver function test is indocyanine green (ICG) clearance.

1.3.4.1 Indocyanine green clearance for the assessment of liver function

ICG is an organic dye that is excreted by the liver unmetabolised via organic anion transporting polypeptides OATP1B3 and Na-taurocholate transporting polypeptide (NTCP). These transport polypeptides are exclusive to hepatocytes. ICG clearance depends on both the hepatic function and hepatic blood flow and so changes with both the functional changes of liver damage and the

haemodynamic changes of portal hypertension. ICG clearance is used in some centres, particularly in the Far East, to estimate liver function prior to hepatic surgery. ICG clearance may also be a prognostic marker in cirrhosis [116, 117] and a marker of portal hypertension [118].

ICG clearance and most of the other liver function tests can only estimate the function of the liver as a whole, whereas one may be more interested to estimate liver function by region. In liver surgery for example, functional assessment of the planned residual liver segment may be important pre-operatively to identify patients at risk of developing post hepatic liver failure. Such regional analysis requires the incorporation of imaging to the functional assessment. This is possible using MRI and the liver-specific contrast agent gadoxetic acid (gadolinium ethoxybenzyl dimeglumide; gadoxetate; Gd-EOB-DTPA; Primovist®; Eovist®).

1.3.4.2 MRI with gadoxetic acid for the assessment of liver function

Gadoxetic acid is a safe and well tolerated gadolinium-based contrast agent. It has hepatocyte uptake via organic anion transporting polypeptides OATP1B1 and B3 (similar to ICG), followed by excretion unchanged into bile via multidrug resistance-associated proteins MRP2.

Gadoxetic acid is usually excreted 50% by the liver and 50% by the kidneys [119], but the balance of excretion changes to compensate for hepatic or renal impairment [120]. The reduction in parenchymal liver T1 with gadoxetic acid has been correlated with the degree of liver function as measured with Child Pugh score [121], MELD [122, 123], (13)C-methacetin breath test [124] and ICG clearance [125]. In rats, the hepatocyte concentrations of gadoxetic acid have been correlated with bilirubin and the degree of hepatobiliary transporter inhibition in a model of drug induced liver injury [126].

Like other gadolinium-based contrast agents, gadoxetic acid lowers T1 and diffuses freely between the vascular and extracellular spaces in non-hepatic organs. It could therefore be used to assess spleen ECV, liver function and liver lesions simultaneously.

1.4 A bit more about MRI physics

In order to understand the measures to be employed in this thesis, I will discuss MRI physics further.

1.4.1 The principles of MRI and relaxation times

Images are acquired in MRI by exploiting the magnetic properties of tissues rather than by using radiation, unlike CT. Protons naturally precess (spin), which gives them a magnetic moment. The frequency of the spins is called the Larmor frequency. When there are many protons together such as in tissue, however, these spins are in all different directions, so there is no net magnetisation.

The MRI scanner has a strong magnetic field (B_0), aligned longitudinally along the bore of the magnet. When a person lies in the magnet bore, the sum of magnetic moments from their constituent proton spins, aligns with the magnetic field as this is their low energy, resting state.

A radiofrequency pulse (B_1) is then applied at an angle to B_0, which excites the protons, making them spin coherently to produce a magnetic moment at an angle to B_0. The transverse component (90^o to B_0) of this magnetic moment is the signal that can be recorded by the scanner.

The Larmor frequency is dependent on magnetic field strength and so small magnetic field inhomogeneities cause the protons to precess at slightly different rates. Over time, the coherence of the magnetic spins reduces until no signal can be recorded. The time taken for the signal to disappear is the T2 or T2* relaxation time. The magnetic field inhomogeneity that dictates T2 is the Brownian motion of protons in the very slightly varying magnetic fields they all create for each other, whilst T2* is also affected by static magnetic field inhomogeneity e.g. caused by the shape of the body in the magnet bore or presence of iron in tissues.

Hydrogen protons in water are the most common type of proton in the body, so the majority of MRI signal comes from water. In the liver, iron is the main paramagnetic molecule that causes small magnetic field inhomogeneities and so dictates T2*.

1.4.2 Factors that affect T1

T1 is the time taken for longitudinal magnetisation to return from 180° to baseline (0°) following a 180° radiofrequency pulse. A number of factors can affect the measured T1 of a tissue other than the degree of fibrosis and inflammation. Some biases are useful, such as those of T2 and magnetisation transfer on the method used to measure T1 in cT1, which may contribute to the detection of pathology [127]. The influence of iron to lower T1 has already been discussed, but of particular importance to the liver is the effect of fat on T1. In phantoms and patients, T1 measured using the same T1-mapping sequence as for cT1 becomes longer with increasing tissue fat fractions [128]. This could explain why in an external validation cohort of 50 patients with NAFLD, liver cT1 was unable to differentiate between histological stages of fibrosis and or correlate with collagen proportionate area ($r = 0.142$, $p = 0.324$) [129]. A method to correct for

the effects of fat and magnetic field inhomogeneity on cT1 was developed recently [130], although not in time for use in this thesis.

Magnet strength and model of MRI scanner also affect T1. To overcome this, cT1 has been standardised across scanners and magnet strengths, which is the cT1 method used within this thesis. The reproducibility of cT1 in participants across magnet strengths and scanners has 3.3% coefficient of variance and -76.3 – 89.2 ms 95% limits of agreement [76].

A phenomenon that is yet to be explored fully is the observation that in healthy volunteers, higher liver cT1 has been measured in young women than in older women or men [131]. Similarly, young women have also been found to have higher T1 measured at 0.08 T [132] and T1rho [133] than other sex and age groups. These differences may be due to the effect of female sex hormones and will be explored further in this thesis.

1.4.3 Extracellular volume fraction measurement by MRI

The extracellular volume fraction (ECV) has the benefit over T1 quantification in that it is a proportion rather than an absolute value. ECV self-controls for the many influences on T1 values by using the patient's own blood. In support of these theoretical benefits, ECV had better correlation than T1 with collagen volume fraction in a dog model of myocardial fibrosis [134].

ECV can be measured using T1 measurements in the organ of interest and the blood pre and post contrast and the patient's haematocrit. The contrast agent must reach a dynamic steady state of free diffusion between the blood and interstitium, and must not enter the tissue's cells. When a bolus of contrast is administered, steady state is usually achieved by 15 minutes [135].

ECV measurements have correlated well with proportion of histological area comprising interstitial fibrosis in myocardium ($r = 0.78$, $p < 0.001$) [136], collagen proportionate area in the liver ($r = 0.64$, $p < 0.001$) [137] and amyloidosis in the spleen ($r = 0.57$) [109]. I hypothesise that spleen ECV should therefore correlate with the degree of splenic fibrosis and venous congestion that occur in portal hypertension.

1.5 General aims

The overall aim of this thesis is to improve the non-invasive assessment of liver disease. MRI techniques developed at this institution can assess liver fibrosis/inflammation, iron and fat, whilst MRI with gadoxetic acid can assess liver lesions and liver function. This thesis will explore factors that influence liver T1 in healthy young women. Specifically, this thesis also aims to build on the capabilities of current MRI techniques to add gadoxetic acid administration to:

- Identify and quantify (a) portal hypertension and (b) liver dysfunction
- Assess dynamic changes in portal pressure in response to therapy
- Explore if the degrees of liver fibrosis, liver function and portal hypertension differ in different clinical phenotypes of liver disease
- Predict liver-related clinical events

The performance of MRI will be compared to the ability of other non-invasive tests/scores for the factors above.

2 General methods

The studies within this thesis were designed by me under the supervision of Ellie Barnes, Stefan Neubauer and Matthew Robson and help from Michael Pavlides. The concept for the women's hormone study (chapter 3) was conceived by Michael Pavlides and refined further by myself. I screened and recruited all participants at all sites in liaison with research nurses and local clinical staff. I performed all study procedures including the MRI scans in Oxford, whilst MRI scans in Southampton were acquired by Southampton radiographers under my supervision. Pre-contrast, baseline T1 and T2* maps in transverse thoracic vertebra 11 level scans were analysed by Perspectum Diagnostics, whilst I analysed all other MRI data. I also performed all statistical analyses and interpretations.

There are two main studies within this thesis. Chapter 3 was part of the non-invasive and comprehensive liver assessment (NICOLA) study and chapters 4 – 7 were from the MRI assessment of portal hypertension and liver function (MAPLE) study. The studies were granted NHS regional ethics committee (MAPLE 16/SC/0110; NICOLA 13/SC/0243) and local trust approvals, and were conducted in accordance with the Declaration of Helsinki.

An overview of the MAPLE study methods is described here. Some of these methods are also common to the NICOLA study and so will be referred to in chapter 3 where the NICOLA study methods are described in more detail.

The MAPLE study was a multicentre, observational, prospective cohort study registered on the NIHR clinical research network portfolio (CPMS 20866). For the purposes of this study, portal hypertension in subjects is defined as the presence of portosystemic collaterals (varices) on endoscopy or cross-sectional imaging, or ascites. Liver dysfunction is defined as at least one of bilirubin above the upper limit of normal (ULN) or international normalized ratio (INR) above 1.1

of presumed liver cause. Acute liver injury is defined as alanine aminotransferase (ALT) >5 times the upper limit of normal (ULN) and liver dysfunction occurring in the absence of chronic liver disease or obstructive jaundice[1].

2.1 Participants and recruitment

2.1.1 Patient groups

The thesis objectives were addressed using a factorial design of patient groups depending on cirrhosis, portal hypertension and liver function status to allow group comparisons for each factor. The groups comprised (A) healthy volunteers, and patient groups with (B) acute liver injury and (C) cirrhosis with (i) normal liver function, (ii) liver dysfunction alone, (iii) ascites, (iv) hepatic encephalopathy (HE) and (v) planned transjugular intrahepatic porto-systemic shunt (TIPSS). Healthy volunteers acted as a control group. Cirrhosis was present in groups C (i - v) and absent in groups A and B. Portal hypertension was present in groups C (iii - v) and a proportion of groups C (i - ii), whilst absent in groups A, B and the remained of groups C (i - ii). Liver dysfunction was present in groups B and C (ii - v) and liver function was normal in groups A and C (i) (table 2).

The patient groups were chosen to enable comparison of subjects with and without cirrhosis, portal hypertension and liver dysfunction to test the diagnostic accuracy of MRI markers of factors, respectively. The groups also reflect different clinical phenotypes of liver disease, enabling investigation of how different pathophysiological factors contribute to disease

1 Although there is no consensus in the literature for the definition of acute liver injury, the chosen definition captures acute liver injury of most causes and has a sufficiently short timescale to ensure exclusion of chronic liver disease.

phenotype e.g. do different degrees of fibrosis, portal hypertension and liver dysfunction determine the phenotypic manifestations of ascites vs hepatic encephalopathy in patients with cirrhosis? The TIPSS group also have portal pressures measured immediately before and after TIPSS placement, allowing both correlation of candidate portal pressure markers with direct portal pressure measurements and testing how well the candidate portal pressure markers respond to dynamic changes in portal pressure.

Table 2. Factorial design of patient groups by liver function, cirrhosis and portal hypertension status.

<table>
<tr><td rowspan="2"></td><td>No cirrhosis</td><td colspan="2">Cirrhosis</td></tr>
<tr><td colspan="2">No portal hypertension</td><td>Portal hypertension</td></tr>
<tr><td>Normal liver function</td><td>A. Healthy volunteers</td><td colspan="2">C (i). Cirrhosis with normal liver function</td></tr>
<tr><td rowspan="4">Liver dysfunction</td><td rowspan="4">B. Acute liver injury</td><td colspan="2">C (ii). Cirrhosis with liver dysfunction alone</td></tr>
<tr><td rowspan="3"></td><td>C (iii). Cirrhosis with ascites</td></tr>
<tr><td>C (iv). Cirrhosis with hepatic encephalopathy</td></tr>
<tr><td>C (v). Cirrhosis with planned transjugular intrahepatic porto-systemic shunt</td></tr>
</table>

2.1.2 Recruitment

Participants were recruited consecutively from the John Radcliffe Hospital between June 2016 and July 2018. Healthy volunteers were also recruited from the University of Oxford and Oxford University Hospitals NHS Foundation Trust via poster and internet advertisements. Due to the rarity of TIPSS, the TIPSS group were also recruited consecutively from Southampton General Hospital between June 2016 and July 2018 and later Queen Elizabeth Hospital between December 2017 and July 2018.

Recruitment halted from July to October 2017 due to safety concerns regarding linear gadolinium-based contrast agents (GBCAs). GBCAs have either a linear or a cyclical molecular form that holds the gadolinium. Gadoxetic acid is a linear GBCA. Gadolinium was found to accumulate in the brain following repeated administration of some linear GBCAs. Although no clinical consequences of this were evident, the European Medicine's Agency advised that cyclical GBCAs replace linear GBCAs to reduce the risk of gadolinium retention. An exception was made for gadoxetic acid, for which there was no cyclical alternative and also no direct evidence of accumulation. The MAPLE study was halted temporarily whilst the evidence was reviewed. A letter to the ethics committee summarising the issue and evidence is included in appendix A. To hasten recruitment in the remaining months, recruitment at Southampton General Hospital was expanded to include all patient groups from November 2017.

All participants provided written informed consent for participation in the study.

2.1.3 Eligibility criteria

All subjects were aged ≥18 years, gave informed written consent for participation in the study and were haemodynamically stable at the time of assessment. Subjects were excluded if they had contraindications to MRI (e.g. pregnancy, pacemaker, ferromagnetic implant, shrapnel injury, severe claustrophobia); contraindications to gadoxetic acid (hypersensitivity/allergy, estimated glomerular filtration rate <30 ml/min/1.73m^2, severe bronchial asthma, severe cardiac disease, prolonged QT interval, liver transplant listing, breast feeding); current West Haven grade ≥III hepatic encephalopathy [31]; current, suspected or previous primary or secondary liver cancer; current or previous portal vein thrombosis; previous TIPSS; previous liver surgery; previous splenectomy or splenic embolization; or any other significant factor which, in the opinion of the investigator, might influence the participant's ability to participate in the study.

Separate eligibility criteria also existed for each group.

Healthy subjects (A) had good health with no known risk factors for liver disease, body mass index (BMI) 18.5 – 25 kg/m^2, consumed within the recommended limits of alcohol (<14 units/week for females, <21 units/week for males) and had liver function tests (LFTs), albumin, bilirubin, INR, and platelets within the normal range.

Subjects with acute liver injury (B) had an acute liver injury with prior good health, radiological evidence to exclude obstructive jaundice and no known or suspected chronic liver disease. They were excluded if they had significant risk factors for chronic liver disease (BMI >25 kg/m^2, alcohol consumption >14 units/week for females or >21 units/week for males) or histological evidence of fibrosis (Ishak fibrosis score >2).

Groups C (i - v) had cirrhosis of any cause diagnosed using clinical, biopsy, transient elastography, or radiological criteria. They were all being managed as having cirrhosis as part of their clinical

care.

Subjects with cirrhosis and normal liver function (C (i)) had albumin, bilirubin and INR within the normal range; absence of variceal bleeding, ascites or hepatic encephalopathy without the need for specific therapy in >1 year and were due an OGD as part of their clinical care within 1 year of study assessment.

Subjects with cirrhosis with liver dysfunction (C (ii)) alone had cirrhosis, liver dysfunction and absence of ascites and hepatic encephalopathy without the need for specific therapy in >1 year.

Subjects with cirrhosis with ascites (C (iii)) had new onset or recurrent ascites detectable clinically or radiologically within the last 6 months, liver dysfunction and absence of hepatic encephalopathy without the need for specific therapy in >1 year. They were excluded if their ascites resolved without intervention at the time of study assessment, but were still included if controlled with medications.

Subjects with cirrhosis with HE (C (iv)) had an episode of West Haven grade II or more hepatic encephalopathy [31] by clinical and/or electroencephalography criteria within 6 months of study assessment or HE controlled medically.

Subjects with cirrhosis awaiting TIPSS (C (v)) had cirrhosis, liver dysfunction and had been referred for elective TIPSS as part of their clinical care.

Patients were reallocated groups if their study results excluded them from their original group allocation, but made them eligible for another group.

2.2 Study assessments

All participants fasted for at least 4 hours prior to study assessments.

2.2.1 Review of medical history

Medical history was determined by consultation and review of medical records to assess alcohol consumption history and the presence or absence of complications of liver disease including oesophageal variceal bleeding, ascites and hepatic encephalopathy.

2.2.2 Blood tests

Venous blood was taken for full blood count, urea and electrolytes, liver function tests (bilirubin, albumin, protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase), prothrombin time, C-reactive protein, iron studies and lipid profile. The results were used to calculate AST/ALT ratio [49], APRI [50], FIB-4 [51], MELD [40] and Child Pugh [38] scores.

2.2.3 Anthropometric measurements

Participants' weight and height were measured. Their body mass index was calculated as weight (kg)/height $(m)^2$.

2.2.4 Transient elastography of the liver

Transient elastography using Fibroscan® (Echosens, France) is a non-invasive test that measures liver stiffness as a surrogate marker of liver fibrosis and is used commonly in clinical care. Using methods previously described [138] and in accordance with the manufacturer's instructions, at least 10 valid liver stiffness measurements were obtained using either the medium (M) or XL probe. Results were recorded as a median and associated interquartile range, and were deemed acceptable if the interquartile range to median ratio was >0.3 and there was ≥60% success rate in measurement acquisition. Unreliable measurements with the M probe were reattempted with the XL probe unless the patient had ascites.

2.2.5 Multiparametric MRI

A multiparametric MRI scan was performed using a 3 Tesla scanner (Siemens Healthcare, Germany - Tim Trio in Oxford; Skyra in Southampton). Patients recruited in Birmingham were scanned in Oxford. The scan protocol included T1, T2* and proton density fat fraction mapping in a transverse abdominal slice incorporating both liver and spleen at the level of the 11th thoracic vertebra (T11) (figure 3). All scans were acquired with the patients in expiration breath hold, with ECG or peripheral pulse gating and shimmed for the organs of interest.

The scan sequence details were as follows.

2.2.5.1 T1 mapping

Shortened modified look locker inversion (shMOLLI) recovery sequences were used for T1 mapping. The sequence uses balanced steady state free precession to measure the signal

intensity at different times throughout T1 recovery. These signals are then modelled to a T1 recovery curve to give the measured T1.

The details of this method have been described previously [139] and applied to the liver [70]. The sequence parameters were a 400 x 400 mm (or optimised per patient) field of view (FOV); 8mm slice thickness; voxel size 2.1 x 2.1 x 8 mm; 202.37 ms repetition time (TR); 1.02 echo times (TEs); 35° flip angle, GRAPPA acceleration factor 2 and 24 reference lines. Acquisition time was approximately 9 seconds, depending on the patient's heart rate.

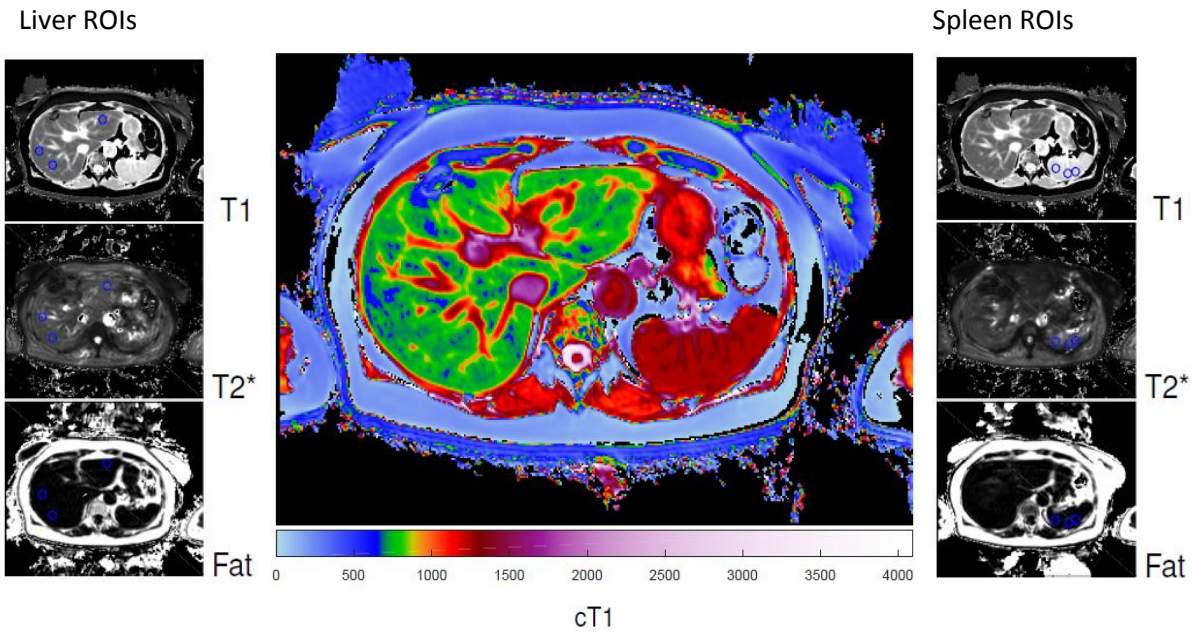


Figure 3. Typical example of a transverse slice at the upper level of T11 through the liver and spleen.

A colour-coded LiverMultiScan cT1 map is shown, with its associated T1, T2* and fat fraction images. Three regions of interest (ROIs) are drawn in areas of the liver (left panels) and spleen (right panels) away from organ boundaries, vessels and bile ducts. The colour scale shows cT1 in milliseconds.

2.2.5.2 T2 and proton density fat fraction*

An MR sequence was used that quantifies T2* for iron measurement and employs Dixon methods for proton density fat fraction (PDFF) quantification using a multi-echo gradient echo acquisition. The Dixon method uses single slice images with fat in-phase and out-of-phase of water. The relative signal contributions of fat only to the total signal gives the tissue's fat fraction.

The same slice position and FOV was used as for the T1-mapping described above. The other parameters were 6 mm slice thickness; voxel size 3.1 x 3.1 x 6.0 mm; 500 ms TR; TEs at 1.25, 2.46, 3.69, 4.92, 6.15, 7.38, 8.61 and 9.84 ms; 20° FA and GRAPPA acceleration factor 2. Acquisition time was approximately 11 seconds.

2.2.5.3 MRI with gadoxetic acid administration

In addition to the transverse T1 map, a pre-contrast T1 map was acquired, incorporating the left ventricular blood pool, liver and spleen (3-point slice; figure 4). Gadoxetic acid 0.25 mmol/ml was then administered as a bolus injection of 0.1 ml/kg to a maximum of 10 ml at 1 ml/sec delivered by automated injection, followed by a 20 ml flush of normal saline. T1 mapping was repeated at 30, 65, 120, 180, 240, 300, 900 and 1200 seconds post contrast, in a transverse slice at the T11 level used previously, and at 330, 630, 930 and 1230 seconds in the 3-point slice. The post contrast scan time was approximately 21 minutes.

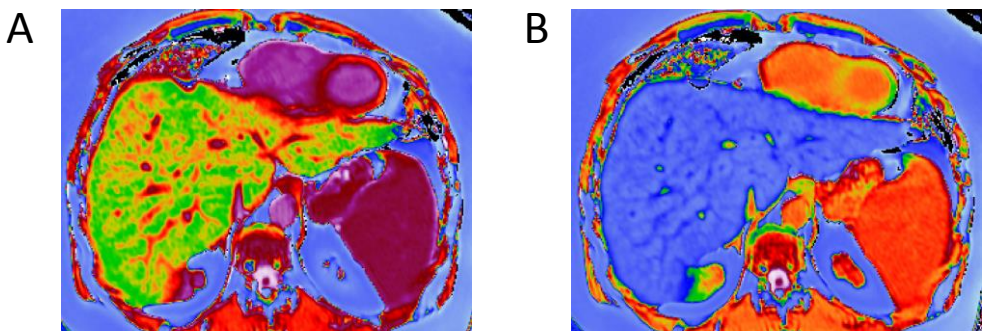


Figure 4. Examples of 3-point slice T1 maps incorporating the left ventricular blood pool, spleen and liver at baseline (A) and post contrast (B).

2.2.5.4 Volumetric interpolated breath hold examination

Three-dimensional volumetric interpolated breath hold examination (3D VIBE) (approximately 11 seconds) was used to image the whole liver and spleen for subsequent organ volume measurement. Voxel size 1.5x1.4x5.0 mm, FOV optimised for the patient; TR 3.31 ms; TE 1.19 ms; 12° FA, fat suppression and GRAPPA acceleration factor 2 were used. This scan sequence could be acquired pre or post contrast, but was acquired at approximately 7 minutes post contrast in most participants.

2.2.6 Varices, ascites and portal pressure results gathered from medical records

All varices and portal pressure assessments were performed on study patients as part of their clinical care. Assessments were performed by senior clinicians blinded to the study results.

OGD results were obtained from patients' medical records following completion of their study assessments. The timing of OGD, grade of varices and presence of red sign was recorded. OGD results were only used in the study if obtained within 1 year of study assessments.

Clinically-indicated imaging results were viewed from patient's medical records to confirm the presence or absence of ascites or non-oesophageal varices within 1 year of study assessments.

Medical records were also searched for the results of HVPG and PPG measurements and their timing. These results were only used for analysis if obtained within 2 months of study assessments and if there was no significant change in the patients' clinical status or portal pressure altering medications between assessments.

2.2.7 Patient follow up for clinical outcomes

All patients with cirrhosis that completed study assessments were followed up for the development of liver-related clinical outcomes. A 6 monthly review of hospital medical records and telephone consultation was conducted following enrolment for the development of a composite primary endpoint, namely liver-related death or new clinical liver event (including: variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular cancer and liver transplantation). Alcohol consumption since enrolment was also reviewed.

2.3 MRI results analysis

2.3.1 LiverMultiScan

LiverMultiScan (LMS) Discovery software version 4.1 (Perspectum Diagnostics, United Kingdom) was used to analyse maps of T1, T2* and PDFF in the same transverse slice (figure 3). LMS also uses the T1 and T2* maps to employ the iron correction algorithm (described in section 1.2.1.3) and produce a map of the iron-corrected T1 (cT1). Three regions of interest (ROI) of 1.5 cm^2 were drawn in the liver and spleen away from organ boundaries, matched on all maps. ROIs were positioned away from large blood vessels or bile ducts and the mean taken of these ROIs per organ. The quality of quantification was considered acceptable if the coefficient of determination (R^2) was ≥99% for T1 and ≥95% for T2*. Results outside these parameters were rejected. The mean of each organ's ROIs was calculated. LMS analysis was performed blind to the participant's clinical results.

2.3.2 Spleen extracellular volume fraction

The extracellular volume fraction (ECV) is the proportion of total tissue fluid outside of cells i.e. a combination of interstitial and intravascular fluid. It can be measured using T1 measurements pre and post contrast and the patient's haematocrit.

LMS was used to obtain the mean of 3 ROIs in the spleen and one ROI in the left ventricular blood pool on the 3-point T1 maps before (native) and after contrast (Gd). The ROIs were matched from the native to the post-contrast 3-point maps using their co-ordinates. Splenic ECV was calculated:

$$\text{Spleen ECV} = (1-\text{Haematocrit}) * \Delta R1_{spleen} / \Delta R1_{blood},$$

$$\text{where } \Delta R1 \text{ (relaxivity change)} = 1/T1_{native} - 1/T1_{Gd}$$

The ECV method used here has been adapted from cardiac use [135, 140] and unless stated otherwise, the reported spleen ECV result was calculated using the 1230 second (20.5 minute) post contrast scan.

2.3.3 Change in liver T1 with gadoxetic acid administration

The T11 vertebral level transverse T1 maps at baseline (T1native) and 1200 seconds (20 minutes) after gadoxetic acid administration (T1Gd) were used to assess the change in liver T1 with gadoxetic acid administration. The co-ordinates of the baseline 3 T1 liver ROIs were used to match the ROI placements on the 20 minute transverse slice using LMS. The percentage reduction in liver T1 with gadoxetic acid administration (%rT1) was calculated using the following formula [122]:

$$\text{Reduction in liver T1 with gadoxetic acid administration} = 100 \times (T1_{native} - T1_{Gd})/T1_{native}$$

2.3.4 Organ size measurements

Semi-automated segmentation was performed using open source ITK-SNAP software to calculate liver and spleen volumes. ITK-SNAP was also used to measure the maximum spleen diameter.

In summary, the MRI outcome measures were:

- Liver T1
- Liver T2*
- Liver cT1
- Liver proton density fat fraction (PDFF)
- Percentage reduction in liver T1 with gadoxetic acid administration (%rT1)
- Liver volume
- Spleen T1
- Spleen T2*
- Spleen cT1
- Spleen ECV
- Spleen volume
- Spleen diameter

2.4 Statistical analysis

Results were described using mean and standard deviation if normally distributed or median and interquartile range (IQR) if non-parametric. Normality was tested using the Shapiro-Wilk test. Differences between groups were assessed using Student's T test for parametric data and Kruskal

Wallis test for non-parametric data. In cases of multiple groups, analysis of variance (ANOVA) was used for parametric data and Kruskal Wallis for non-parametric data. Tukey's multiple comparison test was used to assess differences between group means with the calculation of adjusted p values. Associations between continuous variables were assessed using Pearson's correlation coefficient for linear, continuous variables or Spearman's correlation coefficient for non-linear or ordinal variables. The diagnostic accuracy of candidate biomarkers were assessed by area under the receiver operator curve (AUROC). Cut off values for candidate biomarkers were selected using the Youden index unless stated otherwise and the sensitivity and specificity described. Missing results were reported and excluded from analyses. In all analyses $p < 0.05$ was considered statistically significant. All statistical analysis was conducted using SPSS version 25 (IBM) and GraphPad Prism version 7.02 (GraphPad Software, USA).

2.4.1 Target sample sizes

Sample size calculations were performed to achieve 95% sensitivity with 95% confidence intervals 90 – 100% to identify portal hypertension and liver dysfunction. For this, 73 subjects were needed with and without portal hypertension and liver dysfunction. Estimating 50% prevalence of portal hypertension in cirrhosis [23] and to ensure an 80% chance of recruiting 73 confirmed cases of portal hypertension, the required sample size collectively for groups C and D (patients with cirrhosis) was 157. Specificity assessed using control groups required 20 subjects to achieve 95% specificity with 95% confidence intervals ~75 – 99%.

A target sample size of 20 TIPSS patients would allow a Pearson's correlation coefficient of 0.9 with 95% confidence intervals ~0.76-0.96 for spleen ECV with PPG. This would estimate the correlation with sufficient precision to monitor response to portal hypertension therapies and to

monitor disease progression.

The recruitment targets were therefore 20 healthy volunteers, 20 acute liver injury, 80 compensated cirrhosis, 20 decompensated cirrhosis with liver dysfunction only, 20 ascites, 20 hepatic encephalopathy (HE) and 20 planned TIPSS.

2.5 Validation of methods

The spleen ECV method used in this thesis is novel. Work was therefore undertaken to evaluate the validity of the spleen ECV method. Spleen ECV and other MR methods were also tested for their reproducibility across the two MR scanners used in this thesis and inter-observer repeatability of analysis.

2.5.1 Validation of the spleen ECV method

Although ECV is an established MR technique, it has rarely been applied to the spleen and to the best of my knowledge has not been measured using gadoxetic acid as the contrast agent. Five healthy subjects were therefore rescanned a median of 42 (range 31 – 56) days following their initial study assessment with gadobutrol as the contrast agent. Gadobutrol is used commonly in the assessment of cardiac ECV. Spleen ECV results achieved with the two contrast agents were similar, with 0.0157 (s.d. 0.0254) bias and 95% limits of agreement of -0.034 – 0.065 (figure 5).

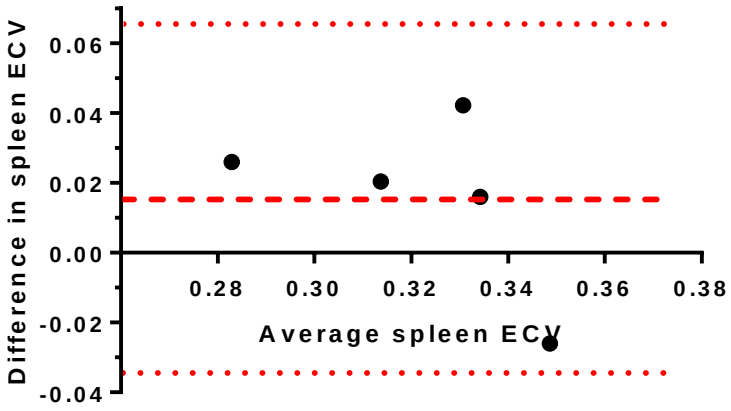


Figure 5. Bland Altman plot showing the reproducibility of spleen ECV measured using gadoxetic acid versus gadobutrol as the contrast agent in 5 healthy volunteers.

There was a 0.0157 (dashed red line) bias between contrast agents and 95% limits of agreement of -0.034 – 0.065 (red dotted line).

ECV measurement is only valid if the relative distribution of contrast agent between the intravascular and interstitial spaces reaches equilibrium. To assess for equilibrium, repeated spleen ECV measurements over time from 5.5 to 20.5 minutes were tested to deviate from a linear regression slope of 0 for each group using the F test. No groups had spleen ECVs that were significantly different from a slope of 0 and were therefore at equilibrium (table 3, figure 6).

Table 3. F test for the equilibrium of spleen ECV for each participant group.

There was no significant deviation in spleen ECV on repeated measures over time from a slope of zero.

	n	F	p
Healthy	24	1.672	0.120
Acute liver injury	7	1.193	0.282
Cirrhosis with normal liver function	66	1.103	0.295
Cirrhosis with liver dysfunction only	39	0.048	0.826
Cirrhosis with ascites	22	0.006	0.939
Cirrhosis with hepatic encephalopathy	11	0.087	0.769

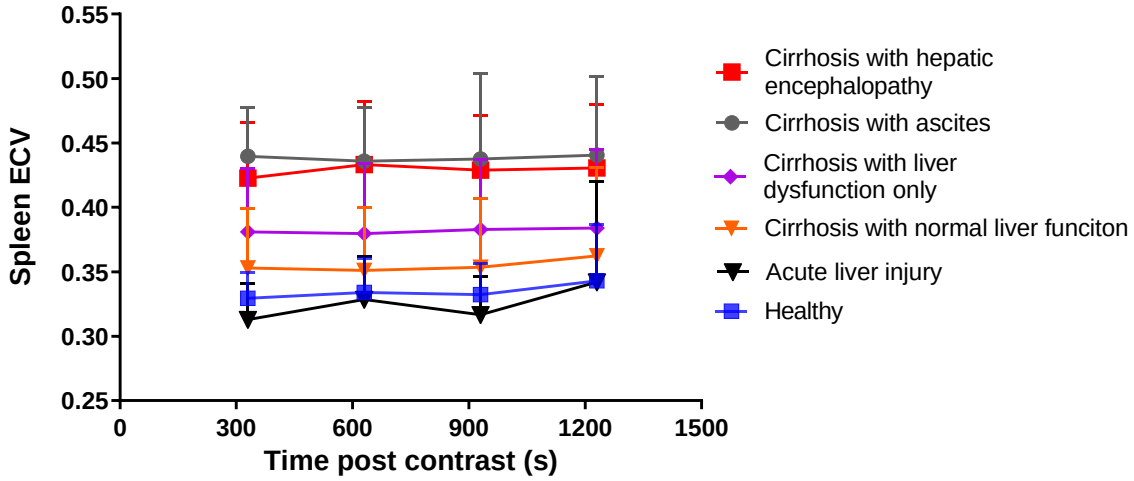


Figure 6. Spleen ECV over time in study participant groups.

Spleen ECV was measured at 330, 630 930 and 1230 seconds post contrast administration in healthy subjects (n = 24) and patients with acute liver injury (n = 7), cirrhosis with normal liver function (n = 66), cirrhosis with liver dysfunction only (n = 39), cirrhosis with ascites (n = 22) and cirrhosis with hepatic encephalopathy (n = 11). The lack of deviation in spleen ECV on repeated measures over time from a slope of zero indicates equilibrium had been reached. Shapes represent mean spleen ECV and error bars represent standard deviation.

2.5.2 Reproducibility of non-invasive MR markers across study sites

The repeatability of MR markers is important in the evaluation of a potential new biomarkers. As subjects were scanned using different MR scanners in Oxford and Southampton in the study, the reproducibility of MR measures was assessed using 5 healthy subjects scanned a median of 44 (IQR 16 – 58) days apart. The coefficients of variation were high for spleen ECV and spleen T2* (table 4). The 95% limits of agreement for spleen ECV, T1, T2*, cT1 and volume, %rT1 and liver T1, T2*, cT1 and volume were wide due to the low number of subjects assessed, however, none of the 95% limits of agreement excluded zero and so no evidence of significant biases between scanners were found. Results from Oxford and Southampton were therefore combined in all analyses in this thesis.

Table 4. Reproducibility of MR measures using healthy subjects (n = 5) repeat scanned in Oxford and Southampton

	Coefficient of variation (%)	Bias (standard deviation)	95% limits of agreement
Spleen ECV	18.48	-0.072 (0.076)	-0.220 – 0.076
Spleen T1 (ms)	3.75	35.0 (64.2)	-90.7 – 160.8
Spleen T2* (ms)	21.79	-3.3 (13.5)	-29.7 – 23.2
Spleen cT1 (ms)	3.20	-1.4 (63.2)	-125.2 – 122.4
Spleen volume (ml)	10.33	-24.3 (20.9)	-65.2 – 16.6
%rT1 (%)	3.39	-1.8 (2.7)	-7.1 – 3.5
Liver T1 (ms)	6.89	24.6 (73.4)	-119.3 – 168.6
Liver T2* (ms)	9.95	2.0 (2.4)	-2.7 – 6.6
Liver cT1 (ms)	6.50	-0.2 (72.6)	-142.6 – 142.1
Liver volume (ml)	3.63	52.4 (65.4)	-75.7 – 180.5

Abbreviations: ECV, extracellular volume fraction; %rT1, percentage reduction in liver T1 post gadoxetic acid administration

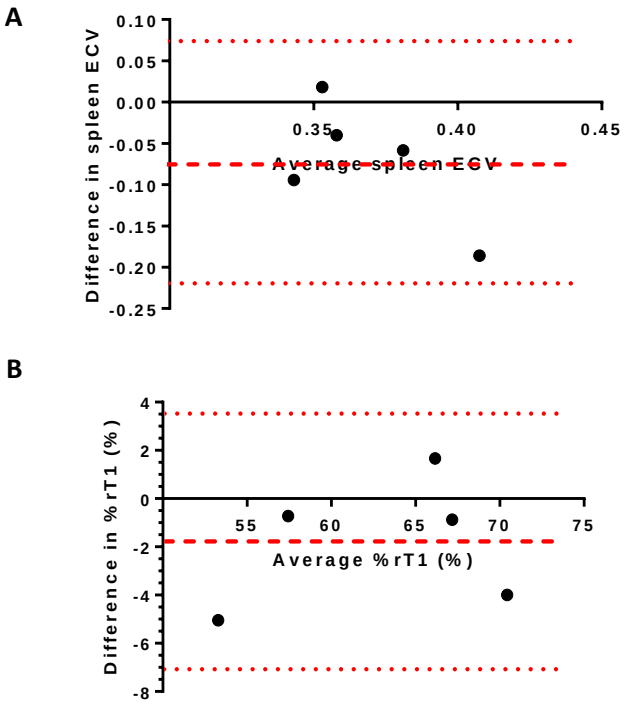


Figure 7. Bland Altman plot of spleen ECV (A) and %rT1 (B) repeated in Oxford and Southampton in 5 healthy subjects.

Red lines shown are the bias (dashes) and 95% limits of agreement (dots).

2.5.3 Inter-observer reproducibility of MR analysis

The inter-observer agreement of MR analysis for spleen ECV, spleen volume, liver volume and %rT1 were assessed using 11 subjects chosen by random number generation from all study participants. These comprised 2 healthy, 8 cirrhosis with normal liver function and 1 cirrhosis awaiting TIPSS subjects. Observer CKL had 3 years experience, observer AJ had 2 years experience and observer FEM had 4 years experience of MR analysis. CKL and AJ analysed spleen ECV and %rT1 and CKL and FEM analysed liver and spleen volumes. All analyses were performed blinded to the other observer's results and the patient's other study results. There were no significant biases between observers and the 95% limits of agreement were sufficiently narrow to detect likely

clinically significant differences in these measures (table 5, figure 8). All coefficients of variation were <4%.

Table 5. Inter-observer reproducibility of MR markers analysis between 2 observers (n = 11)

	Coefficient of variation (%)	Bias (s.d.)	95% limits of agreement
Spleen ECV	1.56	0.002 (0.007)	-0.012 – 0.017
%rT1 (%)	3.19	0.81 (2.46)	-4.01 – 5.64
Liver volume (ml)	3.98	-57.2 (85.7)	-225.2 – 110.8
Spleen volume (ml)	3.44	-10.3 (16.2)	-42.1 – 21.5

Abbreviations: ECV, extracellular volume fraction; %rT1, percentage reduction in liver T1 post gadoxetic acid administration

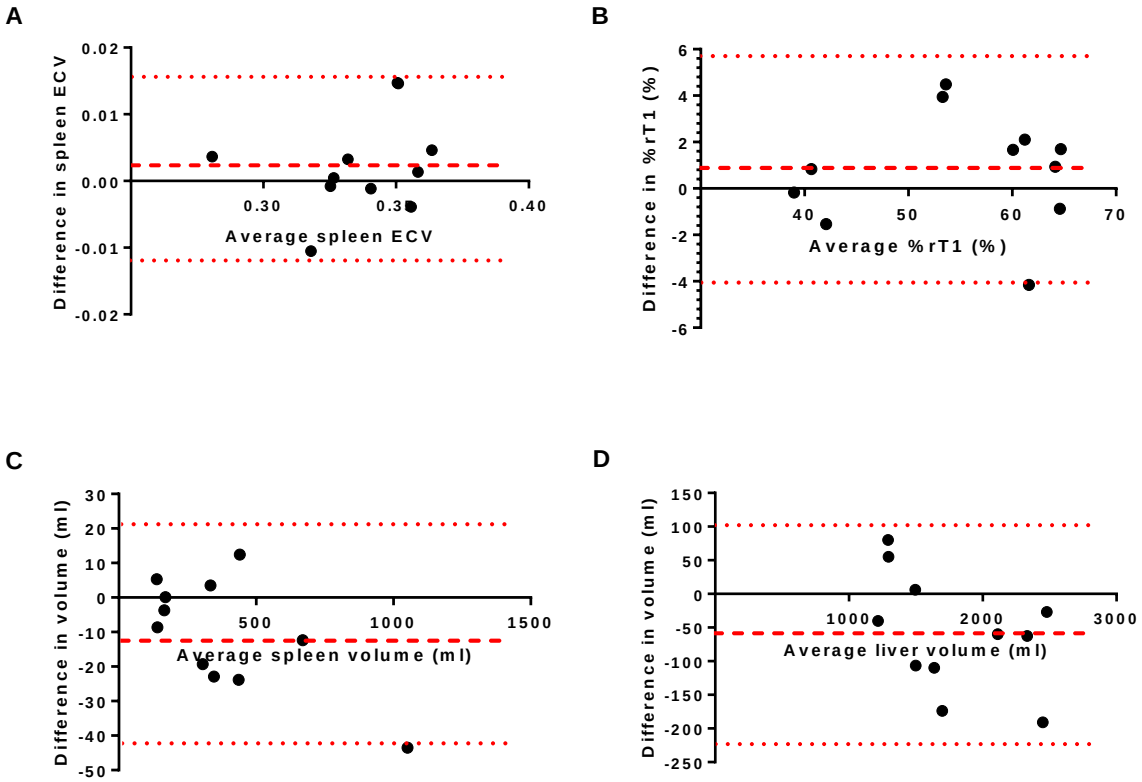


Figure 8. Bland Altman plots for the inter-observer agreement of spleen ECV (A), %rT1 (B), spleen volume (C) and liver volume (D) (n = 11).

Red lines shown are the bias (dashes) and 95% limits of agreement (dots).

3 Influence of the menstrual cycle on liver and spleen T1

3.1 Abstract

The magnetic resonance imaging (MRI) parameter liver cT1 is a non-invasive measure of liver fibrosis that has the potential to replace liver biopsy in clinical practice. Tissue cT1 largely reflects tissue's water content, which increases with fibrosis. Higher and more variable liver cT1 has been observed in young women, compared with males and older women, raising the possibility that it is affected by female hormones, which are known to affect fluid balance. This study explores whether liver and spleen cT1 in young women varies with menstrual stage and hormone levels.

Twelve healthy women aged 16-40 years with regular menstrual cycles and without hormone contraception were assessed 3 times within 1.5 menstrual cycles by MRI for liver and spleen cT1 and T1, liver stiffness measurement, bioelectrical impedance and blood tests. Body water content was estimated from the bioelectrical impedance.

Liver cT1 did not change between follicular and luteal phases (818.3±36.7 vs 805.8±36.4 ms, p = 0.152) or correlate with progesterone (r = 0.03) or oestradiol (r = 0.04) serum levels. Similarly, body water content was unchanged by menstrual phase or hormone levels, but did correlate with liver cT1 (r = 0.54, p = 0.001). Spleen T1 was higher in the follicular than luteal phase (1392±86 vs 1358±76 ms, p = 0.034) and was associated with ferritin (r = -0.486, p = 0.003) and progesterone (r = 0.354, p = 0.037), but not body water content (r = 0.250, p = 0.148).

This study indicates liver cT1 in young women is influenced by total body water content, but this is not dictated by the hormonal changes of the menstrual cycle. Spleen T1 is likely increased by the iron deficiency induced by menses in the follicular phase relative to the luteal phase.

3.2 Introduction

Liver cT1 has been proposed as a non-invasive marker of liver fibrosis, but the normal range of liver cT1 appears to be higher in young women compared with older women and men. At our institution, Pavlides measured the liver cT1 in a cohort of 42 healthy volunteers [131]. He found that the median liver cT1 of women under 52 years old (n = 21, 784 ms (IQR 755 - 871)) was significantly higher than that of men aged under 52 years (n = 10, 742 ms (IQR 703 – 755), p = 0.005) and women over 52 years (n = 7, 741 ms (IQR 726 - 754), p = 0.038). Similarly, Richards et al. observed the liver T1 of young women at 0.08 T has greater variability than that of men or older women, and liver T1 decreases with increasing age [132]. Myocardial T1 has also been found to be higher in women than men and reduces with age [141], so the underlying cause to these T1 differences may be systemic. This is important to know as it could affect spleen cT1 measurements, which have been proposed as a measure of portal hypertension [107].

Pavlides also found that liver cT1 was associated with haematocrit, inferior vena cava area and aorta area, all of which are influenced by intravascular fluid status [131]. A potential explanation for Pavlides' findings could be that the female hormones and menstrual cycle of pre-menopausal women affect total body fluid balance and therefore liver cT1. In support of this theory, Richards et al. also found that young women on the oral contraceptive pill, which raises female sex hormones to supra-physiological levels, have higher liver T1 during the dosed 3 weeks than undosed week on the pill, and that their liver T1 was higher than women without oral contraception [132].

Oestrogen and progesterone both increase extracellular fluid by increasing renal sodium retention. During the normal menstrual cycle, oestrogen usually peaks at the end of the follicular phase to trigger ovulation, whilst progesterone peaks during the luteal phase to prepare the

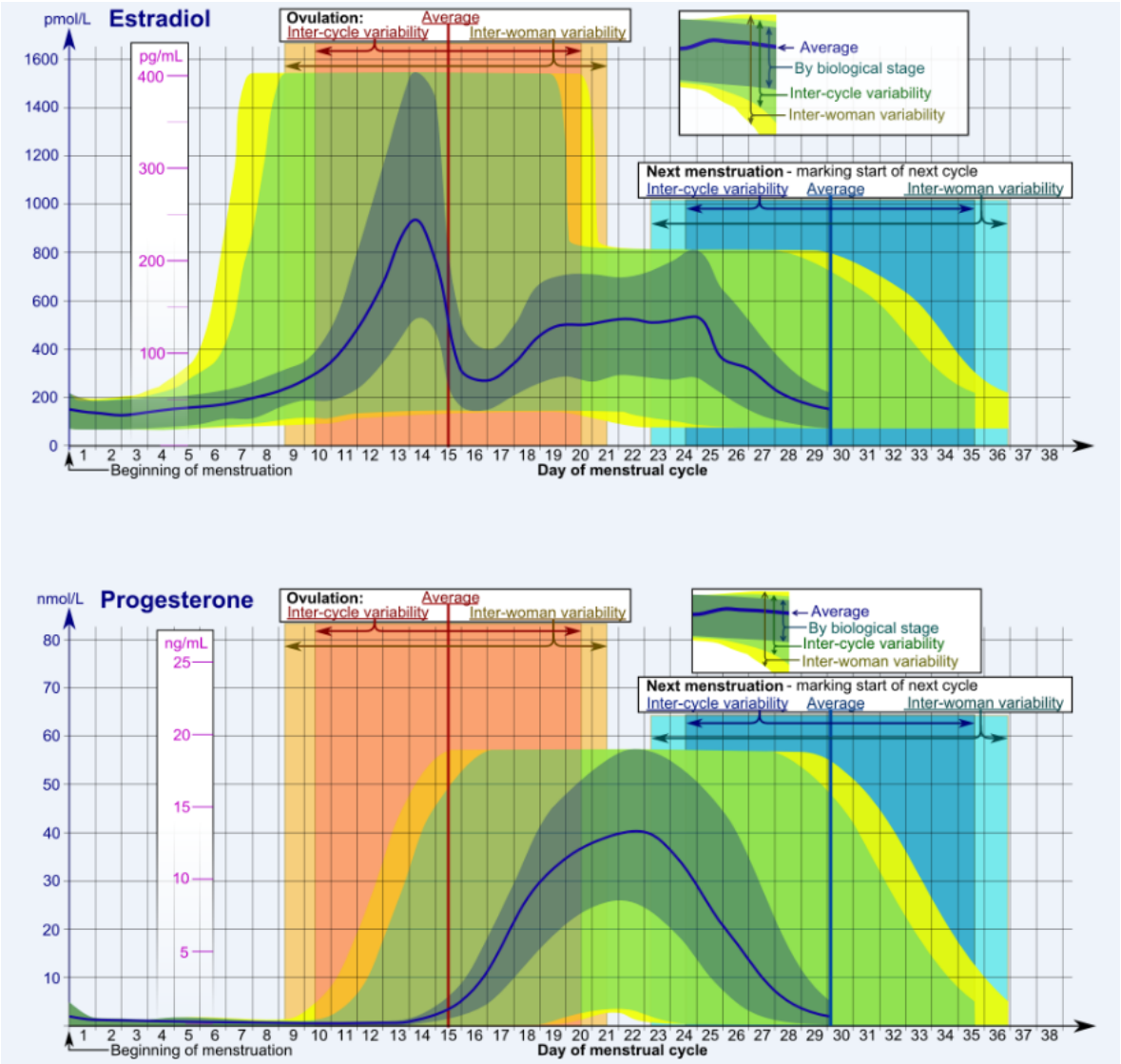


Figure 9. Hormone levels throughout the menstrual cycle.

Figure copied with permission from Stricker et al., 2006 [142].

uterus for pregnancy (figure 9 [142]). Fluid retention symptoms were found to peak on the first day of menses in a 1 year cohort study of healthy women [143]. Plasma volume tends to peak before ovulation when oestrogen is high and progesterone is low, and trough in the mid-luteal phase when progesterone peaks. Oestrogen increases plasma volume by increasing plasma oncotic pressure through a reduction in the transcapillary escape of albumin. In the mid-luteal phase, total circulating plasma proteins decrease, reducing plasma oncotic pressure and shifting

fluid from the intravascular to the interstitial compartment [144]. Correspondingly, a fall in extracellular water content from the menstrual to the pre-ovulatory phase has been observed in young women without oral contraception [145]. There may be little or no changes in total body water content throughout the menstrual cycle [145, 146].

3.3 Hypotheses

It may be that both oestrogen and progesterone cause young women to have higher total body fluid than men or older women and that the high levels of progesterone in the mid-late luteal phase shifts the balance of fluid away from the intravascular to the interstitial compartment (figure 10). This could be what is reflected in the liver cT1 observed by Pavlides et al.

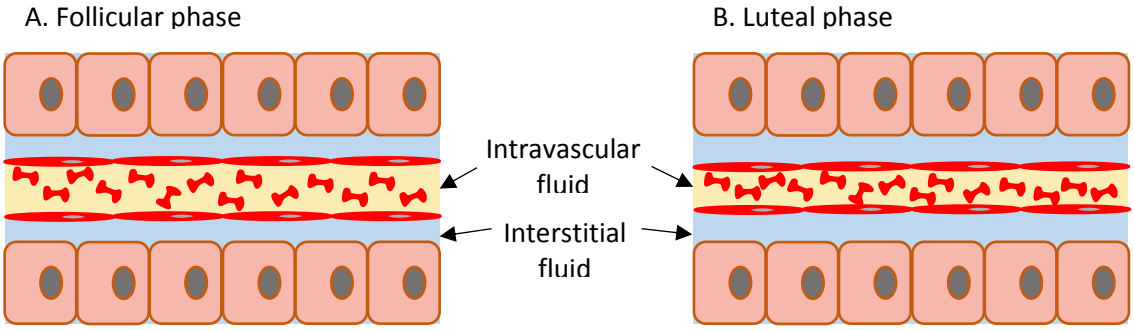


Figure 10. Theoretical changes in extracellular fluid distribution during the menstrual cycle.

A relative expansion of the intravascular fluid compartment may occur in the follicular phase (A), whilst a relative expansion of the interstitial fluid compartment may occur in the luteal phase (B).

3.4 Aims

The aims of this study chapter are to:

1. Observe changes in liver and spleen cT1 between the follicular and luteal phases of the menstrual cycle
2. Correlate liver and spleen cT1 with serum progesterone and oestradiol levels
3. Establish normal liver and spleen cT1 levels for menstruating young women

3.5 Methods

This was an observational cohort study conducted in OCMR in April 2016 – April 2017. The study received region ethics committee approval (13/SC/0243).

3.5.1 Participants and recruitment

Participants were recruited by poster and internet advertisement within the University of Oxford and Oxford University NHS Foundation Trust. Participant inclusion criteria were female; aged 16 – 40 years; regular menstrual cycle; healthy; BMI ≤25 kg/m^2; drink no more than 1-2 units of alcohol per day; and normal liver function tests. Exclusion criteria were contraindications to MRI and hormone contraceptive use (e.g. oral contraceptive pill, Mirena coil).

3.5.2 Study procedures

Participants were assessed 3 times throughout 1½ menstrual cycles to capture the follicular and luteal phases and return to baseline phase. Assessments included menstrual history, height and weight measurement, liver stiffness measurement, body composition estimation using BodyStat® 1500 (Bodystat Ltd., Isle of Man, United Kingdom), blood tests including oestradiol and progesterone levels and a multi-parametric MRI scan (3 Telsa Tim Trio, Siemens). Assessments were performed following a >4 hour fast at a similar time of day for repeat assessments.

3.5.3 Body composition estimation

Body composition estimation was performed using Bodystat® 1500 that measures bioimpedance. The examination was performed as per the manufacturer's instruction manual. Participants were free of metal objects and excess sweat/water and were fasted for >4 hours. They lay flat for 5 minutes without any body parts touching each other and paired electrodes were placed on their right hand and right foot. Anthropometric indices were entered into the Bodystat device and body composition was recorded once per subject. The primary variable of interest was total body water content (%).

3.5.4 Inferior vena cava area

The area of the inferior vena cava (IVC) was also used as a measure of total body fluid content. In states of intravascular depletion, the IVC appears collapsed, whereas it appears full in states of hydration or fluid overload. The IVC area was measured in a transverse slice at the level of T11 by

free-drawing around the edges of the IVC directly on the MR scanner or on open source ClearCanvas DICOM viewer software (Synaptive Medical) (Figure 5). The aorta area was also measured using the same software in the same slice so that an IVC to aorta ratio (IVC/Ao) could be calculated.

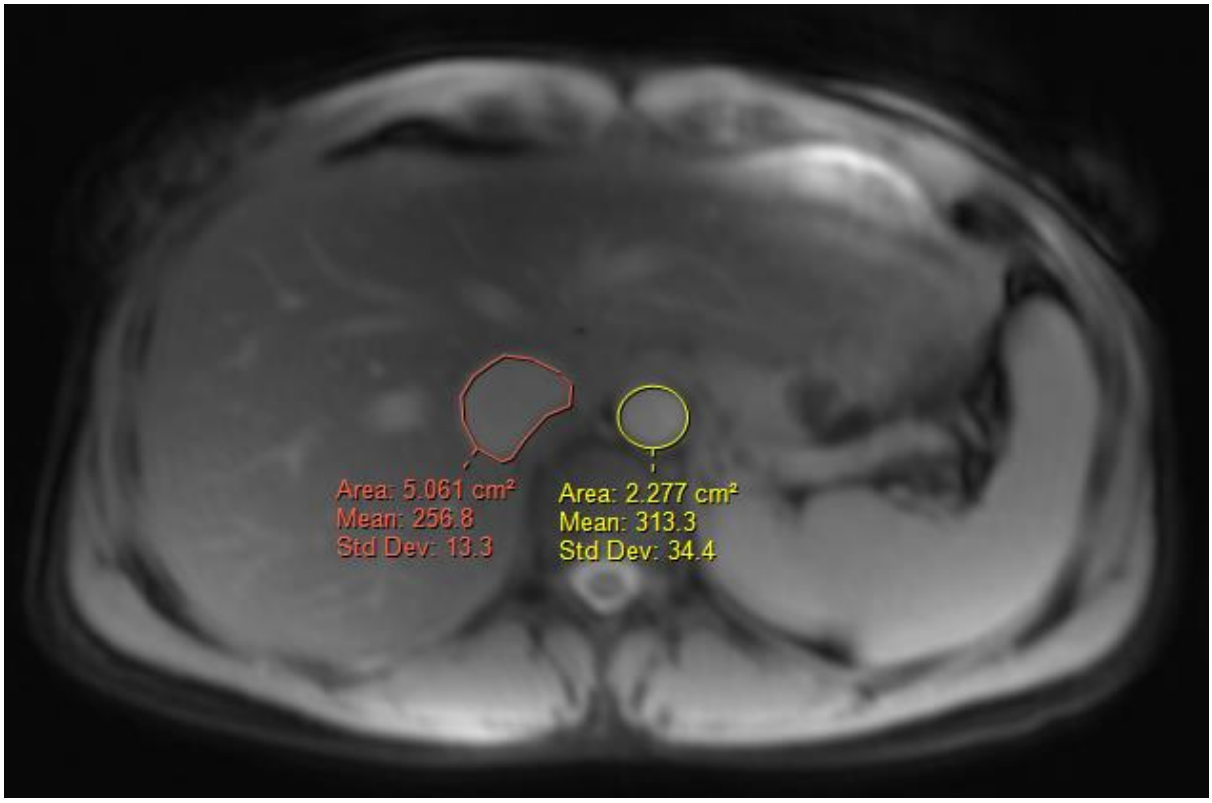


Figure 11. Example of inferior vena cava (orange) and aorta (yellow) area analysis performed using ClearCanvas DICOM viewer.

3.5.5 Sample size calculation

Pavlides et al. observed a ~40 ms liver cT1 difference between young and older women. I assumed that follicular phase women may be similar to the older women and that there was an

equal split between the follicular and luteal phases in Pavlides' cohort. I therefore anticipated that an 80 ms difference between the phases could be observed. A sample size of 12 was needed to observe an 80 ms difference between menstrual phases with 80% power and a 0.05 significance level.

3.5.6 Statistical methods

Results were described using mean and standard deviation if normally distributed or median and interquartile range (IQR) if non-parametric. Normality was tested using the Shapiro-Wilk test. To enable comparison between participants, individual's menstrual cycles were normalised to a 28 day cycle. Comparisons between assessments were made using paired Student's T test for parametric data and Wilcoxon rank test for non-parametric data. Liver cT1 was correlated with hormone levels and other parameters using Pearson's correlation coefficient. In all analyses $p < 0.05$ was considered statistically significant.

3.6 Results

Fourteen women were recruited. Two women were excluded due to loss to follow up (n = 1) and abnormal LFTs (n = 1), leaving 12 in the final analysis (figure 12). Median age was 27 (range 16 – 38) years, BMI 22.5 (IQR 20.8 – 23.3) kg/m^2 and all had normal LFTs (table 6). Eleven women (92%) started their assessments in the luteal phase and one (8%) in the follicular phase. The mean menstrual cycle length was 32±6 days.

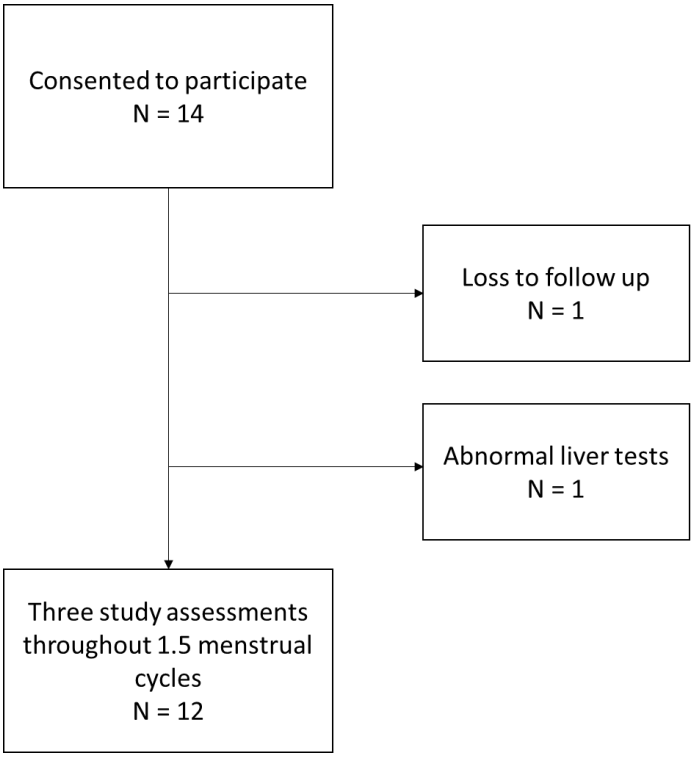


Figure 12. Flow diagram of study participation.

Table 6. Baseline characteristics

Demographics	
Age (years)	27(7)
BMI (kg/m^2)	22.5 (1.9)
Alcohol (units/week)	4.2 (3.0)
Length of menstrual cycle (days)	32 (6)
Blood markers	
Bilirubin (µmol/l)	9.8 (4.35)
ALT (IU/l)	12.5 (10.0 – 16.3)
AST (IU/l)	18.0 (15.5 – 21.8)
ALP (IU/l)	51.5 (42.8 – 61.8)
Albumin (g/l)	38.6 (2.8)
Ferritin (µg/l)	29.9 (13.9 – 57.6)
Transferrin saturation (%)	28.6 (13.0)
Iron (µg/l)	15.8 (6.4)
C reactive protein (mg/l)	0.5 (0.2 – 1.9)
Haemoglobin (g/dl)	129 (6)
Haematocrit (%)	0.388 (0.017)
White cell count (x10^9/l)	6.2 (2.0)
Platelets count (x10^9/l)	239 (48)
Prothrombin time (s)	11.4 (0.6)
Oestradiol (pmol/l)	479 (352)
Progesterone (nmol/l)	10.2 (1.0 – 39.6)
Non-invasive scores	
Liver stiffness (kPa)	5.0 (1.3)
Total body water (%)	52.5 (52.0 – 54.5)
IVC area (cm^2)	3.82 (1.29)
IVC/Ao	1.92 (0.43)
Liver T1	803 (38)
Liver T2*	24.6 (3.5)
Liver cT1	803 (38)
Liver fat fraction (%)	0.65 (0.3)
Spleen T1 (ms)	1347 (100)
Spleen T2* (ms)	42.2 (13.0)
Spleen cT1 (ms)	1347 (100)

The mean (standard deviation) for normally distributed results and median (interquartile range) for non-parametric results.
Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

3.6.1 Changes within the menstrual cycle

Baseline liver cT1 was 803±38 ms. There were no significant changes in liver cT1 between the follicular and luteal phases (mean 818.3±36.7 vs 805.8±36.4 ms, p = 0.152) (figure 13, table 7).

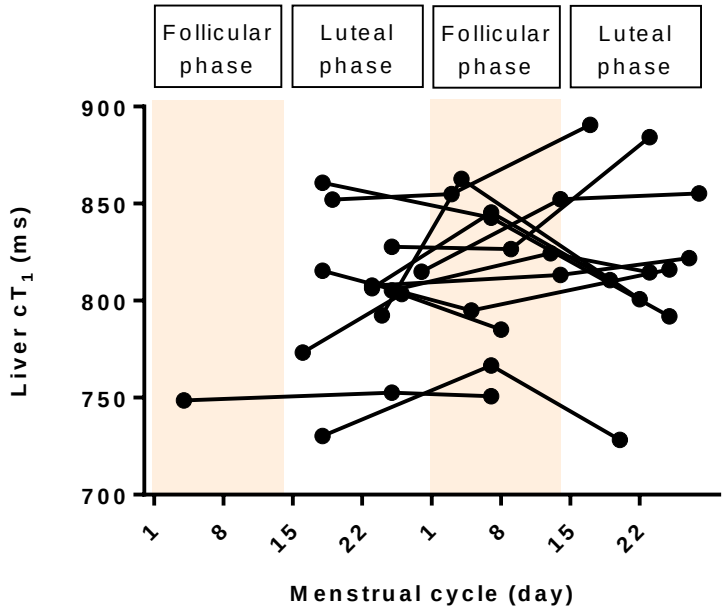


Figure 13. Liver cT1 throughout the menstrual cycle.

Twelve healthy females had liver cT1 measured in 3 sequential menstrual phases. Each line represents an individual participant, with each dot representing a liver cT1 measurement. Participants' menstrual cycles have been normalised to a 28 day cycle. The follicular phase is shaded and the luteal phase is unshaded.

Table 7. Change in parameters in different phases of the menstrual cycle

	Luteal phase N = 12	Follicular phase N = 12	p
Normalised day of cycle	23 (3)	8 (4)	<0.001
Weight (kg)	61.0 (7.4)	61.0 (7.5)	0.964
Blood markers			
Bilirubin (µmol/l)	9.8 (4.4)	9.6 (3.1)	0.910
ALT (IU/l)	13.5 (11.0 – 16.3)	14.0 (13.0 – 16.0)	0.283
AST (IU/l)	18.0 (15.5 – 21.8)	18.0 (16.8 – 22.0)	0.369
ALP (IU/l)	51.5 (42.0 – 61.8)	50.5 (43.5 – 61.5)	0.624
Albumin (g/l)	38.5 (2.7)	39.5 (3.1)	0.123
Ferritin (µg/l)	26.7 (13.4 – 65.5)	15.7 (9.7 – 63.3)	**0.041**
Transferrin saturation (%)	23.5 (16.8 – 36.5)	29.0 (16.6 – 35.0)	0.906
Iron (µg/l)	12.4 (10.3 – 17.7)	15.9 (9.3 – 19.4)	0.695
C reactive protein (mg/l)	0.5 (0.2 – 2.7)	0.5 (0.1 – 1.2)	0.581
Haemoglobin (g/dl)	129.7 (6.3)	129.3 (5.8)	0.782
Haematocrit (%)	0.387 (0.018)	0.392 (0.013)	0.304
Oestradiol (pmol/l)	400.0 (271.5 – 579.0)	184.5 (102.5 – 610.5)	0.433
Progesterone (nmol/l)	19.8 (2.9 – 41.1)	0.5 (0.5 – 0.8)	**0.005**
Non-invasive scores			
Liver stiffness (kPa)	5.0 (1.3)	4.8 (1.1)	0.6108
Total body water (%)	52.5 (52.0 – 54.1)	55.3 (51.2 – 61.7)	0.1763
IVC area (cm^2)	3.69 (1.46)	3.75 (1.70)	0.7353
IVC/Ao	1.87 (0.52)	1.94 (0.78)	0.560
Liver T1 (ms)	806.2 (36.5)	818.5 (36.7)	0.1614
Liver T2* (ms)	24.5 (3.5)	24.1 (4.6)	0.6389
Liver cT1 (ms)	805.8 (36.4)	818.3 (36.7)	0.1519
Liver fat fraction (%)	0.56 (0.37)	0.61 (0.47)	0.6372
Spleen T1 (ms)	1358 (76)	1392 (86)	**0.034**
Spleen T2* (ms)	36.1 (13.8)	35.3 (9.3)	0.864
Spleen cT1 (ms)	1358 (76.4)	1386 (83.5)	0.0618

Results shown are the mean (standard deviation) with the p value of a paired t test for normally distributed results and median (interquartile range) with paired the p value of a Wilcoxon rank test for non-parametric results.
Abbreviations: IVC, Inferior vena cava.

Total body water was also unchanged between follicular and luteal phases (median 55.3 (51.2 – 61.7)% vs 52.5 (52.0 – 54.1)%, p = 0.176). Similarly, other measures of fluid balance, including IVC

area, IVC/Ao and weight were unchanged. As expected, progesterone was significantly higher in the luteal than the follicular phase, but oestradiol was not significantly different between phases. There was a significant difference in spleen T1 between phases, but not spleen cT1. There was also a difference in ferritin between phases, but not transferrin saturation, iron or CRP.

Table 8. Comparison of markers over time at the first and last study assessments in the same menstrual phase.

	1st visit	3rd visit	Difference between 1st and 3rd visits p
Weight (kg)	60.9 (7.3)	61.0 (7.4)	0.486
Blood markers			
Albumin (g/l)	38.6 (2.8)	38.7 (2.3)	0.871
C reactive protein (mg/l)	0.5 (0.2 – 1.9)	0.5 (0.2 – 1.9)	0.624
Ferritin (µg/l)	29.9 (13.9 – 57.6)	18.5 (11.3 – 56.8)	**0.041**
Haemoglobin (g/dl)	130 (6)	128 (9)	0.341
Haematocrit (%)	0.390 (0.017)	0.383 (0.028)	0.345
Oestradiol (pmol/l)	479 (352)	431 (279)	0.672
Progesterone (nmol/l)	10.2 (1.0 – 39.6)	3.6 (0.6 – 39.2)	0.248
Non-invasive scores			
Liver stiffness (kPa)	5.0 (1.3)	4.7 (1.3)	0.228
Total body water (%)	54.1 (5.2)	55.9 (6.2)	0.369
IVC area (cm^2)	3.82 (1.29)	3.64 (1.54)	0.361
IVC /aorta ratio	1.92 (0.43)	2.02 (0.73)	0.505
Liver T1 (ms)	803 (38)	813 (48)	0.310
Liver T2* (ms)	24.6 (3.5)	24.1 (2.7)	0.597
Liver cT1 (ms)	803 (38)	813 (48)	0.304
Liver fat fraction (%)	0.65 (0.32)	0.53 (0.45)	0.254
Spleen T1 (ms)	1347 (100)	1379 (85)	0.099
Spleen T2* (ms)	42.2 (13.0)	34.6 (13.2)	0.075
Spleen cT1 (ms)	1347 (100)	1378 (84)	0.105

Results shown are the mean (standard deviation) with the p value of a paired t test for normally distributed results and median (interquartile range) with paired the p value of a Wilcoxon rank test for non-parametric results.
Abbreviations: IVC, Inferior vena cava.

To test that changes observed between the follicular and luteal phases were not attributable to progressive changes over time that were independent of the menstrual cycle, participants had repeat study assessments back in their baseline menstrual stage. There were no significant changes in MRI, hormone or fluid balance-related measures between participants' first and last visits, which were in the same menstrual phase (table 8). The only measured parameter to change significantly between the first and last study visits was the serum ferritin level, which decreased from median 29.9 to 18.5 µg/l (p = 0.041).

3.6.2 Correlations with liver cT1

When all results were analysed together (n = 36), liver cT1 did not correlate with either progesterone or oestradiol (table 9). Age also had no association with liver cT1. Liver cT1

Table 9. Correlation of liver cT1 with other factors.

	Pearson's correlation coefficient (r)	p
Age (years)	0.065	0.764
Weight (kg)	-0.187	0.276
Blood markers		
Albumin (g/l)	0.036	0.836
Ferritin (µg/l)	-0.257	0.130
Haemoglobin (g/dl)	-0.260	0.137
Haematocrit (%)	-0.179	0.312
Oestradiol (pmol/l)	0.038	0.824
Progesterone (nmol/l)	0.033	0.824
Non-invasive scores		
Liver stiffness (kPa)	0.232	0.173
Total body water (%)	0.544	**0.001**
IVC area (cm^2)	0.309	0.067
IVC /aorta ratio	0.201	0.241
MRI fat fraction (%)	0.151	0.378

increased with total body water content (r = 0.544, p = 0.001; figure 14) measured by bioelectrical impedance, however, other markers of fluid balance (including weight, IVC/Ao, haematocrit) did not reach statistically significant correlations with liver cT1, with IVC area just approaching significance (r = 0.309, p = 0.067).

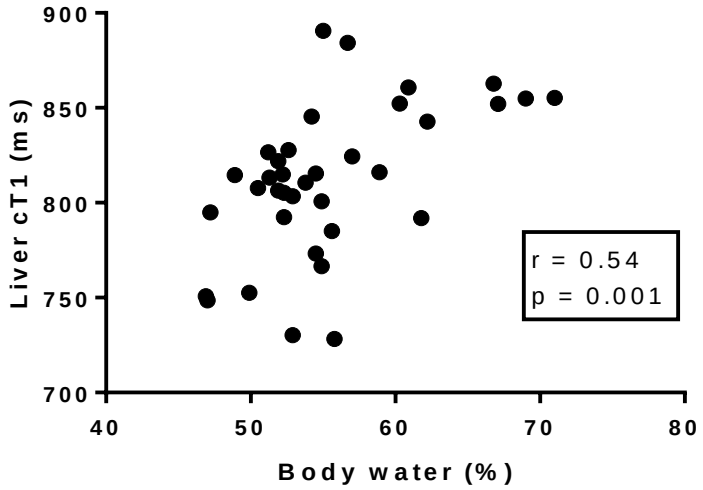


Figure 14. Correlation of liver cT1 with body water content as estimated by bioelectrical impedance (r = 0.544, p = 0.001).

Twelve healthy females were assessed 3 times each, represented by each dot.

3.6.3 Associations of spleen MRI parameters

The systemic effects of total fluid balance and hormones should affect liver and spleen T1 measures similarly. Given spleen T1 reduced from the follicular to the luteal phase, but liver cT1 did not, I analysed this further. Spleen T1 was correlated highly with spleen cT1 (r = 0.992, p <0.001), showing that only small changes were made to the measured T1 following iron correction. As spleen cT1 is of more clinical interest than spleen T1, both were analysed for

associations (table 10). Spleen cT1 tended to have stronger correlations with other variables than spleen T1. Spleen cT1 had a modest association with progesterone (r = 0.37, p = 0.027; figure 15), but not oestradiol. Although spleen cT1 correlated well with liver cT1 (r = 0.63, p <0.001), it did not correlate with total body fluid content (r = 0.27, p = 0.114).

Table 10. Correlation of spleen T1 and cT1 with other factors.

	Spleen T1 Pearson's correlation coefficient (r)	P	Spleen cT1 Pearson's correlation coefficient (r)	P
Weight (kg)	0.043	0.808	0.018	0.918
Blood markers				
Albumin (g/l)	-0.121	0.488	-0.147	0.398
Ferritin	-0.486	**0.003**	-0.478	**0.004**
Haemoglobin (g/dl)	-0.558	**0.001**	-0.559	**0.001**
Haematocrit (%)	-0.400	**0.021**	-0.408	**0.018**
Oestradiol (pmol/l)	0.053	0.762	0.070	0.689
Progesterone (nmol/l)	0.354	**0.037**	0.374	**0.027**
Non-invasive scores				
Liver cT1 (ms)	0.616	**<0.001**	0.625	**<0.001**
Liver stiffness (kPa)	0.000	1.000	-0.037	0.834
Spleen T2* (ms)	0.417	**0.013**	0.440	**0.008**
Total body water (%)	0.250	0.148	0.272	0.114
IVC area (cm^2)	0.182	0.296	0.188	0.278
IVC /aorta ratio	0.089	0.613	0.047	0.790

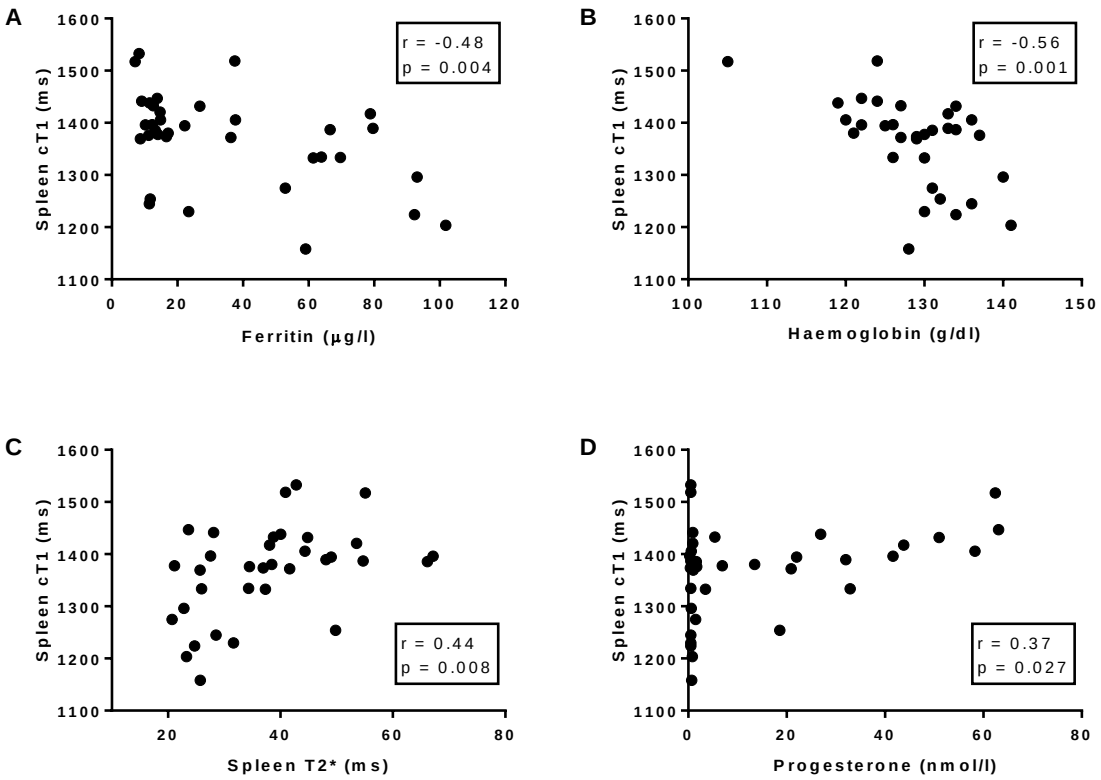


Figure 15. Spleen cT1 correlations with (A) ferritin (r = -0.478, p = 0.004), (B) haemoglobin (r = -0.56, p = 0.001), (C) spleen T2* (r = 0.44, p = 0.008) and (D) progesterone (r = 0.37, p = 0.027).

Twelve healthy females were assessed 3 times each, represented by each dot.

Both spleen T1 and cT1 correlated with blood-based markers of iron status. There were negative correlations of spleen cT1 with ferritin (r = -0.48, p = 0.004), haematocrit (r= -0.41, p = 0.018) and haemoglobin (r = -0.56, p = 0.001), indicating that increased splenic iron content from the blood pool, was reducing spleen cT1, despite the iron correction. This assertion was supported by a positive correlation between spleen cT1 and spleen T2* (r = 0.44, p = 0.008), which reflects splenic iron content inversely.

3.7 Discussion

This study did not demonstrate a difference in the liver cT1 of young women in the follicular versus luteal phase of their menstrual cycles. The results indicate that the follicular versus luteal menstrual phase are not responsible for the higher and more variable liver cT1 in young women. Estimates of total body water measured by bioimpedance correlated with liver cT1, suggesting variance in total fluid balance may explain the variance in liver cT1. Unlike Pavlides et al.'s previous work, there was no correlation of liver cT1 with IVC area or IVC/aorta ratio as measures of fluid status. The lack of correlation between body water and oestrogen or progesterone, indicates these hormones do not have a large influence on fluid balance.

The cohort in this study were assumed to have no liver fibrosis or inflammation. This assumption was supported by the lack of risk factors for liver disease, normal LFTs, normal liver synthetic function and normal liver stiffness. As demonstrated by Pavlides et al previously, the women in this study had high median liver cT1 (807 ms) relative to the previously reported values for healthy men (752 ms) and women aged >52 years (741 ms). Similarly, studies using other liver T1 measures report higher values in women than men [132, 147]. A cohort of 1037 individuals aged 40 - 69 years with BMI <25 and liver fat fraction <5% from the UK-Biobank showed median liver cT1 at 1.5 T for men (672 ms) and women (665 ms) with no statistically significant differences for age and sex in this age range [75]. These data support that young women specifically have physiologically different livers to other age and sex groups. In clinical practice liver cT1 in young women should therefore be interpreted with a slightly higher normal range than other adults. The association of liver cT1 with fibrosis should be studied in this population specifically in order to define diagnostic cut-off values.

In contrast to the liver, an increase in spleen T1 from the luteal (1358 ms) to the follicular phase (1392 ms) was observed, but no significant increase in spleen cT1. Spleen T1 and cT1 were not

associated with body water content, but they correlated negatively with blood-based iron markers (ferritin; haematocrit; haemoglobin) of which ferritin had a significant decrease between the luteal and follicular phases. These associations could be explained by menstrual blood loss in the early follicular phase, which cause a fall in ferritin and haemoglobin that corrects by the luteal phase. No changes in iron were reflected in spleen or liver T2* between menstrual phases, however, indicating our T2* method may not be able to detect these subtle changes. If T1 is being under-corrected for iron (present in ferritin and haemoglobin), the spleen may reflect these blood changes detectably because it is so vascular compared with the liver and other organs. The iron correction algorithm used for spleen cT1 was designed for the liver and so a spleen-specific iron correction algorithm should be sought.

Spleen T1 and cT1 also increased with progesterone, particularly in the luteal phase (figure 15). This association was not strong enough, however, to make spleen T1 higher in the luteal than the follicular phase. The effect of progesterone at the levels seen within the normal menstrual cycle are therefore unlikely to affect the interpretation of spleen cT1, whereas the normal range for spleen T1 and cT1 should be higher in the follicular than the luteal phase.

Of note, the median spleen cT1 (1379 (IQR 1241 - 1415) ms) in this cohort exceeded the diagnostic cut off for clinically significant portal hypertension (1348 ms) proposed previously [148] and the median spleen cT1 of heathy volunteers (1256.7, IQR 1181.6 – 1304.3 ms, n = 20, median age 56 years, 60% male) and patients with portal hypertension (1328.1, IQR 1256.9 – 1371.8 ms) described later in this thesis (section 4.6.3). Further exploration of the associations of spleen cT1 with iron deficiency, age and sex is warranted and existing interpretations of spleen cT1 should not be applied to women aged <40 years.

This chapter's results compliment the findings of Stachenfeld and Taylor, who also found little change in total body water content with changing hormone status [144] and Keevil et al. who observed no change in liver T1 or T2 throughout the menstrual cycle of 5 women imaged weekly

[149]. In muscle, a study of gastrocnemius T2 signal showed greater diurnal variation during the menstrual phase than other phases of the menstrual cycle when oestrogen, progesterone and aldosterone levels were at their lowest [150].

Our results do not support Richards et al.'s observed higher liver T1 at 0.008 T in women taking the oral contraceptive pill than those who did not [132]. The hormone dosing or serum levels were not stated in his paper, but progesterone +/- oestrogen were likely at supra-physiological levels. It may be that at these extreme levels, female sex hormones do exert a detectable effect on total body water content.

3.7.1 Limitations

Our study was powered to detect an 80 ms difference in liver T1 between the follicular and luteal phases of the menstrual cycle, which was not observed. A larger sample size may have enabled detection of smaller, perhaps more realistic changes.

Another limitation is that the participants were scanned at slightly different times within their menstrual cycle due to irregularities in their expected menstrual cycle and logistical issues for study visit timings. This made comparisons difficult. More frequent study visits would have overcome these issues, but affected the practicality of the study negatively.

3.7.2 Conclusions

In conclusion no significant differences in liver cT1 across the menstrual cycle of healthy young women have been demonstrated. The relatively high liver cT1 in this groups is related to body water content, independent of oestradiol and progesterone levels. Spleen T1 is lower in the luteal

than the follicular phase, most likely due to iron loss during menses. Normal ranges for liver and spleen cT1 are different in young menstruating females than in males and older women.

4 Assessment of portal hypertension and oesophageal varices

4.1 Abstract

There is an unmet need for non-invasive markers of portal hypertension and to identify more patients who can safely avoid oesophagogastroduodenoscopy (OGD) screening for oesophageal varices needing treatment (VNTs) than is achievable currently with Baveno VI criteria. Splenic venous congestion, hyperplasia and fibrosis in portal hypertension may be detectable with magnetic resonance imaging (MRI) to provide markers of portal hypertension and VNTs.

Spleen extracellular volume fraction (ECV), T1, cT1, volume and diameter, and the platelet/spleen volume ratio were measured in 20 healthy subjects and 122 subjects with cirrhosis using 3T MRI with gadoxetic acid administration and blood tests, and assessed against clinically indicated OGD, portal pressure and imaging results obtained within 1 year of MRI. Diagnostic accuracy was assessed by area under the receiver operator curve (AUROC) for the identification of portal hypertension and VNTs. Correlations of portal pressure measurements (n = 7) with candidate markers of portal pressure were assessed visually. New and established criteria to identify patients with compensated chronic advanced liver disease (n = 91) able to avoid OGD screening for VNTs were evaluated.

Both spleen ECV and platelet/spleen volume ratio had 0.79 AUROC for the identification of portal hypertension, but no visual correlation with portal pressure (n = 7). A combination of spleen T1 and spleen diameter identified VNTs with the highest accuracy (AUROC 0.87) of all markers tested and could save 66% of screening OGDs without missing any VNTs – 3.3-fold more than Baveno VI criteria.

In conclusion, spleen ECV can identify clinically apparent portal hypertension with good accuracy, but was outperformed by spleen T1 and spleen diameter criteria for the identification of VNTs in patients with cirrhosis and those who can avoid OGD screening for VNTs.

4.2 Introduction

Portal hypertension causes many of the complications of liver disease including oesophageal varices, ascites and hepatic encephalopathy. The hepatic venous pressure gradient (HVPG) is the gold standard measure of portal pressure, which correlates well with direct portal pressure gradients (PPG) [17]. HVPG can be used to predict complications of liver disease [15, 16], but HVPG's invasiveness, cost and expertise required for measurement mean that non-invasive alternatives are needed. This chapter explores MRI-based non-invasive markers of portal hypertension. Such markers should be able to identify patients with and without portal hypertension and correlate with invasive portal pressure measurements.

An ability of non-invasive markers to reflect rapid changes in portal pressure would be useful to monitor response to portal hypertension therapies in both clinical trials and clinical practice. Patients having transjugular intrahepatic portosystemic shunt (TIPSS) placement have PPG measured during their procedure, capturing the fall in portal pressure that the TIPSS create. These patients will be used in this chapter to correlate MRI with invasive portal pressure measurements and test the ability of MRI measures to reflect changes in portal pressure.

MRI measures of portal hypertension will also be applied for the detection and exclusion of oesophageal varices needing treatment (VNTs). VNTs are grade II or III varices or grade I varices with red sign that have high risk of life-threatening bleeding and so require prophylactic β-blockers or variceal band ligation. In patients with compensated advanced chronic liver disease

(cACLD, which includes compensated cirrhosis), the prevalence of VNTs is just 5-17% [110, 112], yet patients are screened for VNTs 2-3 yearly by oesophagogastroduodenoscopy (OGD), an invasive procedure. To limit unnecessary OGDs, the Baveno VI consensus recommends patients with cACLD can avoid OGD if they have platelets >150x10^9/l and liver stiffness <20 kPa [96]. Although this avoids 10-34% of all screening OGDs performed in cACLD [110, 111], a large proportion of unnecessary OGDs are still performed.

The Baveno VI consensus also suggested that <5% of VNTs should be missed if OGD screening is avoided, but they did not state whether the 5% refers to the population with VNTs, the population meeting their OGD avoidance criteria or the cACLD population to which the criteria may be applied. A subsequent analysis of these different interpretations suggests 5% missed VNTs of all VNTs (1-sensitivity) is the most significant interpretation to understand the criteria's performance across studies, whilst 5% missed VNTs of all patients meeting criteria to avoid OGD (1- negative predictive value) is the most relevant clinically [114]. The Baveno VI criteria were found in a meta-analysis to have a pooled risk of 4% missed VNTs in all patients meeting the criteria, which equates to missing 7% of all VNTs [151]. This leaves 2% more VNTs undetected than the most conservative interpretation of the Baveno VI consensus' recommended miss rate.

Newer criteria have been sought to avoid more OGDs than Baveno VI criteria, whilst missing <5% of all VNTs. The expanded Baveno criteria proposed by Augustin et al. classifies cACLD patients with platelets >110 x10^9/l and liver stiffness <25 kPa at low risk of VNTs. These criteria have been tested in multiple cohorts and found to avoid 32-58% of OGDs [110, 111, 113], but missed 6.8% of all VNTs in an Asian validation cohort [113] and 17.7% of all VNTs in a NAFLD cirrhosis cohort [111, 114]. The addition of MELD=6 to the Baveno VI criteria (Baveno/MELD=6) avoids 28-38% of OGDs with <5% of VNTs missed [112] and Abraldes at al. have suggested a liver stiffness and platelet count/spleen diameter ratio score (LSPS) <1.33 would miss 5% of VNTs, although it is unclear whether the 5% is of all VNTs, all patients with LSPS <1.33 or all patients in the cohort.

Pertinent to centres without Fibroscan, the criteria platelets >150x10^9 cells/L and MELD=6 proposed by Jangouk et al. avoids 30-54% of OGDs [112]. MRI may be able to improve on these criteria.

In portal hypertension, splenic venous congestion and fibrosis increase splenic water content and splenic hyperplasia also increases spleen size. MRI may be a useful tool for measuring these changes non-invasively. A pilot study indicated spleen cT1, which reflects whole tissue water content, correlates well with HVPG [148]. Further exploration of spleen T1-based measures is therefore warranted. Extracellular volume fraction (ECV) is more specific than T1 for venous congestion and fibrosis as it excludes the intracellular fluid volume, so spleen ECV will be the primary candidate marker of portal hypertension tested here.

Platelet count/spleen diameter ratio is one of the most established non-invasive markers of oesophageal varices of any size in patients with compensated cirrhosis [99]. Calculation of spleen volume is possible on cross-sectional imaging such as MRI or CT and so may improve the performance of platelet count/spleen ratio as a marker of oesophageal varices and portal hypertension. I therefore propose that platelet count to spleen volume ratio (platelet/spleen volume ratio) may be an accurate marker of portal hypertension and identifier of VNTs.

Spleen T1, cT1, ECV and size measurements can be incorporated into multiparametric MRI with gadoxetic acid contrast, which could also be used to assess liver function, fibrosis, fat and iron and liver lesions also. This would enable a comprehensive non-invasive assessment of liver disease.

4.3 Hypotheses

Portal hypertension increases splenic extracellular water content and spleen size, which can be measured using multiparametric MRI with gadoxetic acid administration. These splenic changes are sensitive to changes in portal pressure.

MRI-derived measures of portal hypertension can be used to identify varices needing treatment in patients with compensated advanced chronic liver disease. MRI-derived measures of portal hypertension can also identify a large proportion of patients with compensated advanced chronic liver disease who can avoid oesophagogastroduodenoscopy screening for varices, whilst missing <5% of varices needing treatment.

4.4 Aims

This chapter aims to use multiparametric MRI with gadoxetic acid administration to:

- differentiate patients with and without portal hypertension
- quantify portal pressure by correlating candidate markers against portal pressure measurements
- reflect dynamic changes in portal pressure in patients receiving a transjugular portosystemic shunt (TIPSS)
- identify the presence of varices needing treatment in patients with compensated advanced chronic liver disease
- exclude varices needing treatment in patients with compensated advanced chronic liver disease

The principle MR measure is spleen ECV, with secondary measures being spleen T1, spleen cT1, spleen volume and platelet/spleen volume ratio.

4.5 Methods

This chapter forms part of the MAPLE study and uses methods described in chapter 2. Within this chapter portal hypertension is defined as the presence of varices of any grade, ascites or HVPG >5 mmHg.

4.5.1 Participants

All subject groups were used, except acute liver injury patients. Subjects were grouped into healthy subjects, cirrhosis without portal hypertension and cirrhosis with portal hypertension. Healthy subjects were assumed to have no significant portal hypertension and no oesophageal varices.

Patients with cirrhosis with normal liver function and cirrhosis with liver dysfunction only who had not had variceal bleeding, jaundice, ascites, hepatic encephalopathy or ascites were combined into a group termed compensated advanced chronic liver disease (cACLD). This is the group to which the Baveno VI consensus recommendations for safe avoidance of OGD screening for varices apply [96].

Subjects were excluded from analysis if they did not have a spleen ECV measurement using the 3 point slice (figure 3), which was only adopted part way through the MAPLE study and if patients with cACLD did not have an OGD within 1 year of study assessments.

4.5.2 Study assessments

Most participants had one study visit. The TIPSS group had their study assessments within 4 weeks prior to TIPSS and repeated within 4 weeks following TIPSS with at least 7 days between assessments to ensure full washout of contrast.

Subjects were assessed by clinical history, physical examination, anthropometrics, blood tests, liver stiffness measurement and MRI scan with gadoxetic acid as detailed in the general methods chapter 2.

OGDs and portal pressure measurements were performed blinded to the study results as part of the patients' clinical care. The patients' clinical records were searched for oesophageal varices grade and presence of red sign, and portal pressure (HVPG or PPG) measurements following the completion of study assessments. VNTs were grade ≥II or grade I with red sign. Portal pressures were obtained in Oxford and Southampton using a straight catheter method and in Birmingham using a balloon catheter method.

4.5.3 Calculation of non-invasive markers of portal hypertension

Spleen ECV was the principle test variable and was calculated as follows:

$$ECV = (1\text{-}Haematocrit) * \Delta R1spleen / \Delta R1blood,$$

$$where\ \Delta R1\ (relaxivity\ change) = 1/T1native - 1/T1post\ gadoxetic\ acid$$

Additional candidate markers of portal hypertension were platelet/spleen volume ratio and platelet/spleen diameter ratio. Their calculations were:

Platelet/spleen diameter ratio = platelets (x10^9/l) / spleen volume (mm)

Platelet/spleen volume ratio = platelets (x10^9/l) / spleen volume (ml)

4.5.4 Application of criteria to exclude VNTs

Established Baveno (platelets >150 x10^9/l and liver stiffness <20 kPa) [96], extended Baveno (platelets >110 x10^9/l and liver stiffness <25 kPa) [110], MELD platelet (platelets >150x10^9 cells/L and MELD=6) [112] and liver stiffness and platelet count/spleen diameter ratio score (LSPS) criteria (liver stiffness (kPa) x spleen diameter (cm)/platelets (x10^9/l) <1.33) [152] were applied and tested in cACLD patients for the exclusion of VNT. New criteria were explored using MR and other variables to miss <5% of patients with VNTs (>95% sensitivity), whilst avoiding OGDs in the maximum number of patients.

4.5.5 Statistical methods

Differences between groups were tested using unpaired t test for parametric results and Kruskal Wallis test for non-parametric results. Healthy and cirrhosis without portal hypertension subjects were compared for variables associated with cirrhosis, and cirrhosis with and without portal hypertension subjects were compared for markers of portal hypertension. Variables in cACLD with and without VNT were compared. The performance of markers with significant differences between groups were assessed using area under the receiver operator curve (AUROC) for the identification of portal hypertension and VNTs. Cut off values were chosen using the Youden index and sensitivity and specificity reported.

Associations between candidate markers of portal hypertension and HVPG or PPG measurements were assessed visually. The percentage change in PPG and candidate markers of portal hypertension following TIPSS was calculated and their associations assessed visually.

The percentage patients fulfilling VNT exclusion criteria (potential OGDs avoided), missed VNTs within patients fulfilling criteria (1 – negative predictive value (NPV)) and missed VNTs of all patients with VNTs (1 – sensitivity) were calculated. These analyses were performed per protocol (i.e. on patients with successful application of criteria) rather than on an intention to diagnose basis given the inclusion and exclusion criteria selected for patients able to have the study MRI.

4.6 Results

4.6.1 Participants

One hundred and seventy-two subjects consented to the study. Participants were excluded due to no left ventricular blood pool T1 obtained for ECV measurement due to a methods change early in the study (n = 10), inability to calculate spleen ECV from the MR data obtained (n = 3), inability to tolerate the MRI (n = 6), withdrawal of consent for venous cannulation (n = 1), insufficient fast duration (n = 1), a healthy volunteer being diagnosed with hepatitis B (n = 1), misdiagnosis of cirrhosis (n = 1), and OGD not performed within 1 year of study visit for participants with cirrhosis (n = 7) (figure 16). This left 142 study participants included for analysis comprising 20 healthy volunteers, 59 patients with cirrhosis with normal liver function, 34 patients with cirrhosis with liver dysfunction only, 18 patients with cirrhosis with ascites, 11 patients with cirrhosis with hepatic encephalopathy and 4 patients with cirrhosis referred for TIPSS.

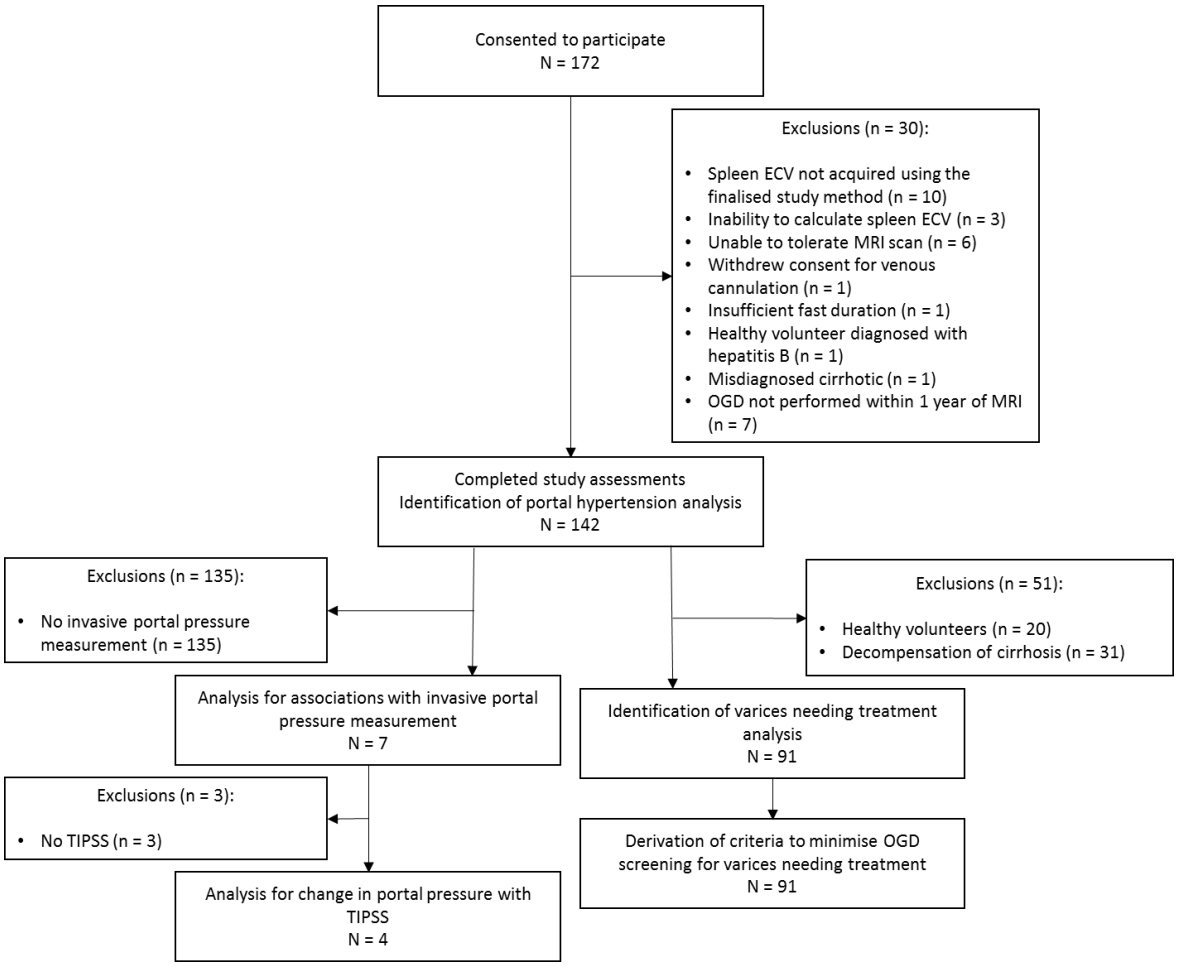


Figure 16. Flow diagram of participants in the portal hypertension study.

Median interval between MRI and OGD for patients was 31 (IQR 12 – 93) days. Patients were classified as having portal hypertension due to HVPG or PPG >5 mmHg (n = 7), varices (n = 68) and ascites (n = 27), with some patients having more than one factor. In total, 73 patients had portal hypertension and 49 did not, meaning the cirrhosis without portal hypertension group fell 23 subjects short of the target recruitment. Under-recruitment of patients awaiting TIPSS (4/20) meant aims to correlate candidate portal pressure measures against PPG and assess dynamic changes in portal pressure could not be addressed.

Healthy volunteers had median age 56 years, mean BMI 22.9 kg/m^2 and 60% were male (table 11). All of their blood parameters were within the normal range.

Both cirrhosis groups with and without portal hypertension had median age 61 years and similar BMI and alcohol consumptions. The portal hypertension group tended to have a higher proportion of males, alcohol-related liver disease (ArLD) and NAFLD than those without portal hypertension. Liver function-related blood tests and scores and liver fibrosis non-invasive scores tended to be higher with than without portal hypertension.

Table 11. Baseline characteristics by participant groups used in the analysis of portal hypertension

	Healthy N = 20	Cirrhosis without portal hypertension N = 49	Cirrhosis with portal hypertension N = 73
Demographics			
Age (years)	56 (50 – 66)	61 (54 – 65)	61 (54 – 70)
Male (%)	12 (60%)	30 (61%)	57 (78%)
BMI (kg/m^2)	22.9 (2.0)	29.8 (5.9)	28.7 (5.7)
Alcohol (units/week)	6 (3 – 12)	0 (0 – 5)	0 (0 – 1)
Aetiology			
ArLD	-	20 (41%)	36 (49%)
NAFLD	-	11 (22%)	18 (25%)
Viral	-	10 (20%)	11 (15%)
Autoimmune	-	10 (20%)	5 (7%)
Other	-	4 (8%)	6 (8%)
Liver-related parameters			
Bilirubin (µmol/l)	10.0 (8 – 14)	11 (8 – 17)	21 (15 – 31)
Albumin (g/l)	39 (37 – 41)	37 (35 – 39)	35 (30 – 38)
Prothrombin time (s)	11.2 (10.9 – 11.6)	11.4 (11.0 – 12.1)	12.8 (12.1 – 13.6)
Child Pugh score	5 (5 – 5)	5 (5 – 6)	6 (5 – 8)

Results shown are median (interquartile range) if non-parametric, mean (standard deviation) if normally distributed or count (percentage).
Abbreviations: BMI, body mass index; ArLD, alcohol-related liver disease; NAFLD, non-alcohol-related fatty liver disease.

4.6.2 Success of non-invasive measurements

Liver stiffness was measured successfully in 119 (84%) patients, of which the XL probe was used for 23 (19%). Unreliable liver stiffness measurements (n =23) occurred in 14% of cirrhosis patients without portal hypertension and 22% with portal hypertension.

Liver T2* and so cT1 were unreliable in 1 (2%) patient with cirrhosis without portal hypertension. Spleen T2* and therefore spleen cT1 was unreliable in 3 (6%) of cirrhosis without portal hypertension and 2 (3%) of cirrhosis with portal hypertension.

Spleen volume and so platelet/spleen volume ratio could not be measured in 1 patient with portal hypertension due to an MR scanner fault causing VIBE sequence failure.

4.6.3 Non-invasive markers in subjects with and without portal hypertension

Median spleen ECV was higher in patients with than without portal hypertension (0.402 (IQR 0.364 – 0.457) vs 0.343 (IQR 0.319 – 0.368), $p<0.001$) (table 12, figure 17). Significant differences between those with and without portal hypertension also existed for all other spleen-related markers (platelets, spleen T1, spleen cT1, spleen volume, spleen diameter, platelet/spleen volume and platelet/spleen diameter) ($p <0.001$ for all) and liver-related markers (MELD, AST/ALT, APRI, FIB-4, liver T1, liver cT1 and liver stiffness).

Table 12. Comparison of subject groups with and without portal hypertension

	Healthy N = 20	Cirrhosis without portal hypertension N = 49	Cirrhosis with portal hypertension N = 73	Difference between healthy and cirrhosis without portal hypertension P value	Difference between cirrhosis with and without portal hypertension P value
Candidate MR markers					
Spleen ECV	0.338 (0.324 – 0.352)	0.343 (0.319 – 0.368)	0.402 (0.364 – 0.457)	0.302	<0.001
Spleen T1 (ms)	1307.3 (1206.2 – 1362.7)	1294.5 (1213.8 – 1328.5)	1358.1 (1284.9 – 1416.3)	0.475	<0.001
Spleen cT1 (ms)	1256.7 (1181.6 – 1304.3)	1236.8 (1174.0 – 1291.8)	1328.1 (1256.9 – 1371.8)	0.580	<0.001
Spleen volume (ml)	174 (128 – 235)	400.7 (288.7 – 548.2)	679.5 (486.7 – 949.0)	<0.001	<0.001
Platelet/spleen volume	1.29 (1.06 – 2.01)	0.40 (0.27 – 0.60)	0.17 (0.09 – 0.30)	<0.001	<0.001
Other markers					
Platelets count ($x10^9$/l)	247 (220 – 279)	155 (139 – 218)	113 (92 – 151)	<0.001	<0.001
Spleen diameter (mm)	106 (98 – 115)	133 (121 – 172)	157 (140 – 175)	<0.001	<0.001
Platelet/spleen diameter	2.40 (1.92 – 2.72)	1.13 (0.89 – 1.70)	0.69 (0.51 – 1.08)	<0.001	<0.001
MELD	7 (6 - 7)	7 (6 – 7)	9 (8 – 11)	0.097	<0.001
AST/ALT	1.48 (1.12 – 1.67)	1.10 (0.93 – 1.27)	1.50 (1.18 – 1.91)	0.003	<0.001
APRI	0.23 (0.18 – 0.26)	0.50 (0.37 – 0.76)	0.91 (0.59 – 1.55)	<0.001	<0.001
FIB-4	1.27 (1.03 – 1.68)	2.24 (1.81 – 3.17)	4.17 (3.17 – 6.65)	<0.001	<0.001
Liver cT1 (ms)	693.9 (636.4 – 755.7)	823.8 (780.6 – 922.4)	892.2 (814.3 – 979.1)	<0.001	0.009
Liver stiffness (kPa)	4.5 (3.4 – 5.2)	20.1 (13.5 – 33.9)	25.7 (17.5 – 59.3)	<0.001	0.029

Results shown are median (interquartile range) if non-parametric or mean (standard deviation) if normally distributed results.

Abbreviations: MELD, modified end stage liver disease score; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

To identify markers that may reflect cirrhosis rather than portal hypertension, healthy subjects were compared with cirrhosis patients without portal hypertension. All markers had significant differences between groups except spleen ECV, spleen T1, spleen cT1, and MELD.

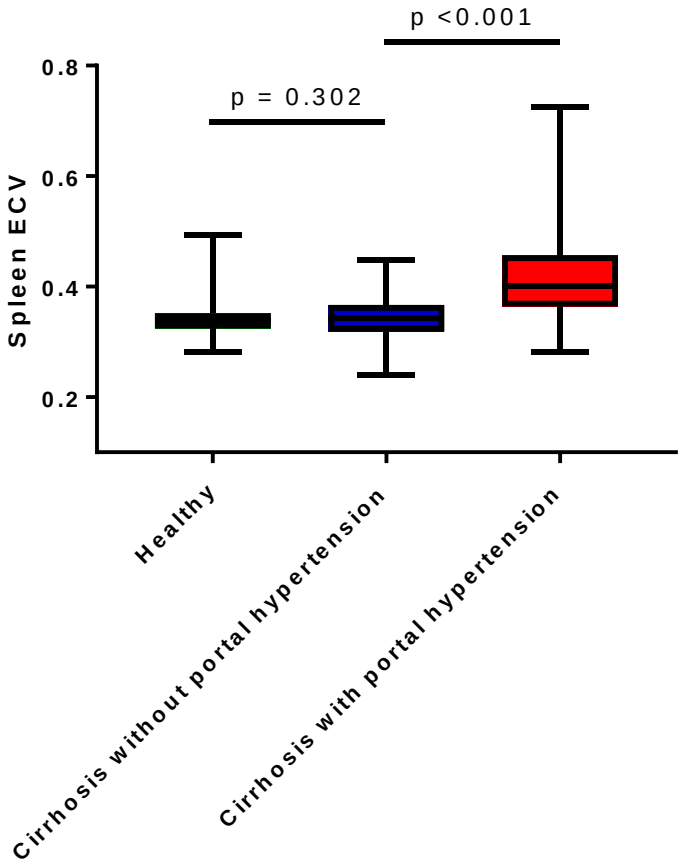


Figure 17. Box and whisker plot of spleen ECV in healthy subjects (n = 20), cirrhotic subjects without portal hypertension (n = 49) and cirrhotic subjects with portal hypertension (n = 73).

Spleen ECV was higher in cirrhotic subjects with than without portal hypertension, but not significantly different between healthy and cirrhotic subjects without portal hypertension. Lines represent median, interquartile range, maximum and minimum values.

4.6.4 Diagnostic accuracy of non-invasive markers for the identification of portal hypertension in cirrhosis

Spleen ECV identified portal hypertension in cirrhosis with AUROC 0.79 (95% CI 0.71 – 0.87) (p <0.001; table 13, figure 18). A cut off of 0.36 had 78% sensitivity and 72% specificity for the diagnosis of portal hypertension. Platelet/spleen volume ratio and MELD performed similarly well with AUROCs 0.79 each. By comparison, platelets, spleen T1, spleen cT1, spleen volume, spleen

Table 13. Diagnostic accuracy of non-invasive markers for the identification of portal hypertension in patients with cirrhosis

	AUROC (95% CI)	p	Cut off	Sensitivity (%)	Specificity (%)
Candidate MR markers					
Spleen ECV	0.79 (0.71 – 0.87)	<0.001	0.36	78	72
Spleen T1 (ms)	0.71 (0.62 – 0.80)	<0.001	1332	63	79
Spleen cT1 (ms)	0.73 (0.63 – 0.82)	<0.001	1274	73	73
Spleen volume (ml)	0.77 (0.69 – 0.86)	<0.001	480	77	67
Platelet/spleen volume	0.79 (0.71 – 0.87)	<0.001	0.28	74	71
Other markers					
Platelets ($x10^9$/l)	0.73 (0.64 – 0.81)	<0.001	117	55	84
Spleen diameter (mm)	0.74 (0.65 – 0.83)	<0.001	152	58	82
Platelet/spleen diameter	0.76 (0.68 – 0.85)	<0.001	0.71	53	94
MELD	0.79 (0.70 – 0.87)	<0.001	8	77	78
AST/ALT	0.71 (0.62 – 0.81)	<0.001	1.28	63	76
APRI	0.75 (0.67 – 0.84)	<0.001	0.55	81	61
FIB-4	0.81 (0.72 – 0.89)	<0.001	3.39	73	84
Liver stiffness (kPa)	0.63 (0.52 – 0.74)	0.029	32.5	46	75
Liver cT1 (ms)	0.64 (0.54 – 0.74)	0.011	808	81	46

Abbreviations: AUROC, area under the receiver operator curve; MELD, modified end stage liver disease score; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

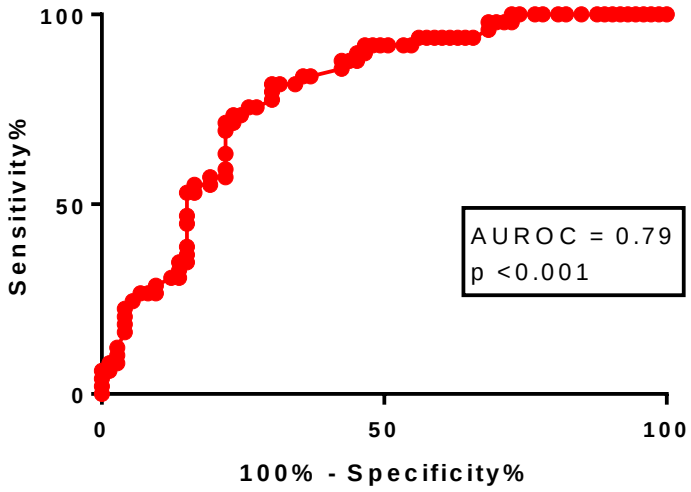


Figure 18. Receiver operator curve for the identification of portal hypertension in subjects with cirrhosis using spleen ECV.

diameter, platelet/spleen diameter ratio, AST/ALT and APRI had smaller AUROCs ranging between 0.71 and 0.77. FIB-4 had the highest accuracy for the identification of portal hypertension (AUROC 0.81) with a 3.39 cut off having 73% sensitivity and 84% specificity. The cirrhosis measures liver stiffness and liver cT1 performed poorly (AUROCs 0.63 and 0.64, respectively).

4.6.5 Association of non-invasive markers with portal pressure measurements

HVPGs were obtained as part of clinical care in 3 participants at 1, 27 and 40 days from MRI assessment. Direct portal pressure gradients (PPGs) were obtained in 4 patients before TIPSS, a median of 3 (range 1 – 7) days following study assessments (figure 16). The results are underpowered for the calculation of correlation coefficients, but visual inspection of scatter

graphs does not indicate any strong associations between candidate MR portal pressure markers with HVPG or PPG (figure 19).

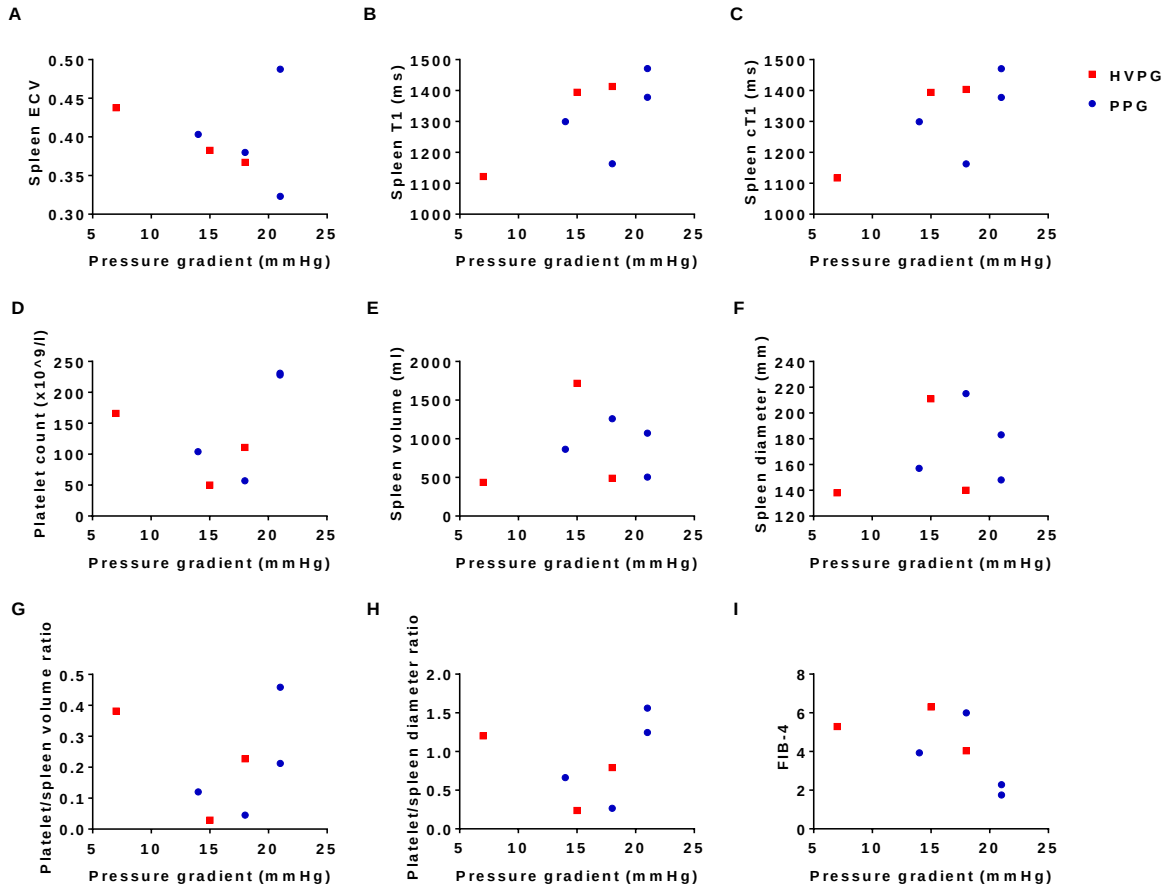


Figure 19. Associations of candidate portal pressure markers with portal pressure measurements in 7 subjects.

Scatter plots show spleen ECV (A), spleen T1 (B), spleen cT1 (C), platelet count (D), spleen volume (E), spleen diameter (F), platelet/spleen volume ratio (G), platelet/spleen diameter ratio (H) and FIB-4 (I) versus hepatic venous gradient (HVPG, red squares) or portal pressure gradient (PPG, blue dots).

4.6.6 Non-invasive assessment of dynamic changes in portal pressure

TIPSS participants had mean age 53 (s.d. 6.4) years and 3 (75%) were male. Their liver disease aetiologies were alcohol (n = 2), chronic hepatitis C virus (n = 1) and small vessel veno-occlusive disease (n = 1). Indications for TIPSS were intractable ascites (n = 2), recurrent bleeding from stomal varices (n = 1) and prevention of liver disease progression due to small vessel Budd Chiari syndrome (n = 1).

The TIPSS group were re-assessed a median 13 days (range 11 – 14) post TIPSS. The PPGs measured during TIPSS fell by median 11.5 mmHg (range 7 - 13) and all patients achieved a post-TIPSS PPG <12 mmHg (figure 20). As only 4 patients were recruited, no statistical analyses were performed. The relative changes in non-invasive markers before and after TIPSS versus the change in PPG are shown in figure 21.

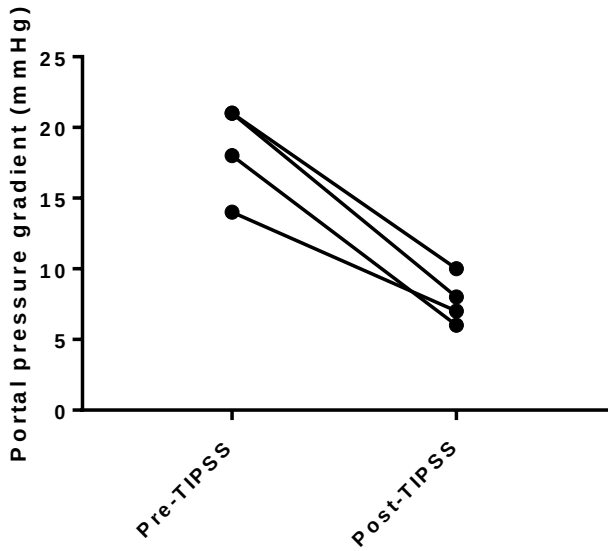


Figure 20. Portal pressure gradient in patients pre and post TIPSS measured during the procedure (n = 4).

Each line represents one patient.

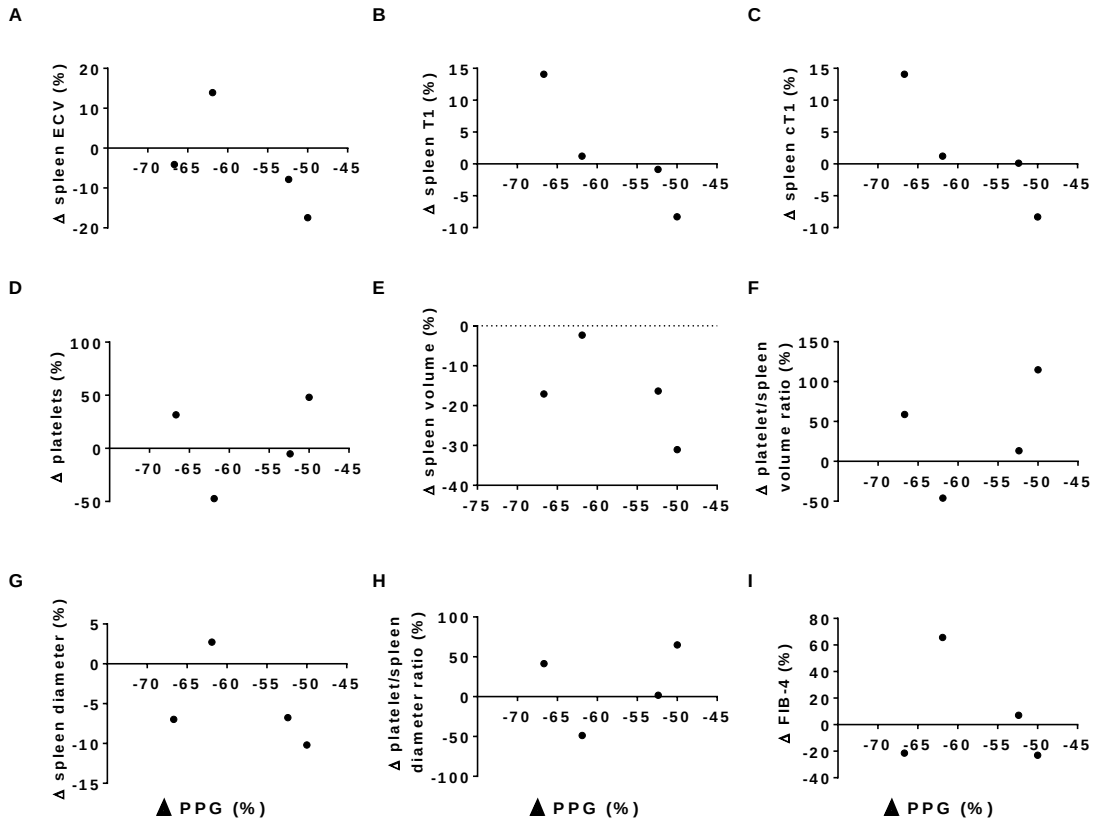


Figure 21. Scatter plots of the percentage change in non-invasive markers versus the percentage change in portal pressure gradient (PPG) following TIPSS (n = 4).

Results are shown for spleen ECV (A), spleen T1 (B), spleen cT1 (C), platelet count (D), spleen volume (E), platelet/spleen volume ratio (F), spleen diameter (G), platelet/spleen diameter ratio (H) and FIB-4 (I).

4.6.7 Non-invasive markers in compensated advanced chronic liver disease with and without significant varices

Ninety-one patients had compensated advanced chronic liver disease (cACLD; figure 16), namely the cirrhosis with normal liver function and cirrhosis with liver dysfunction only groups. They had OGD screening for varices within a median of 30 days (IQR 11 – 117) of their study assessments. VNT prevalence was 14%.

Liver stiffness measurement was unreliable in 10 patients and spleen T2* measurement and therefore spleen cT1 calculation was unreliable in 4 patients.

Table 14. Baseline characteristics of cACLD subjects with and without varices needing treatment (VNTs)

	No VNTs N = 78	VNTs N = 13
Age (years)	61 (55 – 66)	63 (55 – 71)
Male (%)	52 (67%)	12 (92%)
BMI (kg/m^2)	30.0 (5.8)	29.0 (4.0)
Aetiology		
ArLD	32 (41%)	4 (31%)
NAFLD	20 (26%)	7 (54%)
Viral	17 (22%)	0
Autoimmune	12 (15%)	1 (8%)
Other	5 (6%)	1 (8%)
Blood tests		
Bilirubin (µmol/l)	13 (9 – 18)	19 (13 – 26)
Albumin (g/l)	37 (35 – 39)	37 (33 – 38)
Prothrombin time (s)	11.7 (11.2 – 12.5)	12.5 (11.8 – 13.7)

Results shown are median (interquartile range), mean (standard deviation) or count (percentage).
Abbreviations: BMI, body mass index; ArLD, alcohol-related liver disease; NAFLD, non-alcohol-related fatty liver disease.

The VNT group had a higher proportion of males and NAFLD than the no VNT group and slightly higher bilirubin and prothrombin time (table 14).

All candidate MR markers for portal hypertension (spleen ECV, T1, spleen cT1, spleen volume, platelet/spleen volume ratio,) differed significantly between groups (table 15, figure 22). Patients with VNTs also had lower platelets and platelet/spleen diameter ratio and higher spleen diameter, blood-based fibrosis markers (AST/ALT ratio, APRI, FIB-4) and MELD than those without VNTs. Liver stiffness and liver T1 measures did not differ significantly between groups.

Table 15. Comparison of non-invasive markers in compensated advanced chronic liver disease (cACLD) patients with and without varices needing treatment (VNTs)

	No VNTs N = 78	VNTs N = 13	P value
Candidate MR markers			
Spleen ECV	0.35 (0.32 – 0.38)	0.38 (0.36 – 0.46)	**0.019**
Spleen T1 (ms)	1302.2 (1225.8 – 1343.3)	1395.4 (1337.5 – 1447.1)	**<0.001**
Spleen cT1 (ms)	1249.4 (90.7)	1356.3 (76.3)	**<0.001**
Spleen volume (ml)	449 (314 – 670)	797 (592 – 1428)	**<0.001**
Platelet/spleen volume	0.33 (0.20 – 0.55)	0.15 (0.03 – 0.22)	**<0.001**
Other markers			
Platelets ($x10^9$/l)	147 (116 – 185)	104 (64 – 112)	**<0.001**
Spleen diameter (mm)	141 (122 – 156)	167 (157 – 194)	**<0.001**
Platelet/spleen diameter	1.07 (0.77 – 1.53)	0.63 (0.28 – 0.77)	**<0.001**
MELD	7 (6 – 8)	8 (7 – 10)	**0.028**
AST/ALT	1.17 (0.94 – 1.38)	1.53 (1.09 – 1.87)	**0.043**
APRI	0.56 (0.42 – 1.04)	1.66 (0.77 – 2.08)	**0.001**
FIB-4	2.63 (1.85 – 3.77)	6.65 (3.92 – 8.97)	**<0.001**
Liver stiffness (kPa)	19.9 (12 – 32.8)	22.1 (15.5 – 60.1)	0.163
Liver cT1 (ms)	840.0 (96.5)	867.7 (108.3)	0.350

Results shown are median (interquartile range) if non-parametric or mean (standard deviation) if normally distributed. P values are for differences between groups by unpaired t test for parametric results and Kruskal Wallis test for non-parametric results.
Abbreviations: ECV, extracellular volume fraction; MELD, modified end stage liver disease score; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

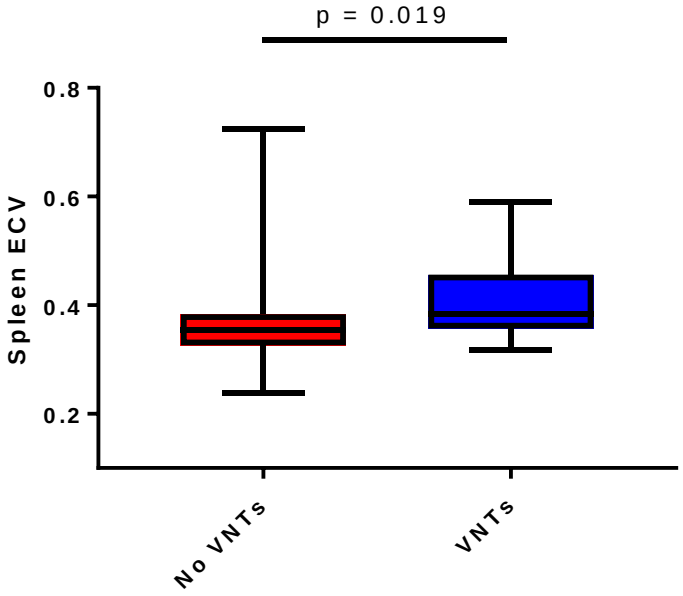


Figure 22. Comparison of spleen ECV in compensated advanced chronic liver disease with and without varices needing treatment (VNTs).

Spleen ECV was significantly higher in patients with (n = 13) than without (n = 78) varices needing treatment (p = 0.019). Lines represent median, interquartile range, maximum and minimum.

4.6.8 Diagnostic accuracy for the detection of varices needing treatment

Markers with significant differences with and without VNTs were analysed for their ability to identify VNTs. Of the pre-defined candidate MR markers, platelet/spleen volume ratio had the greatest ability to identify participants with VNTs with AUROC 0.84 and a 0.267 cut off having 100% sensitivity and 63% specificity for VNTs (table 16, figure 23). Spleen T1 had AUROC 0.83, which improved to 0.87 when spleen T1 was multiplied by spleen diameter, making it the best of all markers tested. Spleen T1 x spleen diameter >210194 msmm had 83% sensitivity and 79% specificity for the identification of VNTs. Spleen ECV performed poorly, with AUROC 0.70. Platelet

count performed well with AUROC 0.82, as did scores incorporating its use - APRI 0.80, platelet/spleen diameter ratio 0.83 and particularly FIB-4 0.85.

Table 16. Diagnostic accuracy of biomarkers in compensated advanced chronic liver disease patients for the identification of varices needing treatment.

	AUROC (95% CI)	p	Cut off	Sensitivity (%)	Specificity (%)
Candidate MR markers					
Spleen ECV	0.70 (0.55 – 0.86)	0.019	0.367	77	62
Spleen T1 (ms)	0.83 (0.73 – 0.93)	<0.001	1389	69	87
Spleen cT1 (ms)	0.81 (0.70 – 0.92)	0.001	1330	75	80
Spleen volume (ml)	0.81 (0.69 – 0.92)	<0.001	570	85	67
Platelet/spleen volume	0.84 (0.75 – 0.93)	<0.001	0.267	100	63
Spleen T1 x spleen diameter (msmm)	0.87 (0.79 – 0.95)	<0.001	210194	85	78
Liver disease markers					
Platelets ($x10^9$/l)	0.82 (0.70 – 0.95)	<0.001	113	85	79
Spleen diameter (mm)	0.81 (0.71 – 0.92)	<0.001	155	85	74
Platelet/spleen diameter	0.83 (0.72 – 0.95)	<0.001	0.87	92	67
MELD	0.69 (0.54 – 0.84)	0.029	8.2	69	76
AST/ALT	0.68 (0.52 – 0.83)	0.044	1.45	62	77
APRI	0.80 (0.66 – 0.94)	0.001	1.57	62	95
FIB-4	0.85 (0.74 – 0.96)	<0.001	3.69	92	76

Abbreviations: ECV, extracellular volume fraction; MELD, modified end stage liver disease score; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

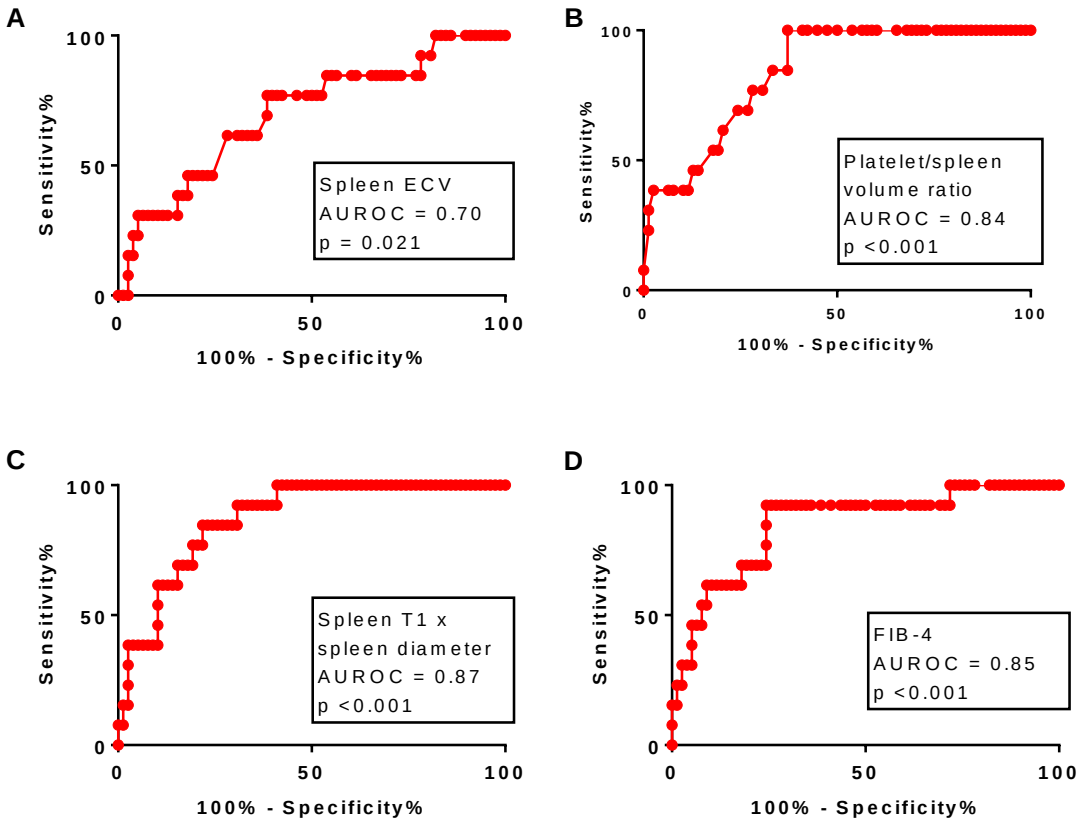


Figure 23. Receiver operator curves of spleen ECV (A), platelet/spleen volume ratio (B), spleen T1 x spleen diameter (C) and FIB-4 (D) for the diagnosis of varices needing treatment in compensated advanced chronic liver disease (n = 91).

4.6.9 Identification of patients to avoid screening OGDs for varices

New criteria were chosen to miss <5% of all VNTs. As 1 patient with VNTs accounted for 8% of all patients with VNTs in this cohort, all new criteria had 100% sensitivity and NPV. Unreliable liver stiffness measurements meant the Baveno, expanded-Baveno, liver stiffness and LSPS criteria could not be applied in 10 (11%) patients.

Of the established criteria, a screening OGD could have been avoided in 20% of patients using Baveno VI criteria and 16% of patients using MELD-platelet criteria without missing any VNTs (table 17). The expanded Baveno criteria, however, would have missed an unacceptable 18% of VNTs and spared 48% of OGDs. LSPS <1.33 would also have missed one patient (8%) with VNTs and would have saved 25% of OGDs.

None of the new criteria missed varices. Spleen T1 <1307 ms was the best single MR criterion, saving 45% of OGDs and being acquired successfully in all participants. It outperformed spleen cT1. Spleen T1 x spleen diameter <185088 msmm improved spleen T1's performance further to avoid 51% of OGDs. Platelet/spleen volume <0.267 saved 54% of OGDs. Spleen ECV <0.317 performed poorly, with just 14% of OGDs spared.

Multiple combination criteria were tested, of which the criteria, spleen T1 <1307 ms or spleen diameter <137 mm, spared the most OGDs (66%). Only 23% of patients without VNTs would have had OGD screening using these criteria.

Table 17. Ability of non-invasive criteria to avoid OGD screening for varices needing treatment (VNTs) in compensated advanced chronic liver disease. VNT prevalence was 14%.

Criteria	N	OGDs avoided (%)	VNTs missed	
			(1-NPV %)	(1-sensitivity %)
Established criteria				
Baveno VI	81	16 (20%)	0	0
Expanded Baveno	81	39 (48%)	2 (5%)	2 (18%)
MELD = 6, platelets >150 $x10^9$/l	91	15 (16%)	0	0
LSPS <1.33	81	23 (28%)	1 (4%)	1 (8%)
New criteria				
Spleen ECV <0.317	91	13 (14%)	0	0
Spleen T1 <1307 ms	91	41 (45%)	0	0
Spleen cT1 <1252 ms	87	37 (43%)	0	0
Spleen T1 x spleen diameter <185088 msmm	91	46 (51%)	0	0
Spleen volume <403 ml	91	33 (36%)	0	0
Platelet/spleen volume <0.267 $x10^9$/l/ml	91	49 (54%)	0	0
Spleen diameter <137 mm	91	36 (40%)	0	0
Platelet/spleen diameter <1.27 $x10^9$/l/mm	91	24 (26%)	0	0
Platelets >213 $x10^9$/l	91	15 (16%)	0	0
Liver stiffness <4.8 kPa	81	1 (1%)	0	0
APRI <0.39	91	16 (18%)	0	0
FIB-4 <1.95	91	22 (24%)	0	0
New combination criteria				
Spleen T1 <1307 ms or spleen diameter <137 mm	91	60 (66%)	0	0
Spleen T1 <1307 ms or platelets >213 $x10^9$/l	91	47 (52%)	0	0
Spleen diameter <159 mm and platelets >112 $x10^9$/l	91	52 (57%)	0	0

Abbreviations: NPV, negative predictive value; MELD, modified end stage liver disease score; LSPS, liver stiffness and platelet count/spleen diameter ratio score; ECV, extracellular volume fraction; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

4.7 Discussion

This study has found promising MRI-based markers for the assessment of portal hypertension and oesophageal varices needing treatment (VNTs). The best of the MRI-based markers tested were spleen ECV and platelet/spleen volume ratio for the identification of portal hypertension, spleen T1 x spleen diameter for identifying VNTs in cACLD and the criteria spleen T1 <1307 ms or spleen diameter <137 mm for excluding VNTs in cACLD.

The primary candidate MR marker of portal hypertension was spleen ECV. Spleen ECV had good diagnostic accuracy for the identification of portal hypertension in all cirrhosis patients (AUROC 0.79), but both spleen ECV's sensitivity (78%) and specificity (72%) for portal hypertension fell short of the ≥90% sensitivity and ≥75% specificity that were targeted for the study. No markers tested for portal hypertension met both these targets. Spleen ECV and all other markers tested also had poor visual correlation with HVPG and PPG. The spleen ECVs recorded in healthy volunteers (median 0.338, IQR 0.324 – 0.352) were consistent with those reported by Bandulla et al. at 1.5 Tesla (median 0.34, IQR 0.32 – 0.35) [109], supporting the reproducibility of spleen ECV across magnet strengths. Contrary to the study hypothesis, spleen ECV performed poorly in the identification and exclusion of VNTs in cACLD (AUROC = 0.70). This indicates that spleen ECV may rise relatively late in the progression of portal hypertension, when ascites and hepatic encephalopathy occur.

Platelet/spleen volume ratio performed the best overall of the pre-defined candidate MR markers of portal hypertension. It identified portal hypertension in cirrhosis with AUROC 0.79, 74% sensitivity and 71% specificity; VNTs in cACLD with AUROC 0.84; and could avoid 54% of screening OGDs for VNTs in cACLD. Platelet/spleen volume ratio performed better numerically than platelet/spleen diameter ratio in these analyses (AUROC 0.76, AUROC 0.83 and 26%, respectively), indicating the more labour-intensive measurement of spleen volume versus

diameter may be worthwhile, particularly for the exclusion of VNTs in cACLD. In comparison with spleen ECV as a marker of portal hypertension, platelet/spleen volume ratio takes longer to analyse, but has more reliable acquisition and no contrast requirement. Spleen ECV could not be calculated for 3 patients and one withdrew consent for cannulation.

Unlike spleen ECV and T1 measures, platelet/spleen volume ratio accounts for the splenic hyperplasia that occurs in portal hypertension. Such hyperplasia may occur relatively early in the progression of portal hypertension, hence why platelet/spleen volume ratio can assess VNTs accurately in patients without other portal hypertensive complications. It is also recognised, however, that spleen volume increases with some haematological disorders and infections and so is not specific to portal hypertension.

Pavlides et al. had found previously that spleen cT1 in 19 patients with suspected cirrhosis had AUROC 0.91 for the identification of HVPG >5 mmHg and >10 mmHg [148]. The results presented here had lower diagnostic ability for portal hypertension (AUROC 0.73), perhaps due to the different patient population, sample size and definition of portal hypertension used.

Pavlides et al. also found spleen cT1 performed better than spleen T1 for diagnosing portal hypertension. These findings were supported numerically for the identification of portal hypertension (spleen T1 AUROC 0.71), but spleen T1 outperformed cT1 for the identification of VNTs (AUROC 0.83 vs 0.81) and exclusion of VNTs (45% vs 41%). This may be because the iron correction in cT1 was derived for the liver [72] and so may not be well-suited for the spleen as suggested in chapter 3. Splenic iron is not known to be raised in liver disease, with possible rare exceptions of hereditary haemochromatosis [153] and transfusion-related iron overload [154]. Also of relevance, spleen T2* acquisition and so cT1 calculation was unreliable in 5 (3.5%) subjects, making spleen T1 a better test on an intention to diagnose basis.

The exploratory marker of spleen T1 x spleen diameter was derived during the study. As it reflects spleen water content and size, it represents all three pathological changes that occur in the

spleen in portal hypertension, namely venous congestion, fibrosis and hyperplasia. Spleen T1 x spleen diameter could identify VNTs with the highest accuracy (AUROC 0.87) of all markers tested. A cut off of 210194 msmm had 85% sensitivity and 78% specificity. Spleen T1 x spleen diameter could also avoid 51% of screening OGDs for VNTs. Spleen T1 x spleen diameter can be acquired in 2 MRI breath-holds (T1 map and VIBE) without the need for contrast and could be analysed quickly on the scanner console.

The detection of VNTs by spleen T1 x spleen diameter compares favourably to spleen transient elastography (AUROC 0.82) [92], liver MRE (AUROCs 0.76 – 0.81) [106, 155] and spleen MRE (AUROC 0.75) [106]. One study reported spleen MRE had AUROC 0.93 for the identification of VNTs, but it only had 36 subjects [88]. A meta-analysis of spleen stiffness measurement by multiple methods reported AUROC 0.80, sensitivity 81% and specificity 66% for detecting VNTs [156]. Measurement of spleen T1 and spleen diameter was also successful in all participants whereas measurements fail or are unreliable in 16% with spleen TE [103] and 11% with liver and spleen MRE [106].

By combining spleen T1 and diameter parameters in a different way, the criteria spleen T1 <1307 ms or spleen diameter <137 mm excluded VNTs in 66% of cACLD patients who therefore could have avoided screening OGDs. This far exceeded the performance of any reported criteria I am aware of to date [97, 110-114] and those tested in this cohort. Even combination of Baveno VI criteria with spleen stiffness measurement by transient elastography avoids just 37-44% of screening OGDs [157]. The success of spleen T1 and spleen diameter measurements compares favourably to the proportion of unreliable measurements for spleen cT1 (4%) and liver stiffness (11%), affecting Baveno, expanded Baveno and LSPS criteria. Application of spleen T1 <1307 ms or spleen diameter <137 mm saved 44 (48%) more screening OGDs than Baveno VI criteria in this cohort. Also, no VNTs were missed, unlike expanded Baveno (18%) and LSPS (8%) criteria.

The necessary technology exists in most hospitals for easy translation of spleen ECV, T1 and diameter and platelet/spleen volume into clinical care. These markers could be incorporated into existing liver lesion MRI protocols without extending the scan duration by more than 5 minutes.

An unexpected observation in this study was how well FIB-4 performed in the identification of portal hypertension (AUROC 0.81) and VNTs (AUROC 0.85). It could also avoid 24% of screening OGDs for VNTs, which was more than Baveno VI criteria (20%). FIB-4 benefits from being a simple composite of age, AST, ALT and platelets, making its use attractive for further exploration, particularly in settings with limited access to transient elastography and imaging modalities.

All patients in this study had cirrhotic portal hypertension, but non-cirrhotic portal hypertension can also occur. Spleen ECV, spleen T1, spleen cT1, and MELD were the only markers higher with than without portal hypertension that were similar between healthy subjects and patients with cirrhosis without portal hypertension. This indicates that these markers may be specific for portal hypertension rather than fibrosis severity. MELD may be an exception as it lacks biological plausibility without liver disease. Application of spleen ECV, spleen T1 and spleen cT1 in non-cirrhotic portal hypertension should be tested in further studies.

4.7.1 Limitations

Although some HVPG measures were recorded as part of patients' clinical care, too few portal pressure measurements were obtained with which to correlate candidate MR markers of portal hypertension. This was partly because HVPG was not a requirement for participation and partly because only 20% recruitment of patients awaiting TIPSS was achieved. Similarly, the ability of candidate portal hypertension markers to follow dynamic changes in portal pressure could not be tested with only 4 TIPSS patients.

Of the portal pressure measurements obtained, a straight catheter method was used for HVPG measurement in two of the three centres, rather than the gold standard balloon catheter method. Potential inaccuracies in the HVPGs may have contributed to the lack of visual correlation seen with any of the candidate portal hypertension markers.

Despite the lack of portal pressure measurements, the definition of portal hypertension used in the study was still meaningful as these patients had the clinical manifestations of portal hypertension that are important to detect (varices, ascites, hepatic encephalopathy). It is, however, difficult to compare the results to those of other studies.

Recruitment of patients with cirrhosis in defined subgroups limited recruitment to the study and left some patients unrepresented e.g. patients with cirrhosis with ascites and normal liver function. This may have biased the diagnostic value of candidate portal hypertension markers. For example, positive selection of patients with liver dysfunction may have positively biased the performance of liver function-related markers such MELD (AUROC 0.79 for the identification of portal hypertension). Selection bias would have been minimal, however, in the identification and exclusion of VNTs in cACLD, who represented the target population well.

The Baveno criteria were published during the study recruitment period and may have been applied to some patients at the recruiting centres. If this were the case, the effect appears minimal as the cACLD cohort had a similar liver stiffness, platelet count and VNT prevalence as seen in other studies [110-112] (table 18).

The majority of VNT screening OGDs occurred prior to study assessment due to the logistics of recruitment and MRI availability. OGDs were done blinded to the study results in all cases, but OGD results were sometimes known during the acquisition of study results, which may have led to some unintentional bias.

Table 18. Reported results from this and other studies for the application of Baveno criteria to exclude VNTs.

	MAPLE N=81	Augustin [110] N = 925	Petta [111] N = 790	Jangouk [112] N = 161
Baveno criteria fulfilled	16 (20%)	198 (21.5%)	219 (27.7%)	25.5%
Liver stiffness (kPa)	25.4 (17)	25.9 (15.0)	25 (16)	20.4 (10 – 75)
Platelets $x10^9$/l	149 (62)	140 (68)	177 (85)	139 (25 – 838)
VNT prevalence	14%	9.9%	11.5%	8.7%

Results shown are median (interquartile range), mean (standard deviation) or count (percentage).

To further this research, the portal pressure markers require prospective validation against HVPG and the VNT markers require external validation.

4.7.2 Conclusion

The study results support the study hypotheses. Spleen ECV and platelet/spleen volume can identify clinically defined portal hypertension in cirrhosis, although without sufficient sensitivity and specificity for adoption in clinical practice or research. In compensated advanced chronic liver disease, spleen T1 x spleen diameter can identify varices needing treatment with high accuracy and the criteria spleen T1 <1307 ms or spleen diameter <137 mm can identify patients at low risk of oesophageal varices needing treatment, who could safely avoid oesophagogastroduodenoscopy screening for varices.

5 MR assessment of liver function and the ability of combined liver fibrosis, portal hypertension and liver function assessments to differentiate clinical phenotypes of liver disease

5.1 Abstract

Liver function is of prognostic importance in liver disease. The percentage reduction in liver T1 with gadoxetic acid administration (%rT1) has been correlated with measures of liver function. This chapter incorporates %rT1 into a magnetic resonance (MR) scan together with assessments of liver fibrosis by liver cT1 and portal hypertension by spleen extracellular volume fraction (ECV) to measure liver function and differentiate liver disease phenotypes.

One hundred and sixty-nine participants of different liver disease phenotypes had MR with gadoxetic acid administration, clinical, blood test and liver stiffness assessments. %rT1, liver cT1, spleen ECV and liver volumes were analysed from the MR scans. Liver dysfunction was defined as at least one of bilirubin above the upper limit of normal or international normalized ratio >1.1 of presumed liver cause.

%rT1 identified participants with liver dysfunction accurately (AUROC 0.86). %rT1 correlated with liver function markers bilirubin (r = -0.64), prothrombin time (r = -0.44) and albumin (r = -0.46), MELD (r = -0.58) and Child Pugh score (r = -0.59) and was modelled best by bilirubin, albumin, aspartate aminotransferase and liver stiffness (R^2 = 0.51). A %rT1 model for MELD improved from R^2 = 0.34 to R^2 = 0.45 by incorporating measured and estimated liver volumes. Combinations of liver cT1, %rT1 and spleen ECV could differentiate participants with healthy livers, acute liver injury, cirrhosis with normal liver function, cirrhosis with liver dysfunction only and cirrhosis with

hepatic encephalopathy, but could not differentiate patients with cirrhosis and ascites from those with cirrhosis with liver dysfunction only or hepatic encephalopathy.

The results indicate %rT1 is a non-invasive measure of liver function that can be improved by incorporation of measured and estimated liver volumes. A single MR scan can assess liver fibrosis by liver cT1, portal hypertension by spleen ECV and liver function by %rT1, which vary according to liver disease phenotype.

5.2 Introduction

Measures of liver function are predictive of clinical events [40, 158]. MRI techniques with the liver-specific contrast agent, gadoxetic acid, have been investigated for the non-invasive quantification of liver function previously [121-125, 159]. Gadoxetic acid is excreted by the liver using a pathway common to many toxins and metabolites. Gadoxetic acid is taken up by OATP receptors on hepatocytes and excreted via MRP2 into bile [160]. The degree of gadoxetic acid uptake therefore reflects the functioning hepatocyte mass and can be quantified using MRI by either the relative change in signal intensity (signal enhancement) or T1 of the liver pre and post gadoxetic acid administration.

MR signal intensity is based on arbitrary grayscales and so the relative change in signal intensity may not be replicable in subjects and between subjects and different MR scanners. Quantitative T1 mapping, however, allows more standardised and exact measurements.

The percentage reduction in liver T1 with gadoxetic acid administration (%rT1) has been correlated with measures of liver function including Child Pugh score [121, 125, 159], MELD [122, 123], indocyanine green (ICG) clearance [125] and ^{13}C-methacetin breath test [124]. These studies observe that %rT1 reduces with worsening liver function. Haimerl et al. have shown that %rT1 has

excellent inter-observer reproducibility with an intra-class correlation coefficient of >0.88 when patients are grouped into 3 categories of liver function, based on ^{13}C-methacetin breath test results [124].

Liver volume may also be an important contributor to overall liver function. Indeed, future remnant liver volume is a well-established predictor of liver function post hepatectomy [158]. Incorporation of liver volume may improve the performance of %rT1 in estimating liver function as a large volume of poorly functioning liver may be equivalent to a small volume of well-functioning liver. The estimated or standard liver volume is the volume of liver expected for an individual in good health with normal liver function. Discrepancies between the measured and estimated liver volumes have been shown to relate to liver function and it is a relatively common clinical finding in end stage liver disease that the liver is shrunken. Chuang et al. found that the signal intensity change of the liver between 3 and 30 minutes post gadoxetic acid multiplied by the total liver volume/standard liver volume was an independent predictor of post hepatectomy liver failure, whereas relative signal enhancement pre and post gadoxetic acid without liver volume correction and ICG clearance were not [161]. Similarly, Haimerl et al. found that the incorporation of liver volume to %rT1 improved its correlation with ICG plasma disappearance rate as a reference standard for liver function from r = 0.86 to r = 0.92 [125].

The studies of %rT1 to date have used different T1-mapping techniques to shMOLLI used in this thesis and my institution. This chapter aims to measure liver function by %rT1 using shMOLLI T1 mapping, incorporated into the same MRI scan as non-invasive measurements of liver fibrosis and fat [70], and portal hypertension and varices (chapter 4). A single MRI scan should therefore be able to give a clinically-relevant, multifactorial assessment of liver disease.

The severity and relative contributions of liver fibrosis, portal hypertension and liver function likely relate to the different clinical phenotypes of liver disease seen in clinical practice [39]. The spectrum of liver disease phenotypes includes fully compensated with normal liver function, liver

dysfunction, ascites and hepatic encephalopathy (HE). Current understanding of the pathophysiology of liver disease and these phenotypes was described in section 1.1. Although there is a general tendency to progress from the former to the latter and the pathological processes are interlinked, some patients can develop severe HE without any ascites or severe ascites without significant liver dysfunction or hepatic encephalopathy. A better understanding of the relative contributions of liver fibrosis, portal hypertension and liver dysfunction to the different liver disease phenotypes may enable prediction of specific liver decompensations and help guide management.

This chapter brings together a comprehensive assessment of liver disease in one MRI scan.

5.3 Hypotheses

The percentage reduction in liver T1 post gadoxetic acid administration using shMOLLI T1-mapping is a measure of liver function.

Incorporation of liver volume improves the percentage reduction in liver T1 post gadoxetic acid administration as a measurement of liver function.

The combined assessment of liver fibrosis, portal hypertension and liver function in one MR scan can differentiate liver disease phenotypes.

5.4 Aims

To identify liver dysfunction using the percentage reduction in liver T1 post gadoxetic acid administration.

To quantify liver function using the percentage reduction in liver T1 post gadoxetic acid administration in comparison to bilirubin, albumin, prothrombin time, MELD and Child Pugh score.

To explore factors that influence the percentage reduction in liver T1 post gadoxetic acid administration.

To explore how liver volume measures and estimates contribute to the percentage reduction in liver T1 post gadoxetic acid administration in the measurement of liver function.

To explore how liver cT1 as a measure of liver fibrosis, spleen ECV as a measure of portal pressure and the percentage reduction in liver T1 with gadoxetic acid administration as a measure of liver function differ with liver disease phenotypes (healthy; acute liver injury; cirrhosis with normal liver function, cirrhosis with liver dysfunction; cirrhosis with ascites and cirrhosis with hepatic encephalopathy).

5.5 Methods

Subjects were recruited prospectively to the MAPLE study from the John Radcliffe Hospital, Oxford, Southampton General Hospital, Southampton and Queen Elizabeth Hospital, Birmingham between June 2016 and July 2018. Subjects were assessed by clinical history, physical

examination, anthropometrics, blood tests, liver stiffness measurement and MRI scan with gadoxetic acid as detailed in the general methods chapter 2.

Liver dysfunction was defined as at least one of bilirubin above the upper limit of normal (ULN) or international normalized ratio (INR) above 1.1 of presumed liver cause. Acute liver injury was defined as alanine aminotransferase (ALT) >5 times the upper limit of normal (ULN) and liver dysfunction occurring in the absence of chronic liver disease or obstructive jaundice.

5.5.1 Participants

All MAPLE study participants were used in this chapter with only their first study visit assessments used for analysis. Patients with cirrhosis who did not have an OGD within 1 year of study assessment were included in analyses. The TIPSS group were incorporated into the cirrhosis with liver dysfunction only (n = 2) and ascites groups (n = 2) that matched their clinical phenotypes.

In the assessment of %rT1 for liver function, all patients with cirrhosis and liver dysfunction (i.e. the dysfunction only, ascites, hepatic encephalopathy (HE) and awaiting TIPSS cirrhosis groups) were combined into one group, termed cirrhosis with liver dysfunction. Four groups were analysed - healthy (no cirrhosis, no liver dysfunction), acute liver injury (no cirrhosis, but liver dysfunction), cirrhosis with normal liver function (cirrhosis, no liver dysfunction) and cirrhosis with liver dysfunction (cirrhosis, liver dysfunction).

5.5.2 MRI assessments

The percentage reduction in liver T1 post gadoxetic acid administration was calculated as:

$$\%rT1 = 100 \times (T1native - T1Gd)/T1native,$$

In this equation, T1native is the liver T1 pre-contrast and T1Gd is the liver T1 20 minutes post gadoxetic acid.

The contribution of liver volume and estimated liver volume in addition to %rT1 was explored for the estimation of liver function using MELD as a reference standard. MELD was chosen because it is used widely as a measure of liver function in clinical practice and has prognostic significance. Liver volume was measured from the MRI scans as described in section 2.3.4. The estimated liver volume was calculated using the method described by Yu et al. [162], which was found in preliminary work to estimate liver volumes in the healthy volunteer group best of the published liver volume estimate equations (data not shown).

$$Estimated\ liver\ volume\ (ml) = 21.585 \times Weight\ (kg)^{0.732} \times Height\ (cm)^{0.225}$$

All participants were analysed for the assessment of MRI measures of liver fibrosis, portal hypertension and liver function to differentiate clinical phenotype.

5.5.3 Statistical methods

The percentage reduction in liver T1 post gadoxetic acid administration (%rT1) was described for each participant group and differences between groups assessed using analysis of variance (ANOVA) and Student's unpaired t test for parametric results and Kruskal Wallis and Mann Witney U tests for non-parametric results. The diagnostic accuracy of %rT1 to identify liver dysfunction in all subjects and in patients with cirrhosis only was assessed by receiver operator curves, sensitivity and specificity. %rT1 was correlated with MELD, bilirubin, albumin and prothrombin time as measures of liver function. Associations with %rT1 were explored with

univariate and multivariable analyses. The contribution of liver volume and estimated liver volume to improve the correlation of %rT1 with MELD as a gold standard of liver function was explored using multiple linear regression.

Liver cT1, spleen ECV and %rT1 were described for each clinical phenotype group. Differences between clinical phenotype groups were assessed by analysis of variance (ANOVA) and Student's unpaired t test for parametric results and Kruskal Wallis and Mann Witney U tests for non-parametric results. Tukey's adjustment was applied for multiple comparisons.

5.6 Results

One hundred and eighty-four participants were recruited to the study (figure 24). There were 15 exclusions due to inability to tolerate the MRI (n = 6), withdrawal of consent for venous cannulation (n = 1), insufficient fast duration (n = 1), a healthy volunteer being diagnosed with hepatitis B (n = 1), acute liver injury resolving beyond inclusion criteria at the time of study assessments (n = 5) and misdiagnosis of cirrhosis (n = 1). This left 169 participants included in the study analysis. The acute liver injury, cirrhosis with normal liver function and HE groups fell short of their target group size by 13 (65%), 14 (17.5%) and 9 (45%) respectively.

5.6.1 Missing results for non-invasive measures

Fibroscan was unreliable in 25 (15%) of study participants. By group these were 1 (14%) with acute liver injury, 8 (12%) with cirrhosis with normal liver function, 3 (8%) with cirrhosis and liver dysfunction only, 6 (27%) in patients with ascites and 7 (64%) with HE.

Spleen ECV was not acquired for 4 healthy, 4 cirrhosis with normal liver function, 2 cirrhosis with liver dysfunction only and 3 ascites patients due to a change in methods early in the study. Spleen ECV was also unreliable in 1 patient with cirrhosis with liver dysfunction only.

Liver volume was missing in 2 patients with cirrhosis with liver dysfunction only and 1 with ascites due to technical problems with the scan sequence at the time of acquisition.

Liver T2* and therefore liver cT1 was unreliable in 1 cirrhosis with normal liver function patient.

Spleen T2* and therefore spleen cT1 was unreliable in 2 patients with cirrhosis with normal liver function, 2 with cirrhosis with liver dysfunction only and 1 with HE.

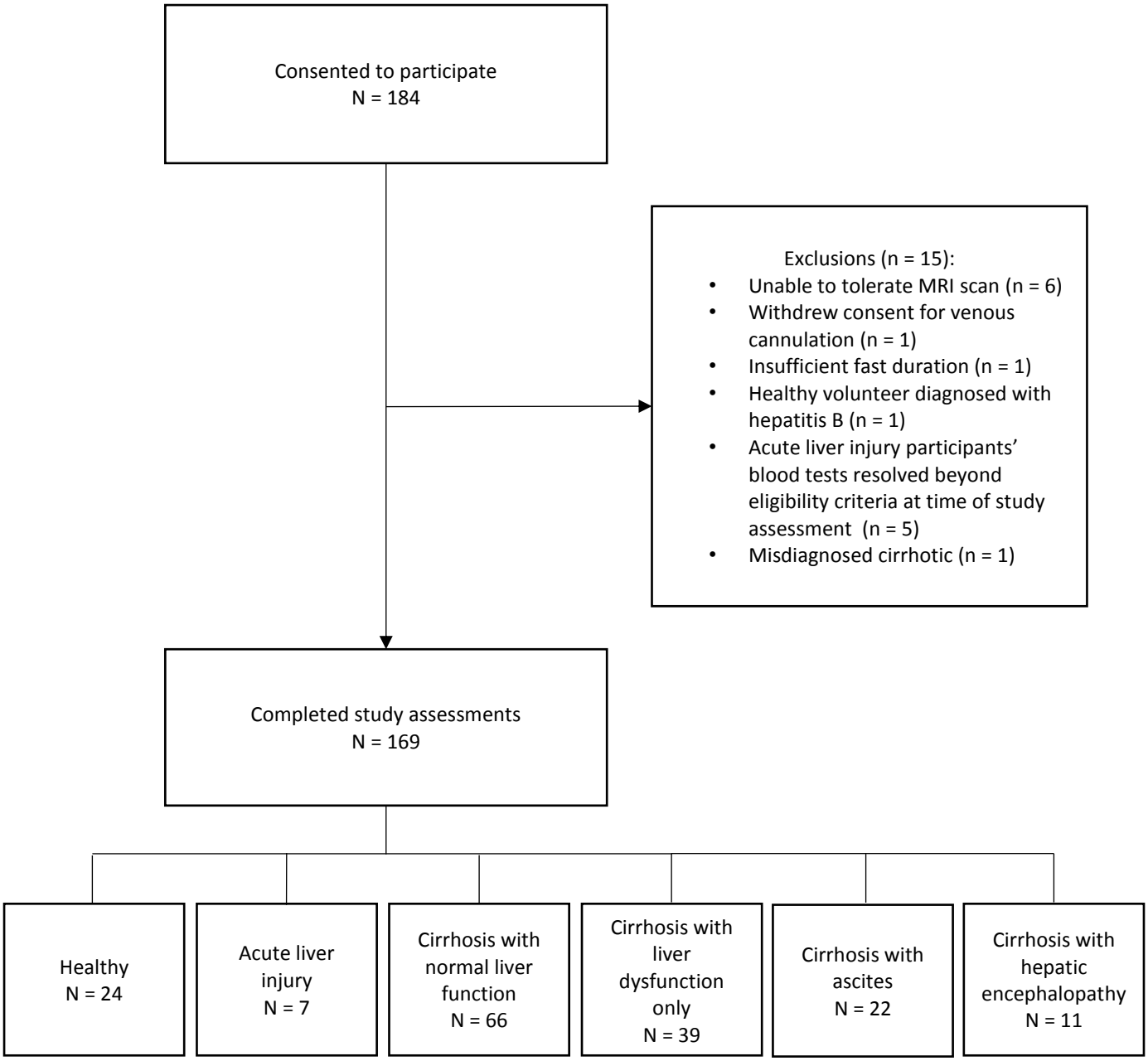


Figure 24. Flow diagram of recruitment for the analysis of liver function and differentiation of liver disease phenotype using MR parameters.

5.6.2 Participant baseline characteristics

Participants had a mean fast of 10±4 hours.

Healthy controls (n = 24) had median age 56 (IQR 47 – 66) years, BMI 23.4 (IQR 21.8 – 24.2) kg/m^2 and 58% were male (table 19). All of their blood parameters, alcohol consumption, liver stiffness and liver fat were within the normal range and they had normal MELD and Child Pugh scores as expected.

Acute liver injury patients (n = 7) were the youngest group with median age 29 years and 86% were male. Their liver disease aetiologies were hepatitis E virus (n = 3), hepatitis A virus (n = 1), Epstein-Barr virus (n = 1), drug-induced liver injury from co-amoxiclav (n = 1) and unknown (n = 1). AST and ALT were higher in acute liver injury than in the other groups, consistent with active inflammation and their defining criteria. The liver fibrosis markers liver stiffness and FIB-4 were higher in acute liver dysfunction than healthy controls, but lower than in cirrhotic patients.

Of the cirrhosis groups combined, 36/122 (30%) had biopsy-proven cirrhosis (Ishak stage 5 or 6) and 70% were diagnosed and being managed as cirrhotic on clinical grounds. They had median age 61 (IQR 54 – 67) years and 87 (71%) were male. The cirrhosis with normal liver function group had the highest BMI of all groups (29.9 kg/m^2).

Alcohol-related liver disease was common in all cirrhosis groups, but was particularly prevalent in cirrhosis with ascites (73%) and HE (64%). NAFLD, however, was relatively higher in the cirrhosis with normal liver function (25%) and dysfunction only (33%) than in the ascites (14%) and HE (9%) groups. Other liver disease aetiologies were similar across cirrhosis groups.

Table 19. Baseline characteristics of participant groups

	Healthy N = 24	Acute liver injury N = 7	Cirrhosis with normal liver function N = 66	Cirrhosis with liver dysfunction only N = 39	Cirrhosis with ascites N = 22	Cirrhosis with hepatic encephalopathy N = 11
Age (years)	56 (47 – 66)	29 (25 – 52)	60 (53 – 66)	65 (58 – 72)	60 (53 – 68)	64 (53 – 75)
Male (%)	14 (58%)	6 (86%)	41 (62%)	35 (90%)	16 (73%)	9 (82%)
BMI (kg/m^2)	23.4 (21.8 – 24.2)	25.4 (22.4 – 28.2)	29.9 (25.0 – 34.7)	29.6 (26.5 – 33.1)	24.4 (21.7 – 28.2)	33.0 (23.9 – 34.3
Alcohol (units/week)	6 (3 – 12)	4 (1 – 10)	0 (0 – 4)	0 (0 – 10)	0 (0 – 0)	0 (0 – 1)
Aetiology						
ArLD	-	-	33 (50%)	13 (33%)	16 (73%)	7 (64%)
NALFD	-	-	15 (23%)	13 (33%)	3 (14%)	1 (9%)
Viral	-	5 (71%))	13 (20%)	7 (18%)	2 (9%)	2 (18%)
Autoimmune	-	-	12 (18%)	3 (8%)	1 (5%)	1 (9%)
Other	-	2 (29%)	4 (6%)	3 (8%)	1 (5%)	1 (9%)
Bilirubin (µmol/l)	10 (9 – 14)	40 (36 – 126)	11 (8 – 14)	23 (18 – 30)	29 (18 – 37)	40 (28 – 65)
Albumin (g/l)	39 (38 – 41)	35 (31 – 38)	38 (35 – 39)	36 (33 – 38)	30 (27 – 35)	27 (26 – 31)
Prothrombin time (s)	11.4 (11.0 – 12.0)	11.3 (11.1 – 12.5)	11.4 (11.0 – 11.9)	13.0 (12.2 – 13.4	13.3 (12.4 – 14.4)	13.5 (12.8 – 15.0)
Platelets (x10^9/l)	237 (216 – 279)	218 (194 – 285)	155 (122 – 218)	122 (88 – 133)	157 (98 – 218)	95 (64 – 106)
AST (IU/l)	23 (20 – 24)	223 (74 – 291)	35 (27 – 45)	43 (24 – 58)	54 (43 – 66)	59 (33 – 69)
ALT (IU/l)	16 (13 – 20)	394 (342 – 1430)	31 (22 – 42)	34 (26 – 42)	25 (21 – 44)	22 (18 – 31)
Child Pugh	5 (5-5)	7 (6 – 8)	5 (5 – 6)	5 (5 – 6)	8 (7 – 9)	10 (8 – 11)
MELD	7 (6-7)	10 (9 – 15)	7 (6 – 7)	9 (8 – 11)	11 (8 – 13)	11 (10 – 15)

Table 19 continued.

	Healthy N = 24	Acute liver injury N = 7	Cirrhosis with normal liver function N = 66	Cirrhosis with liver dysfunction only N = 39	Cirrhosis with ascites N = 22	Cirrhosis with hepatic encephalopathy N = 11
AST/ALT	1.48 (1.16 – 1.67)	0.39 (0.20 – 0.47)	1.10 (0.94 – 1.32)	1.35 (1.03 – 1.68)	1.77 (1.28 – 2.29)	1.9 (1.50 – 2.50)
APRI	0.23 (0.18 – 0.26)	2.30 (0.57 – 8.16)	0.51 (0.38 – 0.77)	1.03 (0.64 – 1.66)	0.81 (0.58 – 1.16)	1.00 (0.88 – 1.58)
FIB-4	1.15 (0.98 – 1.62)	1.64 (0.99 – 2.33)	2.23 (1.66 – 3.38)	4.75 (3.17 – 7.21)	3.69 (2.34 – 5.65)	6.99 (5.42 – 10.45)
Liver stiffness (kPa)	4.6 (3.5 – 5.6)	7.3 (6.5 – 10.0)	18.0 (12.0 – 26.5)	26.7 (13.6 – 44.1)	65.9 (39.0 – 73.9)	43.3 (19.1 – 72.6)
Liver fat fraction (%)	0.6 (0.4 – 1.3)	0.39 (0.08 – 1.76)	2.32 (0.86 – 6.81)	2.07 (0.99 – 5.22)	0.35 (0.07 – 1.41)	0.50 (0.06 – 1.29)

Results shown are median (interquartile range) or count (percentage).

Abbreviations: BMI, body mass index; ArLD, alcohol-related liver disease; NAFLD, non-alcohol-related fatty liver disease; AST, aspartate aminotransferase; MELD, modified end stage liver disease score; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

5.6.3 %rT1 in participants with and without liver dysfunction

Using all participants, the %rT1 was lower in participants with (n = 79) than without (n = 90) liver dysfunction (47.8±1.2% vs 61.4±0.7%, p<0.001). In cirrhosis groups alone, %rT1 was still lower with liver dysfunction (48.9±1.2 %) than with normal liver function (60.2±0.9%, p<0.001; figure

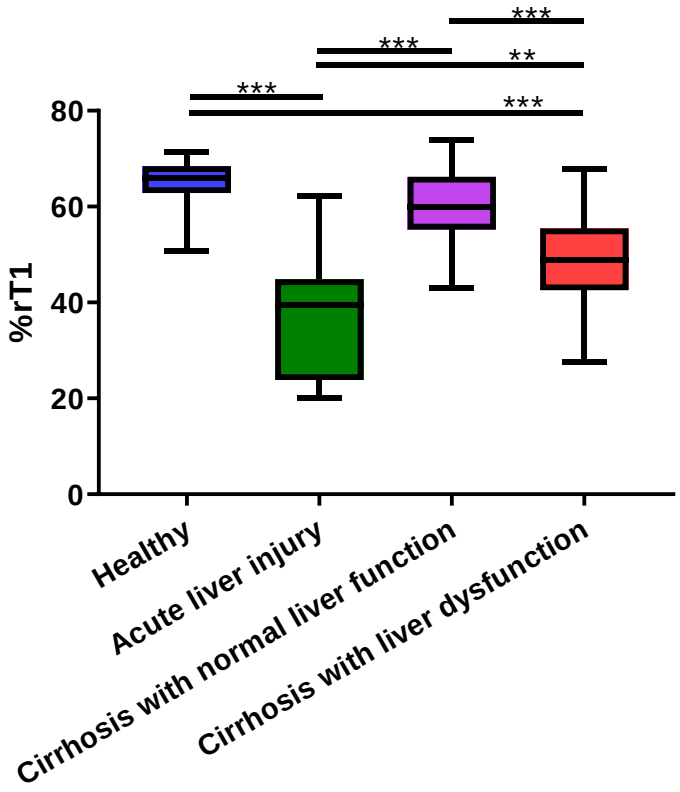


Figure 25. The percentage reduction in liver T1 with gadoxetic acid (%rT1) in healthy volunteers (n = 24) and patients with acute liver injury (n = 7), cirrhosis with normal liver function (n = 66) and cirrhosis with liver dysfunction (n = 72).

There were significant differences between all group pairings except healthy and compensated cirrhosis. Lines represent median, interquartile range, maximum and minimum. Significant differences between groups are shown. *p<0.05, **p <0.01, ***p<0.001.

25). As expected, healthy participants had higher median %rT1 (66.1, IQR 62.8 – 68.4%) than acute liver injury patients (39.4 IQR 23.8 – 44.8%, p <0.001) and decompensated cirrhosis patients (49.0, IQR 42.6 – 44.5%, p<0.001).

Cirrhotic status did not appear to affect %rT1 as healthy participants did not have significantly different %rT1 compared with cirrhosis with normal liver function participants (60.0, IQR 55.3 – 66.3%, p = 0.125), and acute liver injury patients had lower %rT1 than cirrhosis with liver dysfunction patients (p = 0.005).

5.6.4 Diagnostic accuracy of %rT1 to identify liver dysfunction

%rT1 could differentiate participants with and without liver dysfunction with AUROC 0.856 (95% CI 0.80 – 0.91, p <0.001) (figure 26). A %rT1 cut off of <55.05% had 0.76 sensitivity and 0.82 specificity.

Using only patients with cirrhosis, %rT1 could differentiate participants with and without liver dysfunction with AUROC 0.818 (95% CI 0.75 – 0.89, p <0.001) (figure 27). A %rT1 cut off of <55.05% had 0.75 sensitivity and 0.78 specificity.

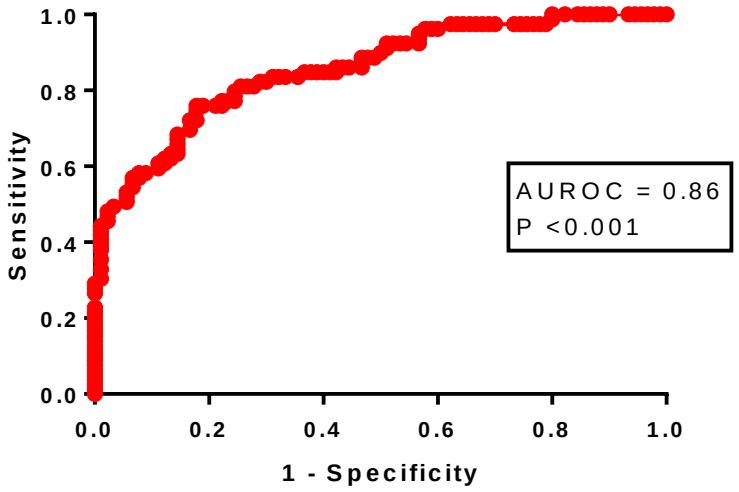


Figure 26. Receiver operator curve of %rT1 in all participants with (n = 79) and without liver dysfunction (n = 90).

%rT1 could identify liver dysfunction with area under the receiver operator curve (AUROC) 0.86, p <0.001.

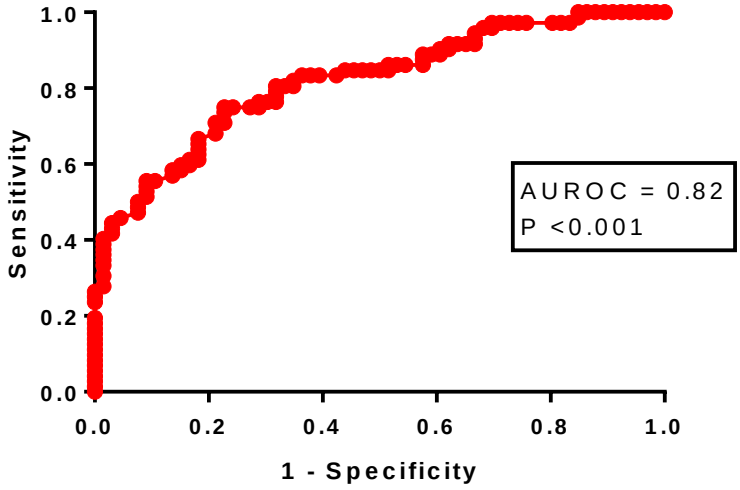


Figure 27. Receiver operator curve of %rT1 in patients with cirrhosis with (n = 72) and without (n = 66) liver dysfunction.

%rT1 could identify liver dysfunction with area under the receiver operator curve (AUROC) 0.82, p <0.001.

5.6.5 Associations with %rT1

The %rT1 reduced with increasing liver dysfunction measures MELD (r = -0.58), Child Pugh score (r = -0.59), bilirubin (r = -0.64) and prothrombin time (r = -0.44), and increased with albumin (r = 0.46) ($p < 0.001$ for all; figure 28, table 20). Significant correlations also existed between %rT1 and AST (r = -0.31), liver stiffness (r = -0.45), liver cT1 (r = -0.34), platelets (r = 0.32), spleen ECV (r = -0.22) and platelet/spleen volume ratio (r = 0.47). There was no significant correlation between %rT1 and liver volume.

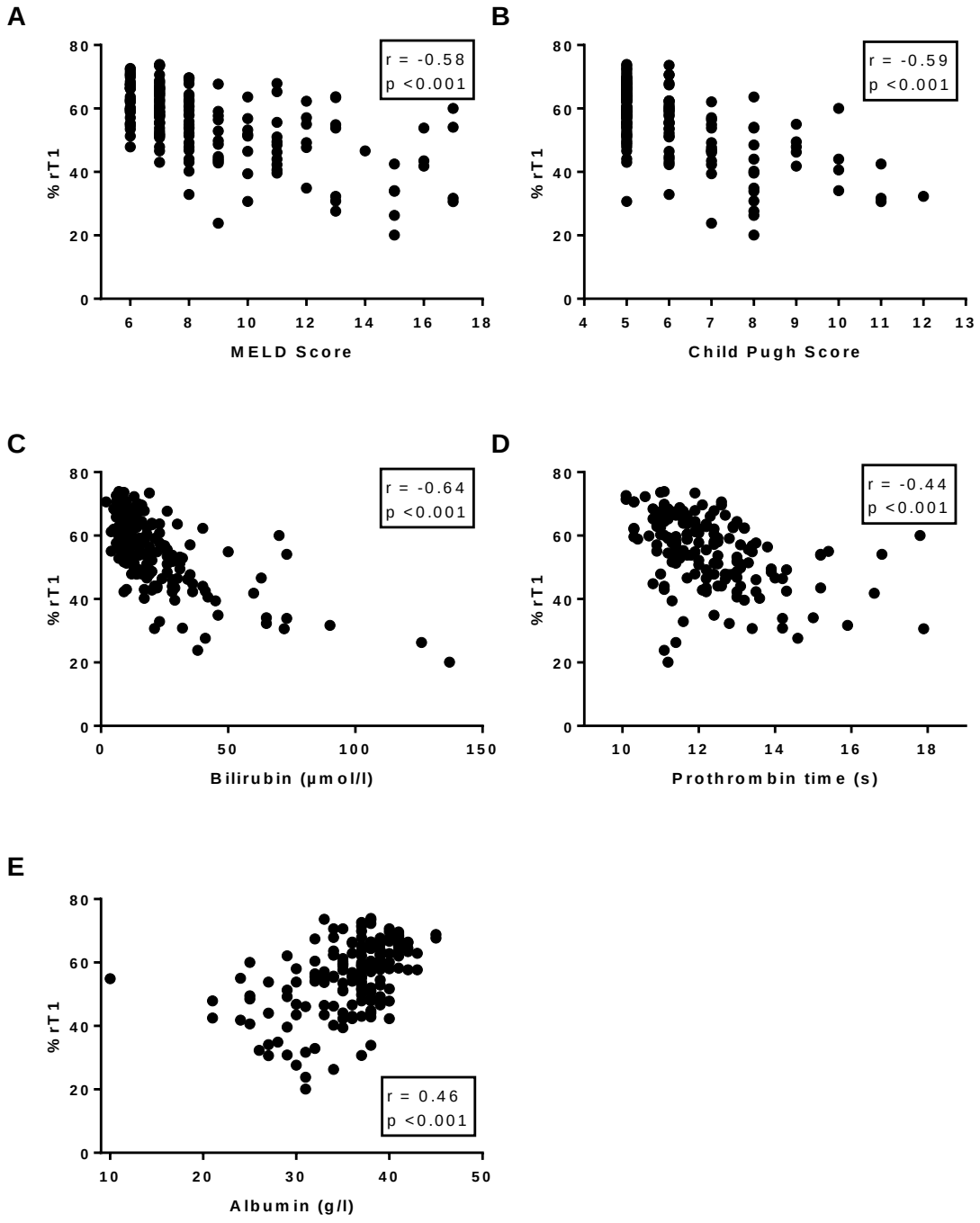


Figure 28. Associations of %rT1 with measures of liver function (n = 169).

%rT1 had significant correlations with (A) MELD (r = -0.58), (B) Child Pugh score (r = -0.59), (C) bilirubin (r = -0.64), (D) prothrombin time (r = -0.44) and (E) albumin (r = 0.46) (p <0.001 for all).

Table 20. Associations of %rT1 with other liver-related measures (n = 169)

	Correlation with %rT1 (r)	p
Liver function measures		
MELD	-0.58	<0.001
Child Pugh	-0.59	<0.001
Bilirubin	-0.64	<0.001
Albumin	0.46	<0.001
Prothrombin time	-0.44	<0.001
Other factors		
Age	-0.04	0.607
BMI	0.06	0.447
Creatinine	0.07	0.349
AST	-0.31	<0.001
Liver stiffness	-0.45	<0.001
Liver cT1	-0.34	<0.001
Liver fat fraction	0.07	0.386
Liver volume	-0.14	0.080
Platelets	0.32	<0.001
Spleen T1	-0.13	0.103
Spleen cT1	-0.11	0.178
Spleen ECV	-0.22	0.006
Platelet/spleen volume	0.47	<0.001

Abbreviations: MELD, modified end stage liver disease score; BMI, body mass index; and AST, aspartate aminotransferase.

The variables with significant correlations with %rT1 were entered into stepwise, backward multiple linear regression. The independent predictors of %rT1 were identified as bilirubin, liver stiffness, AST and albumin and could be used to model %rT1 with $R^2 = 0.51$ ($p < 0.001$; table 21).

Table 21. Multiple linear regression for the prediction of %rT1

Model	B	Std. Error	Beta	t	p
(Constant)	54.86	6.25		8.78	<0.001
Bilirubin	-0.27	0.04	-0.45	-6.99	<0.001
Liver stiffness	-0.14	0.03	-0.29	-4.45	<0.001
AST	-0.03	0.01	-0.20	-3.25	0.004
Albumin	0.32	0.16	0.14	2.08	0.040

The equation for predicting linear %rT1 based on this regression analysis is:
Predicted %rT1 = 54.86 – 0.27*bilirubin – 0.14*liver stiffness – 0.03*AST + 0.32*albumin
Abbreviations: AST, aspartate aminotransferase

5.6.6 The influence of liver volume on %rT1's association with MELD

Multiple stepwise linear regression improved the prediction of MELD using %rT1 from $R^2 = 0.336$ ($p<0.001$) to $R^2 = 0.446$ ($p<0.001$) by adjusting %rT1 for liver volume and estimated liver volume (table 22). The best resultant model was:

MELD = 15.43 – 0.154*%rT1 - 0.001*liver volume + 0.002*estimated liver volume

Table 22. Multivariable linear regression models using %rT1 and liver volume for the estimation of MELD as a gold standard for liver function

	B	p	R^2
Model 1			
%rT1	-0.145	<0.001	0.336
Model 2			
%rT1	-0.135	<0.001	0.396
Liver volume	-0.001	0.007	
Model 3			
%rT1	-0.154	<0.001	0.446
Liver volume	-0.001	<0.001	
Estimated liver volume	0.002	<0.001	
Model 4			
%rT1	-0.158	<0.001	0.424
Liver volume/estimated liver volume	-2.540	<0.001	

5.6.7 Assessment of the contribution of liver fibrosis, liver function, and portal hypertension to different clinical phenotypes of liver disease

All liver disease phenotypes could be differentiated from each other by combinations of liver cT1, spleen ECV and %rT1 apart from patients with cirrhosis and ascites versus liver dysfunction only and HE (table 23, figure 29). Patients with acute liver injury were characterised by significantly lower %rT1 than healthy volunteers ($p < 0.001$), but similar liver cT1 and spleen ECV as expected for the presence of significant liver dysfunction and absence of cirrhosis or portal hypertension. Patients with cirrhosis with normal liver function had significantly higher liver cT1 than healthy volunteers ($p < 0.001$), but similar spleen ECV and %rT1. Higher liver cT1, higher spleen ECV and lower %rT1 was observed in cirrhosis with liver dysfunction only, ascites and HE groups than in healthy volunteers. Patients with cirrhosis with ascites had higher liver cT1 and spleen ECV and lower %rT1 than patients with cirrhosis with normal liver function. Similar trends existed between

patients with cirrhosis with ascites versus patients with cirrhosis with liver dysfunction only, but did not reach statistical significance. Patients with cirrhosis with HE had liver cT1 higher still than patients with cirrhosis with normal liver function and cirrhosis with liver dysfunction only, whilst no significant differences compared with the ascites group.

Ascites was present in 8 (73%) of the HE group. When the 2 patients without ascites in the HE group are excluded, there is a trend towards %rT1 being lower in the HE than the ascites group (39.9±6.7 vs 46.7±9.7, p = 0.066), whereas spleen ECV and liver cT1 were not significantly different between groups (p = 0.946 and p = 0.618, respectively). In order to test the contribution of portal hypertension to HE vs ascites further, platelet/spleen volume was calculated. Platelet/spleen volume was lower in cirrhosis with HE than ascites (p = 0.028), indicating worse portal hypertension in HE than ascites.

Of note, liver cT1 was not significantly lower in acute liver injury than cirrhosis with normal liver function or cirrhosis with liver dysfunction only groups as would be expected for the difference in cirrhotic status. The liver cT1 of the acute liver injury group appears to sit between the healthy and cirrhosis with normal liver function groups, perhaps due to inflammation rather than fibrosis raising liver cT1.

Table 23. MR markers in different phenotypes of liver disease

	Healthy N = 24	Acute liver injury N = 7	Cirrhosis with normal liver function N = 66	Cirrhosis with liver dysfunction only N = 39	Cirrhosis with ascites N = 22	Cirrhosis with hepatic encephalopathy N = 11	P
Liver cT1 (ms)	697.1 (636.4 – 755.7)	730.7 (719.4 – 840.5)	827.8 (782.3 – 922.7)	837.4 (770.4 – 917.4)	982.5 (903.1 – 1034.2)	881.5 (842.3 – 1051.0)	<0.001
Spleen ECV	0.338 (0.324 – 0.352)	0.328 (0.326 – 0.338)	0.344 (0.319 – 0.378)	0.380 (0.347 – 0.407)	0.438 (0.403 – 0.462)	0.451 (0.372 – 0.460)	<0.001
Platelet/spleen volume	1.23 (0.94 – 1.86)	0.64 (0.26 – 0.78)	0.40 (0.23 – 0.72)	0.17 (0.10 – 0.33)	0.23 (0.13 – 0.44)	0.11 (0.07 – 0.14)	<0.001
%rT1	66.1 (62.8 – 68.4)	39.4 (23.8 – 44.8)	59.8 (55.3 – 66.3)	53.5 (46.6 – 58.0)	47.3 (40.5 – 54.1)	42.5 (32.3 – 44.0)	<0.001

Results shown are the median and interquartile range. P values relate to analysis of variance between groups using ANOVA for parametric data and Kruskal-Wallis test for non-parametric data.

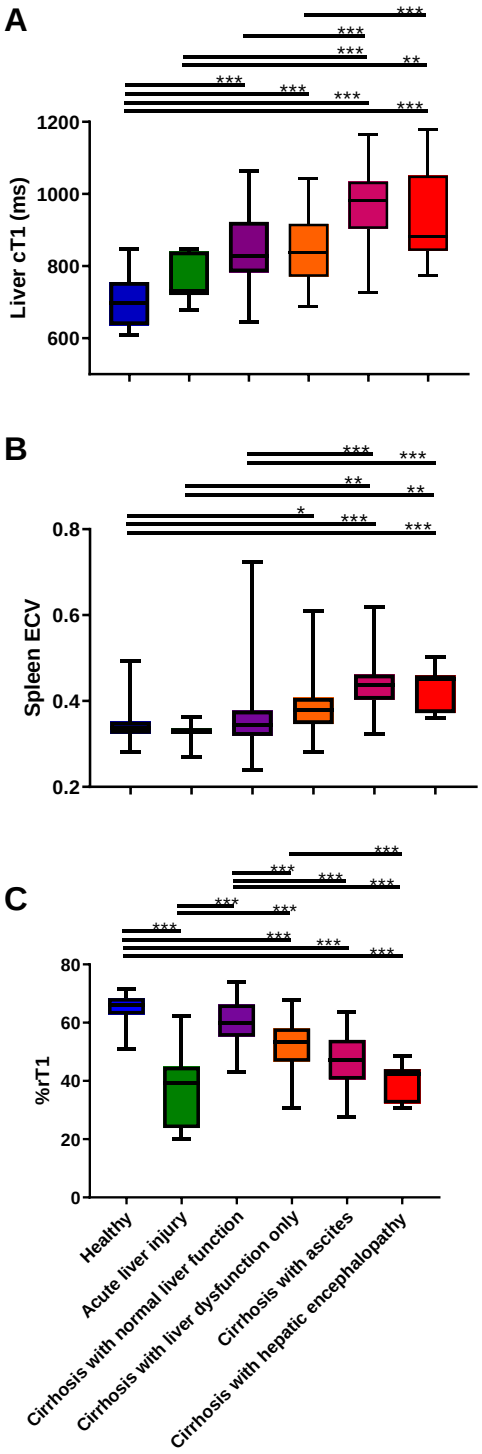


Figure 29. Liver cT1 (A), spleen ECV (B) and percentage reduction in liver T1 with gadoxetic acid administration (%rT1) (C) by liver disease phenotype.

Significant differences between groups are shown for *p<0.05, **p <0.01, ***p<0.001. Lines represent median, interquartile range, maximum and minimum.

5.7 Discussion

The results of this chapter support that %rT1 reflects liver function and can identify patients with liver dysfunction with high accuracy (AUROC 0.86). %rT1 correlated with the simple blood markers of liver function bilirubin (r = -0.64), prothrombin time (r = -0.44) and albumin (r = -0.46) and the composite liver function scores MELD (r = -0.58) and Child Pugh (r = -0.59). Liver function, liver fibrosis and portal hypertension could be assessed in a single MR scan by %rT1, liver cT1 and spleen ECV, respectively.

The observed correlations of %rT1 with liver function scores were similar to those obtained by Besa et al. (r = -0.70 with MELD and r = -0.56 with Child Pugh [163]) and Ding et al. (r = -0.573 with MELD [123]). Such consistent results despite different T1 mapping sequences and MRI scanners indicates %rT1 may be comparable across hospitals if translated into clinical care. This would need testing in future studies.

Of the liver function blood parameters explored, %rT1 correlated closest with bilirubin. This is likely because both gadoxetic acid and bilirubin are transported into hepatocytes via OATP and excreted via MRP2 [164]. ICG clearance uses this pathway also and has moderate (r = 0.46) correlation with liver perfusion [86]. Liver perfusion may therefore contribute to excretory liver function in addition to hepatocyte function. Liver synthetic function parameters correlated less well with %rT1, with prothrombin time being removed from the multivariable regression model for %rT1, whilst bilirubin and albumin remained.

%rT1 also reduced with increasing AST and liver stiffness. Likewise, Verloh et al. found that relative liver enhancement with gadoxetic acid was influenced by AST [165]. AST likely reflects liver inflammation, whilst liver stiffness could reflect liver fibrosis and/or inflammation [59]. Other studies have reported a correlation of r = -0.26 between %rT1 and fibrosis stage [166] and 0.86 AUROC for the identification of cirrhosis [163]. Both of these studies, however, were retrospective and did not account for different liver function in patients with higher fibrosis stage.

My results indicate liver inflammation may be the dominant influence to reduce %rT1 given %rT1 was unchanged by cirrhosis status in group comparisons with normal liver function and liver cT1 as a marker of fibrosis was not an independent predictor of %rT1. Liver function could therefore be underestimated by %rT1 in the presence of liver inflammation.

Asenbaum et al. found relative liver enhancement with gadoxetic acid had a weak ($R^2 = 0.18$), but significant correlation with portal pressure [167]. My results also showed associations of %rT1 with portal hypertension measures (spleen ECV, platelet count to spleen volume ratio), but these were not independent associations, perhaps due to shared elements of the underlying pathophysiology.

The incorporation of liver volume and estimated liver volume improved the performance of %rT1 in a model for MELD from $R^2 = 0.336$ to $R^2 = 0.446$. This likely represents a stronger association with liver function than the correlation with MELD implies, because the renal function component of MELD limits the best possible fit of the model. In the model, a greater discrepancy between the measured and estimated liver volumes worsened liver function in addition to %rT1 itself, indicating both the relative quantity and quality of liver tissue dictate overall liver function. These findings are in keeping with other studies [125, 165, 168] and the common clinical finding of a shrunken liver in end stage liver disease.

%rT1 as a marker of liver function could have future applications in prognostication for patients with liver disease, which will be investigated in the next chapter, or for dose-adjustment of drugs in liver disease. In addition to the assessment of total liver function, %rT1 could also be used to assess regional differences in liver function, which may be particularly useful in planning hepatectomy or liver ablative therapies. This functionality makes %rT1 as an imaging-based technique superior to blood-based or drug clearance-based tests, which can only measure global liver function. Such functionality was not tested here and would need investigating in further studies.

Liver fibrosis by liver cT1, portal hypertension by spleen ECV and liver function by %rT1 were assessed in a single MRI scan. All liver disease phenotypes could be distinguished from others by their liver cT1, spleen ECV and %rT1 in combination except for cirrhosis with liver dysfunction only vs ascites and cirrhosis with ascites vs HE. Although not reaching statistical significance, liver cT1 was numerically lower in patients with acute liver injury than those with cirrhosis with normal liver function and cirrhosis with liver dysfunction only. This may be because significant inflammation, as indicated by high ALT and AST, in acute liver injury increases liver water content. There were apparent trends for worsening of liver fibrosis, portal hypertension and liver function in the progression of cirrhosis with liver dysfunction only to ascites, and worsening portal hypertension and liver function in the progression of cirrhosis with ascites to HE. Greater samples sizes and more complex modelling are needed to explore how liver fibrosis, portal hypertension and liver function measures contribute to the spectrum of liver disease phenotypes.

5.7.1 Limitations

A limitation of this and other studies of liver function assessment is that there is no established definition of liver dysfunction nor a gold standard measure of liver function. A number of blood-based parameters and composite scores were therefore used to correlate with %rT1. MELD was used as the standard to model %rT1, liver volume and estimated liver volume against, but MELD also incorporates renal function, which reduces the best possible fit of a liver function model. MELD has also been found inferior to ICG clearance in the prediction of post hepatectomy liver failure [169], indicating it may not be the best measure of liver function. ICG is excreted by the liver using similar transport pathways to gadoxetic acid and %rT1 and ICG clearance have been correlated closely [125]. The clinical relevance of %rT1 will be tested in the next chapter.

Gadoxetic acid was dosed at 0.1 ml/kg up to a maximum dose of 10 ml as per the manufacturer's instructions. Patients >100 kg therefore would have had a relatively lower dose, which may therefore have affected %rT1 and reduced its correlation with liver function measures.

Renal function is also known to affect the excretion of gadoxetic acid [119], however, creatinine did not have a significant influence on %rT1 in this study. For safety reasons, patients were excluded who had eGFR <30 mL/min/1.73 m^2, which may be why no association with creatinine was seen. This exclusion criterion also reduces the number of patients to which the test can be applied, particularly in more advanced disease where renal dysfunction becomes more prevalent.

The OATP and MRP2 excretion pathway used by gadoxetic acid is common to many molecules. Although some commonly used medications altered liver enhancement by gadoxetic acid in rats [170], no such changes have been found in humans when erythromycin was tested as a drug representative of OATP inhibitors [171]. Drug interactions are therefore unlikely to affect %rT1.

%rT1 was analysed in 3 circular regions of interest (ROI) selected away from organ boundaries, large vessels and bile ducts. As heterogeneity of gadoxetic acid uptake may increase in cirrhosis and diseases such as PSC relative to healthy controls [172, 173], the use of ROIs to measure overall liver function may have been subject to sampling error. The total area sampled was 4.5 cm^2, which is a reasonable size and relatively simple changes to analysis software could enable whole liver slice analysis in the future.

The study was not powered to address differences in MR markers between liver phenotype groups and recruitment fell short of target by 36 patients, so true differences may exist between ascites and liver dysfunction only and HE groups that did not reach statistical significance.

5.7.2 Conclusions

%rT1 is a non-invasive measure of liver function that can be improved by incorporation of liver volume and estimated liver volume, but can be underestimated in the presence of liver inflammation. A single MRI scan can assess liver fibrosis by liver cT1, portal hypertension by spleen ECV and liver function by %rT1, which change in different phenotypes of liver disease.

6 Prediction of clinical outcomes in patients with cirrhosis

6.1 Abstract

Risk stratification for liver-related outcomes is important for the management of patients with cirrhosis. This prospective cohort study aimed to explore the ability of magnetic resonance imaging (MRI)-based markers of a.) liver fibrosis (liver cT1); b.) portal hypertension (spleen ECV, platelet count/spleen volume ratio); and c.) liver function (%rT1) for the prediction of liver-related outcomes.

One hundred and nine patients with cirrhosis of mixed aetiologies were followed up by telephone consultation and medical record review for ≥6 months following MRI with gadoxetic acid administration, clinical, blood and transient elastography assessments. Time to first development of a composite endpoint comprising new variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular cancer, liver transplantation or liver-related death was recorded. The association of MRI and other non-invasive liver disease markers with the composite outcome was assessed by Kaplan Meier and Cox regression univariate and multivariable analyses.

The cohort comprised 75% males and 72% with compensated advanced chronic liver disease (cACLD) aged mean 60 (s.d. 10) years. Twenty-two patients (20%) reached the composite endpoint with median follow up 447 days (IQR 250 – 601). Spleen ECV >0.36 and %rT1 <55.05% were associated with reduced cumulative endpoint-free survival (log rank 8.936, p = 0.003 and log rank 13.219, p<0.001, respectively). MRI-based markers of portal hypertension (spleen ECV, HR 1.108, p <0.001; platelet count/spleen diameter ratio, HR 0.380, p = 0.050; spleen T1 x spleen diameter, HR 1.000, p = 0.034) and liver function (%rT1, HR 0.943, p = 0.002) were predictive of

the composite endpoint in univariate, but not multivariable analysis. The MR marker of liver fibrosis was not predictive of liver-related outcomes (liver cT1, HR 1.003, p = 0.117). The independent predictors of the composite endpoint in multivariable analysis were age (p = 0.003), albumin (p <0.001), sodium (p <0.001) and APRI (p = 0.053).

In conclusion, MRI-based markers of portal hypertension and liver function are predictive of liver-related outcomes, but do not give additional prognostic benefit to age and serum-based markers over the short term.

6.2 Introduction

Risk stratification for clinical outcomes is key to help guide patient management. Important clinical outcomes include the development of varices, variceal bleeding, ascites, hepatic encephalopathy, hepatocellular carcinoma and death. So far, this thesis has concentrated on assessing current liver disease state. Preliminary findings of the ability of MRI-based markers of liver fibrosis, portal hypertension and liver function to predict liver-related clinical outcomes in cirrhosis will be described in this chapter.

Fibrosis stage is well known to predict outcomes in chronic liver disease [4], but even when cirrhosis is established, mortality varies widely from 1 to 57% at 1 year [39] and 1.5 to 88% at 5 years [174]. Clinical staging can help risk stratify patients. In a staging system proposed by D'Amico et al., cirrhosis that is compensated[2] without varices (stage 1); compensated with varices (stage 2); compensated with bleeding varices (stage 3); decompensated by one non-bleeding complication (stage 4); and decompensated by any second complication (stage 5) have 5-year

[2] The term *compensated* used in this staging system refers to the absence of jaundice, variceal bleeding, ascites, hepatic encephalopathy or hepatocellular carcinoma, as used to define compensated advanced chronic liver disease (cACLD).

mortality of 1.5%, 10%, 20%, 30% and 88%, respectively [174]. Only variceal bleeding, moderate-large ascites and hepatic encephalopathy may be detected clinically without the use of additional tests, so there is a need for non-invasive prognostic markers. These should be based on the underlying pathology and be able to predict varices and other liver-related events and survival.

Once cirrhotic, the quantity of liver fibrosis, measured as the collagen proportionate area (CPA), is predictive of oesophageal varices and decompensation events [10, 175]. CPA correlates with HVPG [9, 176] and so this may be part of the mechanism by which it predicts complications. CPA has been correlated with ELF (r 0.51 [177], r 0.41 [77]), liver cT1 (r 0.33) [77] and liver stiffness by transient elastography (R^2 0.56 [178], r 0.52 [77]), ARFI (R^2 0.46) [179] and shear wave elastography (r 0.63) [180]. Non-invasive liver fibrosis markers may therefore be predictive of decompensation events.

Liver stiffness has been shown to predict hepatic decompensation, HCC and death [56]. Serum scores of fibrosis FIB-4, APRI and FibroTest (combines 5 serum markers) have predicted survival in a hepatitis C cohort, but FIB-4 and APRI were less prognostic than liver stiffness [181]. AST/ALT has not been found to predict survival [39]. Liver cT1, categorised into the liver inflammation and fibrosis (LIF) score, can predict clinical events with hazard ratio 9.7, but with wide 95% confidence intervals (2.2 – 43.2) [74], so the prognostic value of liver cT1 requires further investigation.

Portal pressure measurements may allow more specific prognostication in cirrhosis. In support of this, HVPG is predictive of decompensation events [15, 16] and death [182], and has been found to be more predictive of decompensation than fibrosis stage in patients with recurrent hepatitis C post liver transplant [183]. Multiple non-invasive portal hypertension markers such as spleen stiffness have also been reported to be independent predictors of decompensation [102, 184] and survival [39] in compensated cirrhosis. In chapter 5, I found spleen ECV and platelet count/spleen volume ratio to be the most accurate MR markers of portal hypertension and also spleen T1, spleen cT1 and spleen T1 x spleen diameter had AUROC >0.80 for the identification of

varices needing treatment. These non-invasive MR markers need testing for their prognostic significance.

Liver function markers are also predictive of liver-related outcomes. Albumin predicts liver-related outcomes in chronic hepatitis C infection with HR 0.8 [4]. Child Pugh score and its components (albumin, bilirubin, prothrombin time, ascites and hepatic encephalopathy) and age have been found commonly to predict mortality in cirrhosis [39]. MELD, MELD-Na and UKELD are also predictive of mortality (discussed in section 1.2).

Ripoll et al. found that both HVPG and MELD could predict decompensation in patients with compensated cirrhosis, but only MELD could predict death in decompensated patients [42]. This indicates liver and renal function parameters may supersede portal pressure's prognostic ability once decompensation has occurred.

The MRI-based liver function marker %rT1 (chapter 6) has not been investigated for the prediction of liver-related events, but other techniques that also use OATP and MRP2 hepatic excretion pathways have. ICG-15 is predictive of decompensation events in patients with Child Pugh A cirrhosis [116] and of mortality in patients with decompensated hepatitis B, but was not superior to MELD, MELD-Na or Child Pugh [185]. Using MRI, low relative liver enhancement with gadoxetic acid was associated with poor transplant-free survival at 3 years, independent of Child Pugh and MELD [167]. Sandrasegaran et al. found that relative liver enhancement at 15 minutes post gadoxetic acid could predict variceal bleeding and hepatic encephalopathy in cirrhosis, whilst a contrast enhancement spleen index (which uses liver and spleen signal intensities pre and post gadoxetic acid) could predict 2 year mortality, variceal bleeding and hepatic encephalopathy [186]. A combination of liver function and portal hypertension measures may therefore predict liver-related outcomes effectively.

6.3 Hypothesis

Liver fibrosis, liver function and portal hypertension-related measures derived from magnetic resonance imaging with gadoxetic acid administration can risk stratify patients with cirrhosis for clinical liver-related events.

6.4 Aims

I aim to explore if measures derived from magnetic resonance imaging with gadoxetic acid administration in patients with cirrhosis can predict a composite outcome comprising new variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular cancer, liver transplantation or liver-related death. The main MR measures of interest include liver cT1 for liver fibrosis, spleen ECV for portal hypertension and %rT1 for liver function.

I also aim to explore the predictive ability of other MR markers of portal hypertension/varices, serum markers and scores of liver disease for the outcomes listed above.

6.5 Methods

This chapter was part of the MAPLE study. A detailed description of methods is provided in chapter 2. Patients were recruited from the John Radcliffe Hospital and Southampton General Hospital between June 2016 and February 2018. The study is on-going, so this chapter presents preliminary results accrued by August 2018.

6.5.1 Participants

All patients with cirrhosis (cirrhosis with normal liver function, cirrhosis with liver dysfunction only, cirrhosis with ascites and cirrhosis with hepatic encephalopathy) who had at least 6 months follow up were used in this analysis. Patients who had TIPSS were excluded because the TIPSS changes their prognosis.

6.5.2 Assessments and follow up

All patients were assessed by MRI with gadoxetic acid administration, blood tests and transient elastography at baseline. The MR markers of interest were liver cT1 as a measure of fibrosis; spleen ECV and platelet count/spleen volume ratio as markers of portal hypertension and also spleen T1, spleen cT1 and spleen T1 x spleen diameter as markers of varices needing treatment (chapter 5); and %rT1 as a measure of liver function (chapter 6).

Patient's investigations and treatments following the baseline assessments were provided by clinicians as part of routine clinical care, blinded to the baseline study results.

I followed up participants by review of hospital medical records and telephone consultation every 6 months following their baseline assessments for the development of the primary composite endpoint comprising new variceal bleeding, ascites, spontaneous bacterial peritonitis (SBP), hepatic encephalopathy, hepatocellular cancer (HCC), liver transplantation or liver-related death. Patients who had already experienced a component of the primary endpoint at baseline were not considered to reach the primary endpoint if they had a repeat episode of the same condition during follow up e.g. a patient with previous HE who has a further episode of HE during follow up. A composite endpoint was chosen to increase the power of this exploratory study. The

components of the primary endpoint will form secondary endpoints when a longer follow up period has been achieved, but are not analysed here.

Alcohol consumption during the follow up period was also reported by patients and sought from medical records. Alcohol misuse during follow up was defined as >14 units/week for women or >21 units/week for men on a regular basis or at least one period of heavy binge drinking. Alcohol history was obtained as it is known to influence liver-related outcomes.

6.5.3 Statistical methods

Endpoint-free survival analysis was conducted using Kaplan Meier curves to determine the influence of spleen ECV, platelet count/spleen volume ratio and %rT1 categories on the times to the primary endpoint. The cut-off used for spleen ECV was >0.36, for platelet count/spleen volume ratio was <0.28 (both derived in section 4.6.4 to identify portal hypertension) and for %rT1 was <55.05% (derived in section 5.6.4 to identify liver dysfunction). Liver cT1 was not analysed by Kaplan Meier curves as it does not have an established cut-off in the presence of cirrhosis.

Individual non-invasive markers of portal pressure, liver function and liver fibrosis were assessed with univariate Cox regression for their influence on the primary endpoint. In all analyses patients were censored at their first new liver-related endpoint. Markers identified as predictive in univariate analysis ($p<0.05$) were then analysed using multivariable Cox regression to identify independent markers for the prediction of the primary endpoint. In this analysis, markers that did not make significant contributions to the model ($p >0.10$) were excluded stepwise to produce the final model.

6.6 Results

6.6.1 Participants

One hundred and nine patients completed ≥6 months follow up by August 2018. The median follow up duration was 447 days (IQR 250 – 601). All patients had their medical records reviewed and so were included in analysis, however, 4 patients (4%) were lost to their final telephone consultation.

The patient cohort comprised 52 (48%) cirrhosis with normal liver function, 30 (28%) cirrhosis with liver dysfunction only, 18 (17%) cirrhosis with ascites and 9 (8%) cirrhosis with hepatic encephalopathy. Their liver disease aetiologies were 47% alcohol, 23% NAFLD, 17% viral 14% autoimmune and 5% other.

Patients had mean age 60 years and 75% were male. At baseline, 72% had compensated advanced chronic liver disease (cACLD), 52% of patients had liver dysfunction and 61 (56%) had portal hypertension complications (table 24). Seven patients (6%) drank excess alcohol at baseline.

Eighty-one (74%) of the follow up cohort had valid results for all non-invasive markers. Valid non-invasive marker results were unavailable for 1 liver cT1 and T2*, 18 liver stiffness, 2 spleen volume and platelets/spleen volume ratios, 1 spleen diameter and platelets/spleen diameter ratio, 5 spleen cT1s and 8 spleen ECVs.

Table 24. Baseline characteristic of the patients followed up for clinical outcomes

Demographics	Patients with cirrhosis n = 109
Age (years)	60 (10)
Male (%)	82 (75%)
BMI (kg/m^2)	28.6 (5.7)
Alcohol (units/week)	0 (0 – 4)
Decompensation status at or prior to baseline	
cACLD	78 (72%)
Liver dysfunction	57 (52%)
Varices	53 (49%)
Variceal bleed	8 (7%)
Ascites	27 (25%)
Spontaneous bacterial peritonitis	3 (3%)
Hepatic encephalopathy	9 (8%)
Blood markers	
Bilirubin (µmol/l)	17 (10 – 28)
ALT (IU/l)	28 (22 – 41)
AST (IU/l)	40 (29 – 57)
Albumin (g/l)	36 (33 – 38)
Creatinine (µmol/l)	68 (60 – 83)
Platelets count (x10^9/l)	133 (103 – 175)
Prothrombin time (s)	12.1 (11.4 – 13.1)
Non-invasive scores	
Child Pugh	6 (5 – 7)
MELD	8 (7 – 11)
AST/ALT	1.24 (1.00 – 1.67)
APRI	0.75 (0.48 – 1.17)
FIB-4	3.42 (2.15 – 5.54)
Liver stiffness (kPa)	21.8 (13.2 – 47.2)
Liver cT1 (ms)	867.0 (114.0)
Spleen T1 (ms)	1319.8 (116.0)
Spleen cT1 (ms)	1277.5 (105.3)
Spleen ECV	0.368 (0.338 – 0.413)
Spleen volume (ml)	501.4 (331.9 – 749.9)
Platelet count/spleen volume	0.27 (0.13 – 0.48)
Spleen diameter	143.5 (126.3 – 166.8)
Platelet count/spleen diameter	0.92 (0.63 – 1.27)
%rT1	54.9 (47.7 – 60.4)

Results are presented as mean (standard deviation) if normally distributed, median (interquartile range) if non-parametric or count (percentage).

Abbreviations: BMI, body mass index; cACLD, compensated advanced chronic liver disease; ALT, alanine aminotransferase ratio; AST, aspartate aminotransferase; MELD, modified end

stage liver disease score; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

6.6.2 Clinical outcomes during the follow up period

Twenty-two patients (20%) developed the primary endpoint during the follow up period. They comprised new variceal bleeding (n = 4), ascites (n = 7), SBP (n = 4), hepatic encephalopathy (n = 4), HCC (n = 7), liver transplantation (n = 5) and death (n = 5). All deaths were liver-related. Of those who reached the primary endpoint, just 2 had cirrhosis with normal liver function at baseline, one of whom developed ascites and the other HCC. Of the 78 patients classified with cACLD, 10 (13%) developed the primary endpoint.

Four patients (4%) had successful hepatitis C treatment during follow up. No patients had a TIPSS or weight reduction surgery that may have influenced outcomes.

Alcohol misuse during the follow up period was present in 19 (17.4%) of patients and unknown in 1 patient, representing a return to harmful drinking in at least 13 patients. There was no significant difference in alcohol misuse during the follow up period between patients who did and did not reach the primary endpoint (18.2% vs 17.2%, Chi-square = 0.262, p = 0.877). No stratification for alcohol misuse in Kaplan Meier analysis was therefore necessary.

6.6.3 The effect of MRI-determined portal hypertension and liver dysfunction on the primary endpoint-free survival

There was lower cumulative primary endpoint-free survival in patients with MRI-defined portal hypertension than no portal hypertension using spleen ECV >0.36 (log rank 8.936, p = 0.003; figure 30), but not platelet count/spleen volume ratio <0.28 (log rank 0.193, p = 0.660; figure 31).

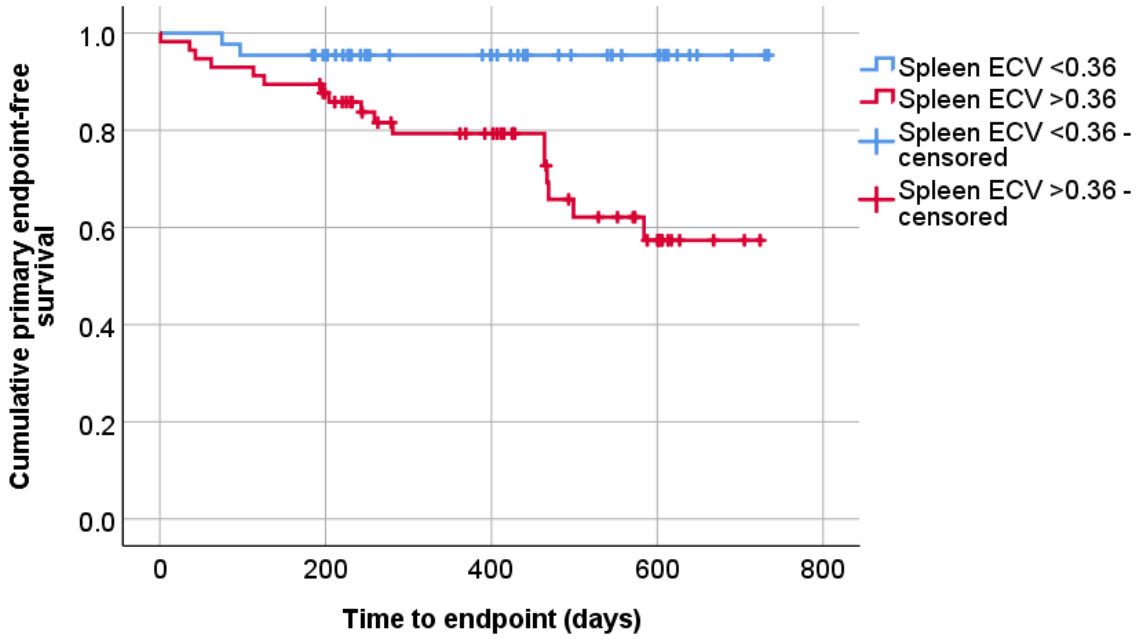


Figure 30. Kaplan Meier curves for patients with cirrhosis with spleen ECV-defined portal hypertension (spleen ECV >0.36; n = 57) and no portal hypertension (spleen ECV <0.36; n = 44) surviving free from the primary composite endpoint.

The cumulative primary endpoint-free survival was lower with spleen ECV >0.36 than spleen ECV <0.36 ($p = 0.003$).

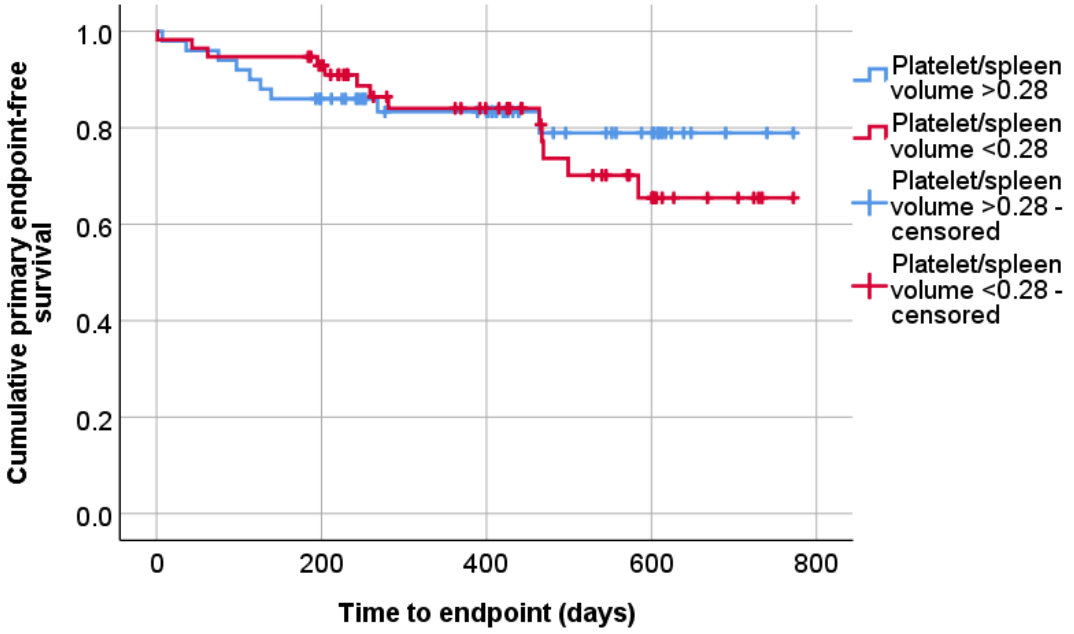


Figure 31. Kaplan Meier curves for patients with cirrhosis with platelet count/spleen volume ratio <0.28 (portal hypertension; n = 57) and platelet count/spleen volume ratio >0.28 (no portal hypertension; n = 50) surviving free from the primary composite endpoint.

There was no difference in the cumulative primary endpoint-free survival between groups (p = 0.660).

Patients identified by MRI to have liver dysfunction (%rT1 <55.05%) had a significantly lower cumulative primary endpoint-free survival than those with MRI-defined normal liver function (%rT1 >55.05%; log rank 13.219, p<0.001) (figure 32).

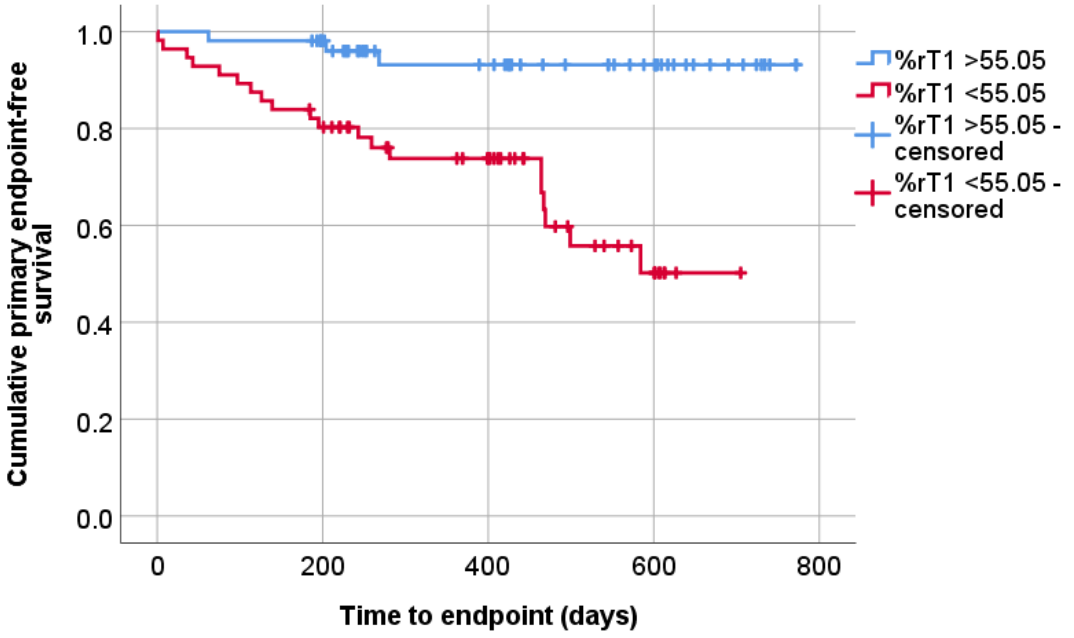


Figure 32. Kaplan Meier curves for patients with cirrhosis with and without liver dysfunction defined by %rT1 >55.05% (n = 53) and %rT1 <55.05% (n = 56) surviving free from the primary endpoint.

There was a significantly lower cumulative primary endpoint-free survival with %rT1 <55.05% than %rT1 >55.05% (p <0.001).

6.6.4 Cox regression analysis for predictors of the primary endpoint

In univariate Cox regression analysis, the MRI-based parameters spleen ECV*100 [3], platelet count/spleen diameter ratio, spleen T1 x spleen diameter and %rT1 had significant influences on primary endpoint occurrence (table 25). Liver cT1 was not associated with primary endpoint occurrence. Age and cACLD status also had significant hazard ratios (HRs) as expected. Blood

[3] The percentage spleen ECV (spleen ECV * 100) was used in Cox regression analyses due to the effect of values <1 in the log transformations

Table 25. Univariate Cox regression analysis for the primary endpoint

	HR	95% CI of HR	Univariate p
Demographics			
Age (years)	1.058	1.006 – 1.113	**0.030**
Sex	0.588	0.199 – 1.741	0.338
BMI (kg/m^2)	0.936	0.865 – 1.013	0.103
Clinical status			
Alcohol at baseline (units/week)	1.003	0.985 – 1.022	0.736
Alcohol misuse during follow up	1.200	0.405 – 3.556	0.742
Diabetes	1.044	0.424 – 2.572	0.925
Hypercholesterolaemia	1.391	0.513 – 3.773	0.517
cACLD	3.562	1.537 – 8.256	**0.003**
Blood and liver stiffness non-invasive measures			
Platelets count (x10^9/l)	0.991	0.983 – 1.000	**0.041**
Bilirubin (µmol/l)	1.021	1.003 - 1.039	**0.022**
Albumin (g/l)	0.854	0.791 – 0.922	**<0.001**
Prothrombin time (s)	1.311	1.069 – 1.607	**0.009**
Creatinine (µmol/l)	1.015	0.999 – 1.031	0.067
Sodium (mmol/l)	0.980	0.967 – 0.994	**0.004**
Child Pugh	1.487	1.221 – 1.790	**<0.001**
MELD	1.199	1.071 – 1.342	**0.002**
AST/ALT	2.304	1.277 – 4.159	**0.006**
APRI	1.483	1.199 – 1.833	**<0.001**
FIB-4	1.270	1.136 – 1.419	**<0.001**
Liver stiffness (kPa)	1.023	1.002 – 1.045	**0.035**
MR non-invasive measures			
Liver cT1 (ms)	1.003	0.999 – 1.006	0.117
Spleen T1 (ms)	1.004	1.000 – 1.007	0.081
Spleen cT1 (ms)	1.003	0.999 – 1.008	0.148
Spleen ECV*100	1.108	1.048 – 1.173	**<0.001**
Spleen volume (ml)	1.001	1.000 – 1.001	0.127
Platelet count/spleen volume	0.128	0.014 – 1.127	0.064
Spleen diameter (mm)	1.010	0.998 – 1.022	0.090
Platelet count/spleen diameter	0.380	0.144 – 1.002	**0.050**
Spleen T1 x spleen diameter (msmm)	1.000	1.000 – 1.000	**0.034**
%rT1	0.943	0.909 – 0.979	**0.002**

Significant p values are highlighted in bold.

Abbreviations: HR, hazard ratio; BMI, body mass index; cACLD, compensated advanced chronic liver disease; MELD, modified end stage liver disease score; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4

parameters of liver function, platelet count, sodium and scores for liver fibrosis also had significant effects. The liver prognostic scores MELD and Child Pugh had HRs of 1.20 and 1.49, respectively.

In multivariable Cox regression analysis, age (p = 0.003), albumin (p <0.001), sodium (p <0.001) and APRI (p = 0.053) were identified as independent prognostic factors (table 26). MRI-based parameters were not found to be independent predictors of the primary endpoint.

Table 26. Multivariable Cox regression analysis model for the primary endpoint

	HR	95% CI of HR	p
Age (years)	1.094	1.032 – 1.160	.002
Albumin (g/l)	0.852	0.780 – 0.931	.000
Sodium (mmol/l)	0.964	0.945 – 0.983	.000
APRI	1.343	0.996 – 1.810	.053

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio

6.7 Discussion

Non-invasive markers are needed to risk stratify patients with cirrhosis for the development of liver-related outcomes. Measures of liver fibrosis, portal hypertension and liver function are known to be prognostic, but the prognostic abilities of non-invasive MR markers of these factors are unknown. In this study, 109 patients with cirrhosis of varying severity who had a baseline MR scan were followed up for ≥6 months for the development of liver-related outcomes. The association of MR and other markers with time to develop liver-related outcomes was assessed.

MRI-derived spleen ECV, platelet count/spleen diameter ratio, spleen T1 x spleen diameter and %rT1 were predictive of liver-related outcomes in patients with cirrhosis. None of these were independent predictors of outcomes when assessed with age and serum-based predictors however. The independent predictors of clinical outcomes were age, albumin, sodium and APRI.

Contrary to the study hypothesis, liver cT1 was not predictive of outcomes in Cox regression analysis. This result contrasts with previous findings that liver cT1-derived LIF[4] was predictive of liver-related outcomes in 112 patients with mixed fibrosis stages (HR 9.7, 95% CI 2.2 – 43.2) [74]. Other studies have also reported liver cT1 ≥825ms to have HR 10.3 (95% CI 4.2 – 25.5; n = 166) for liver-related outcomes in patients with any liver fibrosis stage [187] and native liver T1 >793 ms (measured using fat suppressed spin-echo echo-planar imaging) to reduce clinical outcome-free survival in patients with compensated cirrhosis (p <0.001) [86]. My results indicate liver cT1 may have less prognostic value in established cirrhosis and may be more predictive in the earlier stages of liver disease. As liver cT1's association with CPA is weak (r = 0.33) [77], there may be influences of fat and inflammation that drive on-going liver disease that contribute to liver cT1's prognostic value in the earlier rather than the later stages of liver disease.

APRI (comprising AST and platelets) was the only non-invasive liver fibrosis score to show independent association with clinical outcomes in multivariable analysis. Other studies have shown the prognostic value of APRI in patients with mixed liver fibrosis stages [181, 188], whilst my study indicates its prognostic value continues in the cirrhotic range specifically. APRI has modest association with collagen proportionate area (R^2 = 0.40 [178]), and also fair association with portal hypertension (r = 0.365 with HVPG [83]), perhaps due to its use of platelet count. APRI's reflection of both liver fibrosis and portal hypertension may have given it the advantage over other non-invasive liver fibrosis tests such as liver stiffness, which correlates poorly with HVPG >10 mmHg [79, 80] and was not an independent predictor of clinical outcomes in my cohort.

Of the two MR markers of portal hypertension, spleen ECV >0.36, but not platelet count/spleen volume ratio <0.28 predicted lower cumulative endpoint-free survival in Kaplan Meier analysis. Similarly, spleen ECV was predictive of liver-related outcomes in univariate analysis (HR 1.108,

[4] LIF has continuous values 0-4

95% CI 1.048 – 1.173; p <0.001), but platelet count/spleen volume ratio was not. The poor performance of platelet count/spleen volume ratio was surprising given its strong performance in portal hypertension identification and varices needing treatment identification and exclusion (chapter 5), all of which are known to have prognostic significance. Also tested in univariate analysis were the MR markers of varices needing treatment in cACLD with >0.8 AUROC (chapter 5). Of these markers, platelet count/spleen diameter ratio and spleen T1 x spleen diameter were predictive of liver-related outcomes, although less significantly than spleen ECV (p = 0.050, p = 0.034 and p <0.001, respectively). Spleen T1, cT1, diameter and volume were not predictive of liver outcomes.

A possible explanation why spleen ECV was the most prognostic MR marker of portal hypertension is that splenic extracellular fluid may increase relatively more than splenic hyperplasia at higher portal pressures when decompensation of liver disease is more likely to occur. The extracellular fluid could be from venous congestion and also tissue oedema from hypoalbuminaemia as liver disease progresses. Spleen ECV measures extracellular fluid volume specifically, whereas T1 and cT1 measure total tissue fluid volume, and spleen size measures reflect both fluid expansion of tissues and hyperplasia.

The portal hypertension-related marker that remained prognostic in multivariable analysis was sodium, which has proven predictive of clinical outcomes in other studies [189, 190]. Pathophysiologically, systemic vasodilatation associated with portal hypertension causes salt and water retention, and later impaired water excretion, which causes sodium to fall. Hyponatraemia therefore tends to occur late in cirrhosis, and is associated with ascites, chronic diuretic use, hepatorenal syndrome and bacterial infections [191, 192].

MRI-based liver dysfunction (%rT1 <55.05) identified patients with significantly worse prognosis than those with %rT1 >55.05 (p <0.001). %rT1 was also predictive of liver-related events in univariate analysis (HR 0.943, 95% CI 0.909 – 0.979; p = 0.002). It performed similarly well to other markers of liver function including Child Pugh (p <0.001), MELD (p = 0.002) and

prothrombin time (p = 0.009), and perhaps better than bilirubin (p = 0.022) in univariate analysis. These results support the prognostic value of dynamic tests of the OATP and MRP2-based hepatic excretion pathways demonstrated in other studies [116, 167, 185, 186].

%rT1 did not add to the prognostic significance of albumin, however, which was the only liver function marker that remained predictive of liver-related events in multivariable analysis (p <0.001). Albumin predicted liver-related outcomes in my mixed liver disease cohort slightly less well than reported previously for patients with chronic hepatitis C infection (HR 0.854, 95% CI 0.791 – 0.922 versus HR 0.8, 95% CI 0.75 – 0.85) [4]. Although albumin is a marker of liver synthetic function, albumin is also affected by extra-hepatic disease and states of chronic inflammation and has prognostic value that is not specific to liver disease [193, 194]. The influence of co-morbidities beyond diabetes, hypercholesterolaemia and obesity was not investigated or accounted for in this study, so it is uncertain how much the recorded serum albumin reflects liver function specifically.

This study aimed to find non-invasive markers that transcended clinical phenotypes, but non-invasive markers could have different prognostic values in compensated versus decompensated liver disease. Indeed cACLD status was predictive of clinical outcomes in univariate analysis, although not in multivariable analysis. Portal hypertension markers may be more prognostic in compensated than decompensated cirrhosis as HVPG has been found to predict clinical outcomes in compensated, but not decompensated cirrhosis [42] and the presence of varices in cACLD increases mortality [174]. The liver function measures MELD and Child Pugh, however, have higher prognostic value in decompensated than compensated disease [40], which may explain why they were not significant prognostic markers in multivariable analysis in this predominantly compensated cohort. This study was underpowered to test the prognostic value of non-invasive markers stratified by compensation status, but this strategy should be considered in future studies. None-the-less, markers related to liver fibrosis, portal hypertension and liver function

had significant association with clinical outcomes in this cohort of patients with varying compensation status.

Perhaps surprisingly, alcohol misuse during follow up in my cohort did not influence outcomes, despite continued alcohol consumption being shown to worsen prognosis in other studies [190]. This is likely be due to the low number of patients and the short follow up period.

6.7.1 Limitations

The results presented here are preliminary results only and so may be underpowered to detect significant predictors of clinical outcomes accurately. A composite endpoint was used in order to increase the power of the study, but it is acknowledged that some of the clinical outcomes are less severe than others e.g. the development of new ascites versus death. The number of participants and duration of follow up will increase beyond the confines of this thesis and may allow for analysis of specific outcomes e.g. transplant-free survival.

There may be some small inaccuracies in the follow up results. I was unable to contact 4 patients by telephone at the last follow up and so any complications undocumented in their recruiting hospital's medical records could have been missed. Potential differences between the onset and diagnosis of liver-related outcomes may also have affected the accuracy of results.

Apart from alcohol use, the follow up did not account for factors that may influence clinical outcomes such as weight loss/gain, exercise habits, co-morbidities, medications and prophylactic variceal band ligation. Indeed 4 patients were cured of hepatitis C during follow up. The influence of these factors on outcomes is therefore unknown. Sarcopaenia also has prognostic significance [195], but was not assessed. It could be added easily to the MRI protocol in future studies by extending the anatomical slices (VIBE) down to vertebral level L3.

This study concentrated on the development of new liver-related outcomes, however, recurrence of decompensation events and resolution of liver-related complications would also be of interest and could be investigated in future studies.

6.7.2 Conclusions

My results support the study hypothesis that MR markers of portal hypertension (spleen ECV, platelet count/spleen diameter ratio, spleen T1 x spleen diameter) and liver function (%rT1) are predictive of liver-related outcomes in patients with cirrhosis. At this early follow up time point however, the MR markers did not provide any significant additional prognostic benefit beyond that of age and clinical markers. The MR marker of liver fibrosis, liver cT1, was not predictive of liver-related outcomes in this cohort with established cirrhosis. Any prognostic benefit of MR markers will be evaluated further with the long term follow up planned for this study.

7 General discussion

In this chapter I will summarise the thesis findings and discuss the MR technique's diagnostic accuracy, diagnostic impact and technical feasibility in comparison with other available non-invasive tests. Detailed discussion is found within chapters 3 - 6.

This thesis aimed to improve the non-invasive assessment of liver disease and its complications using MRI techniques. This would build upon the liver fibrosis, fat and iron measures developed previously using liver cT1, proton density fat fraction (PDFF) and T2* measurement respectively, by adding in assessments of portal hypertension and liver function. A previous finding of variable and higher liver cT1 in young women was explored for the association with the menstrual cycle in 12 healthy females aged 16-40. The ability of new and existing MR techniques was explored for the assessment of portal hypertension, oesophageal varices needing treatment (VNTs), liver function and the prediction of clinical outcomes using 20 healthy volunteers, 7 patients with acute liver injury and 138 patients with cirrhosis of varying compensation status. The principle proposed marker for portal hypertension was spleen extracellular volume fraction (ECV) and for liver function was the percentage reduction in liver T1 with gadoxetic acid administration (%rT1).

7.1 Summary of thesis findings

This thesis confirmed that healthy young women have higher liver cT1 than those reported for healthy older women and men, and that liver cT1 is associated with total body water content. Contrary to the hypothesis, liver cT1 was not influenced by the menstrual cycle or sex hormones. The physiological determinants of young women's higher total body water content requires further study.

Spleen T1 was not known previously to share this age and sex association, but was observed in young women to be higher than other heathy volunteers and patients with portal hypertension. Unlike liver cT1, spleen T1 and cT1 were unrelated to body water content, but were associated with the menstrual cycle. Spleen T1 was lower in the luteal than the follicular phase, associated with a fall in ferritin likely due to menstrual blood loss. Although T2* lowers with increasing iron content, spleen T2* measurement did not reflect changes in ferritin and had poor reproducibility in healthy volunteers between Oxford and Southampton (21.8% coefficient of variation). These results cast doubt over the accuracy of T2* as an iron measure in the spleen. The iron correction algorithm, derived originally for the liver, may also under-correct T1 when spleen cT1 is calculated. These results suggest spleen T1 is more sensitive to iron content than liver T1 and that spleen T2* measurement and the iron correction of spleen T1 needs further refinement.

None-the-less, spleen T1-based measures were effective non-invasive markers of portal hypertension and oesophageal varices needing treatment in patients with cirrhosis (table 27), a population in which young women are rare. Spleen ECV and platelet count/spleen volume ratio were the best identifiers of portal hypertension in cirrhosis (both AUROC 0.79). Recruitment was insufficient to assess if MR markers can monitor dynamic changes in portal pressure in response to therapy. Spleen T1 x spleen diameter was the best marker of oesophageal varices needing treatment (VNTs) in compensated advanced chronic liver disease (cACLD; AUROC 0.87) and the criteria spleen T1 <1307 ms or spleen diameter <137 mm could exclude 66% of patients with cACLD from OGD screening without missing VNTs. This represents a 3-fold improvement over Baveno IV criteria and the best performing VNT exclusion criteria I am aware of to date.

In the assessment of liver function, %rT1 could identify patients with liver dysfunction accurately (AUROC 0.86). %rT1 correlated with bilirubin, prothrombin time, albumin, MELD and Child Pugh score, confirming its association with liver function as found in other studies [121-123, 125, 159]. %rT1 was modelled best by bilirubin, albumin, aspartate aminotransferase and liver stiffness (R^2 = 0.51), indicating influences by liver inflammation as well as liver function. Liver cT1, %rT1 and

spleen ECV combined were different between subjects grouped by liver disease phenotype, except between patients with cirrhosis and ascites versus liver dysfunction only or hepatic encephalopathy.

Liver-related outcomes in patients with cirrhosis were associated with spleen ECV, platelet count/spleen diameter ratio, spleen T1 x spleen diameter and %rT1 (table 25). None of these measures were independent predictors of outcomes however, when assessed with age and serum-based markers. The independent predictors of clinical outcomes were age, APRI, albumin and sodium, indicating no additional benefit of MR scanning for the prognostication of patients with established cirrhosis.

Table 27. Results summary for MR markers of portal hypertension.

Marker	AUROC for portal hypertension in cirrhosis (95% CI)	AUROC for VNTs in cACLD (95% CI)	Exclusion of VNTs in cACLD (%)	Univariate HR for clinical outcomes (p)
Spleen ECV	0.79 (0.71 – 0.87)	0.70 (0.55 – 0.86)	14%	<0.001*
Platelet/spleen volume ratio	0.79 (0.71 – 0.87)	0.84 (0.75 – 0.93)	54%	0.064
Spleen volume	0.77 (0.69 – 0.86)	0.81 (0.69 – 0.92)	36%	0.127
Spleen T1 x spleen diameter	0.76 (0.69 – 0.86) **	0.87 (0.79 – 0.95)	51%	0.034
Platelet/spleen diameter ratio	0.76 (0.68 – 0.85)	0.83 (0.72 – 0.95)	26%	0.050
Spleen diameter	0.74 (0.65 – 0.83)	0.81 (0.71 – 0.92)	40%	0.090
Spleen cT1	0.73 (0.63 – 0.82)	0.81 (0.70 – 0.92)	43%	0.148
Spleen T1	0.71 (0.62 – 0.80)	0.83 (0.73 – 0.93)	45%	0.081

Abbreviations: AUROC, area under the receiver operator curve; CI, confidence interval; cACLD, compensated advanced chronic liver disease; VNT, varices needing treatment; HR, hazard ratio; ECV, extracellular volume fraction
*Spleen ECVx100
**Analysed post hoc

The ideal non-invasive markers of portal hypertension should have high diagnostic accuracy at the point of acquisition for portal hypertension and its complications, and have prognostic value. Spleen ECV, the principle candidate marker of portal hypertension, performed well for both the

identification of portal hypertension and prediction of clinical outcomes, but not for the identification and exclusion of VNTs. No marker tested performed best in all analyses. On balance, the markers of interest resulting from this thesis are therefore spleen ECV, platelet/spleen volume ratio and spleen T1 and spleen diameter in combination.

7.2 Technical aspects of MR sequences used

High quality, interpretable liver and spleen T1 maps were achieved for all participants that completed MR assessment. This contrasts to 21% of liver and spleen T1 maps being uninterpretable in 22 patients with portal hypertension in Edinburgh using the same MR sequence and magnet strength [196]. I performed or supervised all scans in this thesis following specific training, but a radiographer, perhaps new to the sequence, performed them in Edinburgh. The disparity in map quality likely represents errors at the point of acquisition due to unfamiliarity with T1 mapping and knowing when the sequence needs to be repeated. This highlights a need for specific training if the sequences are to be used in clinical practice.

All T1 maps were obtained in breath hold with cardiac/pulse gating to minimise motion artefacts. Some patients found this challenging. Options to improve this would be to (a) leave the sequences ungated as pulsatile blood flow effects are likely to be minimal in the liver and spleen, which would shorten the breath hold from 9 heart beats (approximately 8 seconds) to 1.2 seconds; or (b) have respiratory-gated, free breathing scans, which would lengthen acquisition to at least 9 breaths (approximately 45 seconds).

The T1 maps were also in single slices, yet 3D acquisition would be optimal to allow assessment of whole organs and accelerate acquisition. Besa et al. have employed a 3D T1 mapping technique, but it required an 18 second breath hold [163] that may be too long for some patients. Further technical development of 3D T1 mapping sequences is needed.

T1 quantification can differ across different scanners and magnetic field strengths, but a standardisation method to account for these changes was employed in this thesis. This enabled excellent reproducibility of spleen T1 and good reproducibility of liver T1 between Oxford and Southampton (3.75% and 6.89% coefficient of variation (CoV), respectively). Other factors also known to affect liver shMOLLI T1 include magnetisation transfer, fat, iron, heart rate, flip angle and off-resonance frequencies [130]. Relative changes in T1 (%rT1 and ECV) may overcome these effects, but they could have affected spleen T1 and particularly liver T1 measurements. A shMOLLI T1 correction for fat, iron and off resonance frequencies has been developed during the course of this thesis [130].

Inversion recovery T1 mapping was used in this thesis (shMOLLI), whilst alternative T1 mapping sequences exist, which use saturation recovery (e.g. SASHA and SAPPHIRE). In a direct comparison of these sequences applied to the heart, shMOLLI was found to be more precise, but less accurate and equally reproducible to SASHA and SAPPHIRE [197]. When used to derive ECV, shMOLLI produced higher values than SASHA and SAPPHIRE, but with similar reproducibility. This implies T1 mapping sequences cannot be used interchangeably, but further studies are needed to compare the different techniques for liver disease assessment specifically.

Both %rT1 and ECV rely on relative changes in T1 pre and post-contrast. Whilst %rT1 had high reproducibility across sites (3.4% CoV), spleen ECV did not (18.5% CoV). The spleen ECV technique using gadoxetic acid appeared valid when compared with gadobutrol (95% limits of agreement -0.034 – 0.065) and reached equilibrium by the time of measurement in all subject groups. Inter-observer reproducibility was high for both %rT1 and ECV techniques (3.2% and 1.6% CoV, respectively), indicating spleen ECV's variability was at the point of acquisition. Indeed, one spleen ECV was uninterpretable.

The accuracy of spleen ECV and %rT1 could be improved by automated motion correction and image registration, which has improved cardiac ECV mapping [135]. Such automation would also simplify and hasten spleen ECV analysis for the observer.

T2* measurements of the liver and spleen were uninterpretable in 1 and 5 subjects, respectively and had poor reproducibility between Oxford and Southampton (10.0% and 21.8% CoV, respectively). The T2* sequence used is highly sensitive to motion and is not optimised for the high T2*s observed in the spleen, which may explain its high variability. Unreliable spleen T2* acquisition may have also reduced the performance of spleen cT1 as a marker of portal hypertension and varices needing treatment, contrary to its excellent correlation with HVPG [148]. An alternative sequence for T2* and fat measurement called IDEAL (Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation) has been developed with faster acquisition and so may reduce motion artefact [198]. Use of IDEAL may improve T2* and cT1 measurement and diagnostic performance.

Spleen volume measurement between Oxford and Southampton had 10.3% coefficient of variation. The spleen volume could have been affected by image distortion at the point of acquisition or by different states of spleen contraction [199, 200], although this is unlikely as the spleen T1s were similar. Some partial volume affects may have resulted from the 5 mm thick scan slices used to contour the spleen, whereas thinner slices may improve accuracy, but with the compromise of longer analysis. None-the-less, the inter-observer reproducibility of spleen and liver volume was excellent at the analysis stage (both <4% CoV). Similarly, Bradley et al. have reported good inter-observer agreement for spleen and liver volumes using multi-slice balanced turbo-field echo localiser images (both 5% CoV) [86].

7.3 Potential applications of multiparametric MR with gadoxetic acid in clinical practice

The MR techniques employed in this thesis allow for the assessment of liver fibrosis, fat, iron and function and portal hypertension and oesophageal varices in a single MR scan. The sequences needed to achieve these measures could be reduced to 3 breath-holds pre-contrast and 1 breath-hold 20 minutes post-contrast if liver T1 measurements for %rT1 are taken from the 3-point slice (figure 4) used to measure spleen and blood T1.

The MR techniques can be used individually for selected purposes, but as MRI with gadoxetic acid is used routinely in clinical practice for liver lesion assessment, incorporation of HCC surveillance into the scan would be possible and improve cost and time efficiency. MRI with gadoxetic acid enables more accurate detection of HCC than ultrasound and CT in high risk populations, particularly in the detection of <2 cm lesions [37, 201] and so gadoxetic acid is used as the first line contrast agent in the evaluation of liver lesions in Japan and is used commonly in the UK. The routine T1- and T2-weighted imaging used in clinical practice can be used for spleen size measurements. A suggested scan sequence is shown in table 28.

A single MR scan with gadoxetic acid could replace liver biopsy for fibrosis staging, ultrasound for hepatocellular carcinoma surveillance, OGD for varices screening and give prognostic information. Such assessment has particular importance in the assessment of patients with HCC in whom portal hypertension and liver function status change their treatment options [201]. Even in centres that use non-liver-specific contrast agents, the MR techniques used in this thesis could still be employed with the exception of %rT1, but with the potential addition of liver ECV, which shows promise as another marker of liver fibrosis [202].

As MRI with contrast has a relatively high rate of false positives for HCC, the suggested multifactorial MRI assessment may not be suitable or likely cost-effective for 6-monthly HCC

Table 28. Suggested MRI sequence for the multifactorial assessment of liver disease.

Scan time (minutes)	Sequence description	Purpose
0 -19	Localisers	Planning imaging
	T1-weighted imaging	Anatomical, spleen size and liver lesion assessment
	T2-weighted imaging	
	Baseline liver PDFF and T2* mapping in transverse slice	Liver fat and iron assessment
	Baseline liver T1 mapping in transverse slice	Liver fibrosis assessment
	Baseline liver, spleen and blood T1 mapping in 3-point slice	Liver function, portal hypertension and varices assessment
20	Gadoxetic acid administration	
20-39	Dynamic T1-weighted imaging in arterial, portal venous and hepatobiliary phases	Anatomical and liver lesion assessment
	Diffusion-weighted imaging	
	T1-weighted imaging at equilibrium	
40	20 min post contrast spleen and blood T1 mapping in 3-point slice	Liver function and portal hypertension assessment

screening [201]. Rather, it could be used at first presentation of suspected liver disease and at times when OGD screening would be performed in cACLD (2-3 yearly), where it could save 66% of OGDs.

Gadoxetic acid has an excellent safety profile, is not known to have drug interactions in humans [171] and is well-tolerated in most patients. Other gadolinium-based contrast agents have been associated with nephrogenic systemic fibrosis and gadolinium retention in the brain, but gadoxetic acid has little evidence of this (appendix A). If MRI with gadoxetic acid were to be used as a screening test, however, the repeat administration of contrast would need monitoring for these complications.

Although the MR measures of portal hypertension, VNTs and liver function would be applicable to the majority of liver disease patients, there are a few exceptions. The thesis finding of higher spleen T1 in young women than other healthy volunteers means the suggested cut offs for portal hypertension and VNTs are not applicable to young women. MRI may not be a practical technique

for critically ill patients, so may not be suitable for the assessment of acute liver failure. Liver transplant listing and severe renal impairment (eGFR <30) are also contraindications to gadoxetic acid and so should not be used for these patients.

7.4 Comparison of multiparametric MR with other non-invasive tests for the multifactorial assessment of liver disease

Comparisons with other diagnostic methods for portal hypertension, varices assessment and liver function have been discussed in chapters 4 and 5, so methods for the multifactorial assessment of liver disease will be discussed here. Unlike many other liver disease assessment methods, the MR techniques described in this thesis do not require additional equipment or medicinal products that are not already used widely in clinical practice. The greatest competing techniques for the multifactorial assessment of liver disease are transient elastography and MR elastography.

7.4.1 Multifactorial assessments using transient elastography of the liver and spleen

Transient elastography (TE) can be used for the combined assessment of liver fibrosis by liver stiffness, liver fat by controlled attenuation parameter (CAP) and portal hypertension/varices by liver and/or spleen stiffness. In contrast to MRI, TE benefits from being a point of care test that can be performed with minimal training. It is well-tolerated by patients.

TE has a large body of evidence supporting its use as a marker of fibrosis and so its use has been adopted in clinical practice, with Fibroscan machines found in most UK hospitals. In NAFLD, TE liver stiffness has better correlation with collagen proportionate area and fibrosis stage, greater accuracy for the identification of advanced fibrosis and cirrhosis [73, 129] and greater accuracy

for the differentiation of simple steatosis from NASH than liver cT1 [129]. However, TE liver stiffness measurement has variable success rate (77% to 97%) in patients with suspected or known NAFLD [61-63] and it only samples a small tissue volume, risking sampling error. Both TE liver stiffness and liver cT1 have prognostic value [56, 74], although the findings in this thesis indicate liver cT1 is less prognostic once patients have established cirrhosis.

CAP has poorer assessment of liver fat than PDFF, which is the gold standard assessment of liver fat [203], and TE has no assessment of liver iron or liver function. Spleen stiffness and liver stiffness combined with other measures can help risk stratify for oesophageal varices, but no criteria have been found to perform superiorly to the spleen T1 <1307 ms or spleen diameter <137 mm criteria found in this thesis. Spleen stiffness measurement by TE also requires ultrasound guidance, making the technique harder to apply in clinical practice.

TE's major flaw is that it is unsuccessful or unreliable in a large proportion of patients, particularly for spleen stiffness measurement [103, 204]. Reliability declines with increasing obesity, which is increasingly problematic as the prevalence of obesity and NAFLD rises [205]. TE also cannot be performed in the presence of ascites, making it unsuitable for monitoring portal pressure in many patients with portal hypertension.

7.4.2 Multifactorial assessments using magnetic resonance elastography of the liver and spleen

Similar to TE, MR elastography (MRE) has been applied to the liver for fibrosis and portal hypertension assessment and to the spleen for portal hypertension and varices assessment, all with reported high levels of accuracy. MRE liver stiffness is also predictive of decompensation [206]. No studies have compared the performance of MRE with cT1 against a gold standard to date, and no consensus has been reached on MRE cut offs for liver fibrosis stages or portal hypertension. Like TE and liver cT1, MRE liver stiffness can be affected by inflammation [66].

As an MR technique, MRE can assess large areas or whole organs, much greater than TE. Although MRE cannot assess liver function, fat and iron, sequences could be added to the MRI scan for these assessments. MRI scans can also be used for anatomical assessments e.g. for liver lesions.

Equipment additional to the MRI scanner is required to perform MR elastography and each elastogram slice requires a 20-29 second breath hold [207] that some patients may find very challenging. Such breath holds are 3-fold longer than the sequences used in this thesis. Perhaps due to these long MRE acquisitions, 11% of participants in one study did not have sufficient MRE image quality for valid results due to motion artefact [106]. In another study, the technical failure rate of liver MRE has also been found to be higher at 3 T (15.3%) than 1.5 T (3.5%) and is linked to the presence of ascites, high BMI and iron deposition [208]. These issues have been largely overcome by the T1 methods used in this thesis.

7.5 Limitations

Limitations particular to each chapter are discussed within the chapter discussion sections.

Multiple groups in the MAPLE study were designed in order to assess the differences in liver fibrosis, liver function and portal hypertension between patients with different clinical phenotypes of liver disease. The groups did not necessarily represent how patients are classified in clinical practice, particularly the division of patients with cirrhosis with normal liver function versus liver dysfunction only, limiting their clinical translatability. Liver disease represents a continuum and so group-wise analysis may have blurred underlying associations between MRI parameters and pathophysiological factors. The aim to use MRI to assess how liver fibrosis, liver function and portal hypertension contribute to liver disease phenotype was also premature given the MRI biomarkers were not validated.

The division of patients into groups also meant that some patients with cirrhosis did not meet the criteria for any groups and so the total study population with cirrhosis was not entirely representative of the general population of patients with cirrhosis. Over-recruitment to the cirrhosis with liver dysfunction only group occurred due to unexpected liver dysfunction on study blood tests in patients thought to have cirrhosis with normal liver function. The division into groups also made total recruitment more difficult, so some groups were under-recruited and not all patients had 6 months follow up for inclusion in outcome analysis. Many analyses were therefore under-powered.

Given the exploratory nature of this thesis, multiple variables were also tested. Some findings may therefore be subject to chance and so need validation in further studies.

In studies investigating techniques such as liver stiffness, diagnostic cut offs have varied between different liver disease aetiologies. As a mixed group of liver disease patients was used in this study, variance in the investigated parameters by aetiology may have been hidden and so reduced the diagnostic accuracy of the techniques. Of special note, hepatitis C cure rates have soared recently, associated with falls in liver cT1, liver stiffness and spleen parameters post treatment. Further study is needed to investigate the pathophysiology behind these changes and to investigate the MR markers of this thesis in specific liver disease aetiologies.

Standard clinical classification of cirrhosis was used in this thesis. Misclassification of participants as having cirrhosis may have occurred as liver biopsy was not mandatory to confirm cirrhosis. This was the case when one patient thought to have cirrhosis was excluded following a clinically-indicated liver biopsy showing insignificant fibrosis. All patients in cirrhosis groups in the study were being managed as cirrhotic however, and so are representative of the population under hospital care in clinical practice. Healthy volunteers were likely to have had non-fibrotic, healthy livers given the exclusion of volunteers with liver disease risk factors and abnormal liver tests.

As portal pressure measurement was not a study requirement, there was no gold standard comparison for the proposed spleen MRI markers of portal hypertension. Instead, the presence of varices and/or ascites defined portal hypertension mostly, which arguably is the most important definition clinically. Endoscopy was performed as part of patient's clinical care by a range of clinicians. There may have been some variation in their classification of varices, whereas a single operator would have ensured consistency, although difficult to deliver in a multicentre study. Portal hypertension was still deemed to be present in diuretic-controlled ascites, but it is acknowledged that their portal hypertension and need for diuretics might have resolved spontaneously. If spleen MRI markers were to be used as non-invasive surrogate outcomes for clinical trials, comparison with HVPG or PPG would be necessary.

Claustrophobia can make MRI an unpleasant experience for some patients. In this thesis, some patients declined study participation due to anticipated claustrophobia and 7 (3.6%) of all participants could not complete the MRI scan due to claustrophobia or physical discomfort. This was higher than the proportion found in clinical practice (0.88%) [209], perhaps due to a lower threshold to abort scans for research only. My results compare favourably, however, to the 8.8% incompletion due to claustrophobia of a 1 hour research MR scan to assess portal hypertension using 15-20 second breath-hold and free-breathing scans [80] and 14% incompletion of MRE due to movement, claustrophobia or inability to breath-hold [88].

7.6 Consideration of translation of research findings into clinical care

The overall aim of this thesis has been to improve the non-invasive assessment of liver disease with a view to translation into clinical care. Of the thesis findings, I consider the spleen T1 and spleen diameter criteria for the detection and exclusion of VNTs in cACLD to have the most

potential for translation into clinical care. Here, I will discuss the remaining knowledge gaps and practicalities to consider prior to adoption of these criteria in clinical practice.

It is not yet known how well the spleen T1 and spleen diameter criteria perform in centres outside Oxford and Southampton, in different ethnicities and liver disease aetiologies and in young women in whom the spleen T1 likely requires a higher cut off. We do not know what proportion of patients would not tolerate the MRI scan or have insufficient scan quality for application the criteria in clinical practice as patients were excluded with contraindications to MRI in this thesis. It is therefore important to discover how effective the criteria are on an intention to diagnose basis and how acceptable use of the MRI criteria would be to patients in comparison to simpler, albeit likely less accurate tests.

Some evidence of spleen T1 reproducibility was presented here, but requires more robust evidence, particularly with the use of different radiographers and MRI scanners as would be the case in the NHS. Although the test-retest repeatability and reproducibility across a wide array of MRI scanners has been determined for liver cT1 using the same scan sequence as for spleen T1 [210], the same should be done for spleen T1 specifically to ensure the criteria can be applied reliably in different hospitals.

Spleen diameter can be measured using standard radiology reporting software, whereas spleen T1 analysis is not readily available in most hospitals. Perspectum Diagnostics Ltd were used for spleen T1 analysis in this thesis and could also be used for wider healthcare use. The use of Perspectum Diagnostics includes the benefit of their T1 standardisation across scanner strengths and models, help with setting up scan sequences/training at different sites, quality control measures and ability to update analysis processes as advances in technology are made. Although these benefits may come at a financial cost, they would be difficult to achieve without commercial help.

Finally, the economic impact of adoption into clinical care remains unknown. Beyond the direct costs of MRI scans and reporting, the introduction of the MRI criteria may have other health economic ramifications such as the investigation and treatment of incidental findings on MRI scans and necessary expansion of MRI services. Meanwhile, savings could be made in endoscopy and ultrasound. Along with patient benefit, the 'value for money' of the criteria's use would be key to the National Institute of Health and Care (NICE) approval process, which helps guide clinical care in the United Kingdom.

7.7 Suggested further work

The most promising finding of this thesis has been the exclusion of varices needing treatment by spleen T1 <1307 ms or spleen diameter <137 mm that allows safe avoidance of OGD screening for VNTs. These criteria need external validation in multiple centres, assessment of impact on variceal bleeding and health economic analysis. Spleen diameter assessment by ultrasound could be tested to reduce the need for MRI and therefore cost of the criteria as only those with spleen diameter >137 mm (60% in this study) would require spleen T1 assessment.

The potential markers of portal hypertension (spleen ECV, platelet/spleen volume ratio and spleen T1 x spleen diameter) could be tested against gold standard HVPG and validated against OGD assessment of VNTs in large multicentre studies. Normal values for spleen T1, cT1, ECV and spleen T1 x spleen diameter across sex and age ranges should be established.

The MAPLE study is still in its follow up phase for up to 20 years to assess the association of MR measures with clinical outcomes, whilst a randomised trial analysed on an intention to diagnose basis would be required to assess the impact on clinical management and outcomes fully. This would be particularly important for the use of spleen T1 x spleen diameter to identify VNTs, where β-blockers could be initiated without OGD. Likewise, %rT1 could be tested for the

prediction of post-hepatectomy liver failure, which occurs in up to 15% of partial hepatectomies in cirrhosis [211], and decision-making on safe remnant liver volumes. The value of serial monitoring of MR parameters as a method for monitoring treatment response or disease progression and prognostication could also be explored.

Further work could also include optimisation of MR techniques to reduce confounding variables, accelerate acquisition and develop 3D mapping. In particular, given the value of spleen T1 and its sensitivity to ferritin, splenic T2* optimisation and a spleen-specific iron correction algorithm should be developed. At the analysis stage, programmes for image registration and voxel-wise mapping of spleen ECV and %rT1 should be developed.

7.8 Conclusion

The results of this study indicate spleen T1 x spleen diameter is a useful biomarker for VNTs and that the criteria spleen T1 <1307 ms or spleen diameter <137 mm can identify a large proportion of patients at low risk of VNTs who could therefore avoid screening OGDs. These markers do not require contrast administration and can be acquired by one breath hold each during MRI. Spleen ECV and platelet count to spleen volume ratio are likely biomarkers of portal hypertension, and %rT1 reflects liver function well, but neither %rT1 nor MR parameters of portal hypertension translate to predict clinical outcomes in patients with established cirrhosis better than simpler non-invasive parameters.

8 References

[1] R. Williams, R. Aspinall, M. Bellis, G. Camps-Walsh, M. Cramp, A. Dhawan, J. Ferguson, D. Forton, G. Foster, S.I. Gilmore, M. Hickman, M. Hudson, D. Kelly, A. Langford, M. Lombard, L. Longworth, N. Martin, K. Moriarty, P. Newsome, J. O'Grady, R. Pryke, H. Rutter, S. Ryder, N. Sheron, T. Smith, Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis, The Lancet 384(9958) (2014) 1953-1997.
[2] N.e.o.l.c.i. network, Deaths from liver disease: Implications for end of life care in England, http://www.endoflifecare-intelligence.org.uk/resources/publications/deaths_from_liver_disease, 2012.
[3] J.A. Fallowfield, Future mechanistic strategies for tackling fibrosis--an unmet need in liver disease, Clin Med (Lond) 15 Suppl 6 (2015) s83-7.
[4] M.H. Khan, G.C. Farrell, K. Byth, R. Lin, M. Weltman, J. George, D. Samarasinghe, J. Kench, S. Kaba, E. Crewe, C. Liddle, Which patients with hepatitis C develop liver complications?, Hepatology 31(2) (2000) 513-20.
[5] Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group, Hepatology 20(1 Pt 1) (1994) 15-20.
[6] K.P. Batts, J. Ludwig, Chronic hepatitis. An update on terminology and reporting, Am J Surg Pathol 19(12) (1995) 1409-17.
[7] D.E. Kleiner, E.M. Brunt, M. Van Natta, C. Behling, M.J. Contos, O.W. Cummings, L.D. Ferrell, Y.C. Liu, M.S. Torbenson, A. Unalp-Arida, M. Yeh, A.J. McCullough, A.J. Sanyal, N. Nonalcoholic Steatohepatitis Clinical Research, Design and validation of a histological scoring system for nonalcoholic fatty liver disease, Hepatology 41(6) (2005) 1313-21.
[8] K. Ishak, A. Baptista, L. Bianchi, F. Callea, J. De Groote, F. Gudat, H. Denk, V. Desmet, G. Korb, R.N. MacSween, et al., Histological grading and staging of chronic hepatitis, J Hepatol 22(6) (1995) 696-9.
[9] V. Calvaruso, A.K. Burroughs, R. Standish, P. Manousou, F. Grillo, G. Leandro, S. Maimone, M. Pleguezuelo, I. Xirouchakis, G.P. Guerrini, D. Patch, D. Yu, J. O'Beirne, A.P. Dhillon, Computer-assisted image analysis of liver collagen: relationship to Ishak scoring and hepatic venous pressure gradient, Hepatology 49(4) (2009) 1236-44.
[10] E. Tsochatzis, S. Bruno, G. Isgro, A. Hall, E. Theocharidou, P. Manousou, A.P. Dhillon, A.K. Burroughs, T.V. Luong, Collagen proportionate area is superior to other histological methods for sub-classifying cirrhosis and determining prognosis, J Hepatol 60(5) (2014) 948-54.
[11] P. Bedossa, F. Carrat, Liver biopsy: the best, not the gold standard, J Hepatol 50(1) (2009) 1-3.
[12] J.H. Boyum, T.D. Atwell, G.D. Schmit, J.J. Poterucha, C.D. Schleck, W.S. Harmsen, P.S. Kamath, Incidence and Risk Factors for Adverse Events Related to Image-Guided Liver Biopsy, Mayo Clin Proc 91(3) (2016) 329-35.
[13] J.C. Garcia-Pagan, J. Gracia-Sancho, J. Bosch, Functional aspects on the pathophysiology of portal hypertension in cirrhosis, J Hepatol 57(2) (2012) 458-61.
[14] A. Manenti, A. Botticelli, G. Gibertini, L. Botticelli, Experimental congestive splenomegaly: histological observations in the rat, Pathologica 85(1100) (1993) 721-4.
[15] C. Ripoll, R. Groszmann, G. Garcia-Tsao, N. Grace, A. Burroughs, R. Planas, A. Escorsell, J.C. Garcia-Pagan, R. Makuch, D. Patch, D.S. Matloff, J. Bosch, G. Portal Hypertension Collaborative, Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis, Gastroenterology 133(2) (2007) 481-8.
[16] C. Ripoll, R.J. Groszmann, G. Garcia-Tsao, J. Bosch, N. Grace, A. Burroughs, R. Planas, A. Escorsell, J.C. Garcia-Pagan, R. Makuch, D. Patch, D.S. Matloff, G. Portal Hypertension

Collaborative, Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis, J Hepatol 50(5) (2009) 923-8.
[17] U. Thalheimer, G. Leandro, D.N. Samonakis, C.K. Triantos, D. Patch, A.K. Burroughs, Assessment of the agreement between wedge hepatic vein pressure and portal vein pressure in cirrhotic patients, Dig Liver Dis 37(8) (2005) 601-8.
[18] A.J. Sanyal, S.L. Friedman, A.J. McCullough, L. Dimick-Santos, D. American Association for the Study of Liver, F. United States, A. Drug, Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop, Hepatology 61(4) (2015) 1392-405.
[19] A. Zipprich, M. Winkler, T. Seufferlein, M.M. Dollinger, Comparison of balloon vs. straight catheter for the measurement of portal hypertension, Aliment Pharmacol Ther 32(11-12) (2010) 1351-6.
[20] J. Bosch, J.G. Abraldes, A. Berzigotti, J.C. Garcia-Pagan, The clinical use of HVPG measurements in chronic liver disease, Nat Rev Gastroenterol Hepatol 6(10) (2009) 573-82.
[21] P. Tandon, C. Ripoll, D. Assis, S. Wongcharatrawee, R.J. Groszmann, G. Garcia-Tsao, The interpretation of hepatic venous pressure gradient tracings - Excellent interobserver agreement unrelated to experience, Liver Int (2016).
[22] U. Thalheimer, L. Bellis, C. Puoti, A.K. Burroughs, Should we routinely measure portal pressure in patients with cirrhosis, using hepatic venous pressure gradient (HVPG) as a guide for prophylaxis and therapy of bleeding and rebleeding? No, Eur J Intern Med 22(1) (2011) 5-7.
[23] R. de Franchis, Non-invasive (and minimally invasive) diagnosis of oesophageal varices, J Hepatol 49(4) (2008) 520-7.
[24] D. Tripathi, A.J. Stanley, P.C. Hayes, D. Patch, C. Millson, H. Mehrzad, A. Austin, J.W. Ferguson, S.P. Olliff, M. Hudson, J.M. Christie, S. Clinical, G. Standards Committee of the British Society of, U.K. guidelines on the management of variceal haemorrhage in cirrhotic patients, Gut 64(11) (2015) 1680-704.
[25] R.J. Groszmann, J. Bosch, N.D. Grace, H.O. Conn, G. Garcia-Tsao, M. Navasa, J. Alberts, J. Rodes, R. Fischer, M. Bermann, et al., Hemodynamic events in a prospective randomized trial of propranolol versus placebo in the prevention of a first variceal hemorrhage, Gastroenterology 99(5) (1990) 1401-7.
[26] E. Moitinho, A. Escorsell, J.C. Bandi, J.M. Salmeron, J.C. Garcia-Pagan, J. Rodes, J. Bosch, Prognostic value of early measurements of portal pressure in acute variceal bleeding, Gastroenterology 117(3) (1999) 626-31.
[27] V. Jairath, S. Rehal, R. Logan, B. Kahan, S. Hearnshaw, S. Stanworth, S. Travis, M. Murphy, K. Palmer, A. Burroughs, Acute variceal haemorrhage in the United Kingdom: patient characteristics, management and outcomes in a nationwide audit, Dig Liver Dis 46(5) (2014) 419-26.
[28] P. Harrison, B.J. Hogan, L. Floros, E. Davies, G.D. Grp, GUIDELINES Assessment and management of cirrhosis in people older than 16 years: summary of NICE guidance, Bmj-Brit Med J 354 (2016).
[29] L. European Association for the Study of the, EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis, J Hepatol 53(3) (2010) 397-417.
[30] T. Bucsics, M. Schoder, M. Diermayr, M. Feldner-Busztin, N. Goeschl, D. Bauer, P. Schwabl, M. Mandorfer, B. Angermayr, M. Cejna, A. Ferlitsch, W. Sieghart, M. Trauner, M. Peck-Radosavljevic, J. Karner, F. Karnel, T. Reiberger, Transjugular intrahepatic portosystemic shunts (TIPS) for the prevention of variceal re-bleeding - A two decades experience, PLoS One 13(1) (2018) e0189414.
[31] H. Vilstrup, P. Amodio, J. Bajaj, J. Cordoba, P. Ferenci, K.D. Mullen, K. Weissenborn, P. Wong, Hepatic Encephalopathy in Chronic Liver Disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver, Hepatology 60(2) (2014) 715-735.
[32] G. Fattovich, T. Stroffolini, I. Zagni, F. Donato, Hepatocellular carcinoma in cirrhosis: incidence and risk factors, Gastroenterology 127(5 Suppl 1) (2004) S35-50.

[33] L. European Association for Study of, R. European Organisation for, C. Treatment of, EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma, Eur J Cancer 48(5) (2012) 599-641.
[34] F. Vasuri, R. Golfieri, M. Fiorentino, E. Capizzi, M. Renzulli, A.D. Pinna, W.F. Grigioni, A. D'Errico-Grigioni, OATP 1B1/1B3 expression in hepatocellular carcinomas treated with orthotopic liver transplantation, Virchows Arch 459(2) (2011) 141-6.
[35] X. Liu, H. Jiang, J. Chen, Y. Zhou, Z. Huang, B. Song, Gadoxetic acid disodium-enhanced magnetic resonance imaging outperformed multidetector computed tomography in diagnosing small hepatocellular carcinoma: A meta-analysis, Liver Transpl 23(12) (2017) 1505-1518.
[36] J. Guo, Y. Seo, S. Ren, S. Hong, D. Lee, S. Kim, Y. Jiang, Diagnostic performance of contrast-enhanced multidetector computed tomography and gadoxetic acid disodium-enhanced magnetic resonance imaging in detecting hepatocellular carcinoma: direct comparison and a meta-analysis, Abdom Radiol (NY) 41(10) (2016) 1960-72.
[37] S.Y. Kim, J. An, Y.S. Lim, S. Han, J.Y. Lee, J.H. Byun, H.J. Won, S.J. Lee, H.C. Lee, Y.S. Lee, MRI With Liver-Specific Contrast for Surveillance of Patients With Cirrhosis at High Risk of Hepatocellular Carcinoma, JAMA Oncol 3(4) (2017) 456-463.
[38] R.N. Pugh, I.M. Murray-Lyon, J.L. Dawson, M.C. Pietroni, R. Williams, Transection of the oesophagus for bleeding oesophageal varices, Br J Surg 60(8) (1973) 646-9.
[39] G. D'Amico, G. Garcia-Tsao, L. Pagliaro, Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies, J Hepatol 44(1) (2006) 217-31.
[40] P.S. Kamath, R.H. Wiesner, M. Malinchoc, W. Kremers, T.M. Therneau, C.L. Kosberg, G. D'Amico, E.R. Dickson, W.R. Kim, A model to predict survival in patients with end-stage liver disease, Hepatology 33(2) (2001) 464-70.
[41] X. Xiol, P. Gines, L. Castells, J. Twose, A. Ribalta, X. Fuentes-Arderiu, S. Rodriguez, J. Castellote, M. Navasa, R. Deulofeu, Clinically relevant differences in the model for end-stage liver disease and model for end-stage liver disease-sodium scores determined at three university-based laboratories of the same area, Liver Transpl 15(3) (2009) 300-5.
[42] C. Ripoll, P. Lastra, D. Rincon, V. Catalina, R. Banares, Comparison of MELD, HVPG, and their changes to predict clinically relevant endpoints in cirrhosis, Scand J Gastroenterol 47(2) (2012) 204-11.
[43] S.H. Teh, D.M. Nagorney, S.R. Stevens, K.P. Offord, T.M. Therneau, D.J. Plevak, J.A. Talwalkar, W.R. Kim, P.S. Kamath, Risk factors for mortality after surgery in patients with cirrhosis, Gastroenterology 132(4) (2007) 1261-9.
[44] E. Reverter, P. Tandon, S. Augustin, F. Turon, S. Casu, R. Bastiampillai, A. Keough, E. Llop, A. Gonzalez, S. Seijo, A. Berzigotti, M. Ma, J. Genesca, J. Bosch, J.C. Garcia-Pagan, J.G. Abraldes, A MELD-based model to determine risk of mortality among patients with acute variceal bleeding, Gastroenterology 146(2) (2014) 412-19 e3.
[45] S.W. Biggins, W.R. Kim, N.A. Terrault, S. Saab, V. Balan, T. Schiano, J. Benson, T. Therneau, W. Kremers, R. Wiesner, P. Kamath, G. Klintmalm, Evidence-based incorporation of serum sodium concentration into MELD, Gastroenterology 130(6) (2006) 1652-60.
[46] W.R. Kim, S.W. Biggins, W.K. Kremers, R.H. Wiesner, P.S. Kamath, J.T. Benson, E. Edwards, T.M. Therneau, Hyponatremia and mortality among patients on the liver-transplant waiting list, N Engl J Med 359(10) (2008) 1018-26.
[47] K. Barber, S. Madden, J. Allen, D. Collett, J. Neuberger, A. Gimson, S. United Kingdom Liver Transplant, P. Allocation Working, Elective liver transplant list mortality: development of a United Kingdom end-stage liver disease score, Transplantation 92(4) (2011) 469-76.
[48] S.L. Wu, Y.X. Zheng, Z.W. Tian, M.S. Chen, H.Z. Tan, Scoring systems for prediction of mortality in decompensated liver cirrhosis: A meta-analysis of test accuracy, World J Clin Cases 6(15) (2018) 995-1006.
[49] A.L. Williams, J.H. Hoofnagle, Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis, Gastroenterology 95(3) (1988) 734-9.

[50] C.T. Wai, J.K. Greenson, R.J. Fontana, J.D. Kalbfleisch, J.A. Marrero, H.S. Conjeevaram, A.S. Lok, A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C, Hepatology 38(2) (2003) 518-26.
[51] A. Vallet-Pichard, V. Mallet, B. Nalpas, V. Verkarre, A. Nalpas, V. Dhalluin-Venier, H. Fontaine, S. Pol, FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest, Hepatology 46(1) (2007) 32-6.
[52] W.M. Rosenberg, M. Voelker, R. Thiel, M. Becka, A. Burt, D. Schuppan, S. Hubscher, T. Roskams, M. Pinzani, M.J. Arthur, G. European Liver Fibrosis, Serum markers detect the presence of liver fibrosis: a cohort study, Gastroenterology 127(6) (2004) 1704-13.
[53] R. Lichtinghagen, D. Pietsch, H. Bantel, M.P. Manns, K. Brand, M.J. Bahr, The Enhanced Liver Fibrosis (ELF) score: normal values, influence factors and proposed cut-off values, J Hepatol 59(2) (2013) 236-42.
[54] S. Bota, H. Herkner, I. Sporea, P. Salzl, R. Sirli, A.M. Neghina, M. Peck-Radosavljevic, Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis, Liver Int 33(8) (2013) 1138-47.
[55] NICE, Cirrhosis in over 16s: assessment and management, in: N.I.f.H.a.C. Excellence (Ed.) 2016.
[56] S. Singh, L.L. Fujii, M.H. Murad, Z. Wang, S.K. Asrani, R.L. Ehman, P.S. Kamath, J.A. Talwalkar, Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis, Clin Gastroenterol Hepatol 11(12) (2013) 1573-84 e1-2; quiz e88-9.
[57] G.J. Ooi, S. Mgaieth, G.D. Eslick, P.R. Burton, W.W. Kemp, S.K. Roberts, W.A. Brown, Systematic review and meta-analysis: non-invasive detection of non-alcoholic fatty liver disease related fibrosis in the obese, Obesity Reviews 19(2) (2018) 281-294.
[58] P. Schwabl, S. Bota, P. Salzl, M. Mandorfer, B.A. Payer, A. Ferlitsch, J. Stift, F. Wrba, M. Trauner, M. Peck-Radosavljevic, T. Reiberger, New reliability criteria for transient elastography increase the number of accurate measurements for screening of cirrhosis and portal hypertension, Liver Int 35(2) (2015) 381-90.
[59] U. Arena, F. Vizzutti, G. Corti, S. Ambu, C. Stasi, S. Bresci, S. Moscarella, V. Boddi, A. Petrarca, G. Laffi, F. Marra, M. Pinzani, Acute viral hepatitis increases liver stiffness values measured by transient elastography, Hepatology 47(2) (2008) 380-4.
[60] L. Castera, J. Foucher, P.H. Bernard, F. Carvalho, D. Allaix, W. Merrouche, P. Couzigou, V. de Ledinghen, Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations, Hepatology 51(3) (2010) 828-35.
[61] P.J. Eddowes, M. Sasso, M. Allison, E. Tsochatzis, Q.M. Anstee, D. Sheridan, I.N. Guha, J.F. Cobbold, J.J. Deeks, V. Paradis, P. Bedossa, P.N. Newsome, Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease, Gastroenterology 156(6) (2019) 1717-1730.
[62] V.W. Wong, M. Irles, G.L. Wong, S. Shili, A.W. Chan, W. Merrouche, S.S. Shu, J. Foucher, B. Le Bail, W.K. Chan, H.L. Chan, V. de Ledinghen, Unified interpretation of liver stiffness measurement by M and XL probes in non-alcoholic fatty liver disease, Gut 68(11) (2019) 2057-2064.
[63] C. Cassinotto, J. Boursier, V. de Ledinghen, J. Lebigot, B. Lapuyade, P. Cales, J.B. Hiriart, S. Michalak, B.L. Bail, V. Cartier, A. Mouries, F. Oberti, I. Fouchard-Hubert, J. Vergniol, C. Aube, Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy, Hepatology 63(6) (2016) 1817-27.
[64] G. Ferraioli, C. Tinelli, R. Lissandrin, M. Zicchetti, S. Bernuzzi, L. Salvaneschi, C. Filice, G. Elastography Study, Ultrasound point shear wave elastography assessment of liver and spleen stiffness: effect of training on repeatability of measurements, Eur Radiol 24(6) (2014) 1283-9.
[65] G.D. Dodd, 3rd, R.L. Baron, J.H. Oliver, 3rd, M.P. Federle, End-stage primary sclerosing cholangitis: CT findings of hepatic morphology in 36 patients, Radiology 211(2) (1999) 357-62.
[66] S. Singh, S.K. Venkatesh, Z. Wang, F.H. Miller, U. Motosugi, R.N. Low, T. Hassanein, P. Asbach, E.M. Godfrey, M. Yin, J. Chen, A.P. Keaveny, M. Bridges, A. Bohte, M.H. Murad, D.J. Lomas, J.A. Talwalkar, R.L. Ehman, Diagnostic performance of magnetic resonance elastography in staging

liver fibrosis: a systematic review and meta-analysis of individual participant data, Clin Gastroenterol Hepatol 13(3) (2015) 440-451 e6.
[67] J. Cui, B. Ang, W. Haufe, C. Hernandez, E.C. Verna, C.B. Sirlin, R. Loomba, Comparative diagnostic accuracy of magnetic resonance elastography vs. eight clinical prediction rules for non-invasive diagnosis of advanced fibrosis in biopsy-proven non-alcoholic fatty liver disease: a prospective study, Aliment Pharmacol Ther 41(12) (2015) 1271-80.
[68] R. Loomba, J. Cui, T. Wolfson, W. Haufe, J. Hooker, N. Szeverenyi, B. Ang, A. Bhatt, K. Wang, H. Aryafar, C. Behling, M.A. Valasek, G.Y. Lin, A. Gamst, D.A. Brenner, M. Yin, K.J. Glaser, R.L. Ehman, C.B. Sirlin, Novel 3D Magnetic Resonance Elastography for the Noninvasive Diagnosis of Advanced Fibrosis in NAFLD: A Prospective Study, Am J Gastroenterol 111(7) (2016) 986-94.
[69] Q.B. Wang, H. Zhu, H.L. Liu, B. Zhang, Performance of magnetic resonance elastography and diffusion-weighted imaging for the staging of hepatic fibrosis: A meta-analysis, Hepatology 56(1) (2012) 239-47.
[70] R. Banerjee, M. Pavlides, E.M. Tunnicliffe, S.K. Piechnik, N. Sarania, R. Philips, J.D. Collier, J.C. Booth, J.E. Schneider, L.M. Wang, D.W. Delaney, K.A. Fleming, M.D. Robson, E. Barnes, S. Neubauer, Multiparametric magnetic resonance for the non-invasive diagnosis of liver disease, J Hepatol 60(1) (2014) 69-77.
[71] J.C. Wood, M. Otto-Duessel, M. Aguilar, H. Nick, M.D. Nelson, T.D. Coates, H. Pollack, R. Moats, Cardiac iron determines cardiac T2*, T2, and T1 in the gerbil model of iron cardiomyopathy, Circulation 112(4) (2005) 535-43.
[72] E.M. Tunnicliffe, R. Banerjee, M. Pavlides, S. Neubauer, M.D. Robson, A model for hepatic fibrosis: the competing effects of cell loss and iron on shortened modified Look-Locker inversion recovery T1 (shMOLLI-T1) in the liver, J Magn Reson Imaging 45(2) (2017) 450-462.
[73] M. Pavlides, R. Banerjee, E.M. Tunnicliffe, C. Kelly, J. Collier, L.M. Wang, K.A. Fleming, J.F. Cobbold, M.D. Robson, S. Neubauer, E. Barnes, Multiparametric magnetic resonance imaging for the assessment of non-alcoholic fatty liver disease severity, Liver Int 37(7) (2017) 1065-1073.
[74] M. Pavlides, R. Banerjee, J. Sellwood, C.J. Kelly, M.D. Robson, J.C. Booth, J. Collier, S. Neubauer, E. Barnes, Multiparametric magnetic resonance imaging predicts clinical outcomes in patients with chronic liver disease, J Hepatol 64(2) (2016) 308-315.
[75] A. Mojtahed, C.J. Kelly, A.H. Herlihy, S. Kin, H.R. Wilman, A. McKay, M. Kelly, M. Milanesi, S. Neubauer, E.L. Thomas, J.D. Bell, R. Banerjee, M. Harisinghani, Reference range of liver corrected T1 values in a population at low risk for fatty liver disease-a UK Biobank sub-study, with an appendix of interesting cases, Abdom Radiol (NY) (2018).
[76] H. Wilman, J. Jacobs, R. Newbould, M. Halberstadt, M. Gyngell, A. Herlihy, C. Fernandes, C. Kelly, M. Maguire, N. Jayaratne, S. Linden, K. Groves, V. Bachtiar, A. McKay, M. Kelly, S. Neubauer, R. Banerjee, Repeatability and reproducibility of multiparametric magnetic resonance imaging of the liver, Journal of Hepatology 68 (2018) S562.
[77] N. McDonald, P.J. Eddowes, J. Hodson, S.I.K. Semple, N.P. Davies, C.J. Kelly, S. Kin, M. Phillips, A.H. Herlihy, T.J. Kendall, R.M. Brown, D.A.H. Neil, S.G. Hubscher, G.M. Hirschfield, J.A. Fallowfield, Multiparametric magnetic resonance imaging for quantitation of liver disease: a two-centre cross-sectional observational study, Sci Rep 8(1) (2018) 9189.
[78] K.Q. Shi, Y.C. Fan, Z.Z. Pan, X.F. Lin, W.Y. Liu, Y.P. Chen, M.H. Zheng, Transient elastography: a meta-analysis of diagnostic accuracy in evaluation of portal hypertension in chronic liver disease, Liver Int 33(1) (2013) 62-71.
[79] F. Vizzutti, U. Arena, R.G. Romanelli, L. Rega, M. Foschi, S. Colagrande, A. Petrarca, S. Moscarella, G. Belli, A.L. Zignego, F. Marra, G. Laffi, M. Pinzani, Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis, Hepatology 45(5) (2007) 1290-7.
[80] N. Palaniyappan, E. Cox, C. Bradley, R. Scott, A. Austin, R. O'Neill, G. Ramjas, S. Travis, H. White, R. Singh, P. Thurley, I.N. Guha, S. Francis, G.P. Aithal, Non-invasive assessment of Portal Hypertension Using Quantitative Magnetic Resonance Imaging, J Hepatol (2016).
[81] P. Salzl, T. Reiberger, M. Ferlitsch, B.A. Payer, B. Schwengerer, M. Trauner, M. Peck-Radosavljevic, A. Ferlitsch, Evaluation of portal hypertension and varices by acoustic radiation

force impulse imaging of the liver compared to transient elastography and AST to platelet ratio index, Ultraschall Med 35(6) (2014) 528-33.
[82] R. Sartoris, P.E. Rautou, L. Elkrief, G. Pollorsi, F. Durand, D. Valla, L. Spahr, S. Terraz, O. Soubrane, F. Cauchy, V. Vilgrain, M. Ronot, Quantification of Liver Surface Nodularity at CT: Utility for Detection of Portal Hypertension, Radiology (2018) 181131.
[83] V. Verma, S.K. Sarin, P. Sharma, A. Kumar, Correlation of aspartate aminotransferase/platelet ratio index with hepatic venous pressure gradient in cirrhosis, United European Gastroenterol J 2(3) (2014) 226-31.
[84] V. Kirnake, A. Arora, P. Sharma, M. Goyal, R. Chawlani, J. Toshniwal, A. Kumar, Non-invasive aspartate aminotransferase to platelet ratio index correlates well with invasive hepatic venous pressure gradient in cirrhosis, Indian J Gastroenterol (2018).
[85] H. Gouya, S. Grabar, O. Vignaux, A. Saade, S. Pol, P. Legmann, P. Sogni, Portal hypertension in patients with cirrhosis: indirect assessment of hepatic venous pressure gradient by measuring azygos flow with 2D-cine phase-contrast magnetic resonance imaging, Eur Radiol (2015).
[86] C.R. Bradley, E.F. Cox, R.A. Scott, M.W. James, P. Kaye, G.P. Aithal, S.T. Francis, I.N. Guha, Multi organ assessment of Compensated Cirrhosis Patients using quantitative Magnetic Resonance Imaging, J Hepatol (2018).
[87] C.L. Michael Pavlides, Jane Phillips-Hughes, Jane Collier, Rajarshi Banerjee, Jeremy F. Cobbold, Lai Mun Wang, Stefan K. Piechnik, Matthew D. Robson, Stefan Neubauer, Eleanor Barnes Multi-parametric magnetic resonance imaging of the spleen: An accurate and non-invasive biomarker of portal pressure, EASL, Barcelona, 2016.
[88] M. Ronot, S. Lambert, L. Elkrief, S. Doblas, P.E. Rautou, L. Castera, V. Vilgrain, R. Sinkus, B.E. Van Beers, P. Garteiser, Assessment of portal hypertension and high-risk oesophageal varices with liver and spleen three-dimensional multifrequency MR elastography in liver cirrhosis, Eur Radiol 24(6) (2014) 1394-402.
[89] P. Sharma, V. Kirnake, P. Tyagi, N. Bansal, V. Singla, A. Kumar, A. Arora, Spleen stiffness in patients with cirrhosis in predicting esophageal varices, Am J Gastroenterol 108(7) (2013) 1101-7.
[90] L. Ying, X. Lin, Z.L. Xie, Y.P. Hu, K.Q. Shi, Performance of platelet count/spleen diameter ratio for diagnosis of esophageal varices in cirrhosis: a meta-analysis, Dig Dis Sci 57(6) (2012) 1672-81.
[91] A. Berzigotti, S. Seijo, U. Arena, J.G. Abraldes, F. Vizzutti, J.C. Garcia-Pagan, M. Pinzani, J. Bosch, Elastography, spleen size, and platelet count identify portal hypertension in patients with compensated cirrhosis, Gastroenterology 144(1) (2013) 102-111 e1.
[92] V. Calvaruso, F. Bronte, E. Conte, F. Simone, A. Craxi, V. Di Marco, Modified spleen stiffness measurement by transient elastography is associated with presence of large oesophageal varices in patients with compensated hepatitis C virus cirrhosis, J Viral Hepat 20(12) (2013) 867-74.
[93] W. Manatsathit, H. Samant, S. Kapur, T. Ingviya, M. Esmadi, K. Wijarnpreecha, T. McCashland, Accuracy of liver stiffness, spleen stiffness, and LS-spleen diameter to platelet ratio score in detection of esophageal varices: Systemic review and meta-analysis, J Gastroenterol Hepatol 33(10) (2018) 1696-1706.
[94] N. Terayama, K.P. Makimoto, S. Kobayashi, Y. Nakanuma, M. Sasaki, K. Saito, K. Katayanagi, Pathology of the Spleen in Primary Biliary-Cirrhosis - an Autopsy Study, Pathology International 44(10-11) (1994) 753-758.
[95] L. Zhou, T.W. Chen, X.M. Zhang, C.J. Li, Z.F. Yang, N.L. Zeng, L.Y. Wang, T. Li, D. Wang, J. Li, C.P. Li, L. Li, X.Y. Xie, Spleen dynamic contrast-enhanced magnetic resonance imaging as a new method for staging liver fibrosis in a piglet model, PLoS One 8(12) (2013) e83697.
[96] R. de Franchis, V.I.F. Baveno, Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension, J Hepatol 63(3) (2015) 743-52.
[97] J.G. Abraldes, C. Bureau, H. Stefanescu, S. Augustin, M. Ney, H. Blasco, B. Procopet, J. Bosch, J. Genesca, A. Berzigotti, I. Anticipate, Noninvasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: The "Anticipate" study, Hepatology 64(6) (2016) 2173-2184.

[98] E. Giannini, F. Botta, P. Borro, D. Risso, P. Romagnoli, A. Fasoli, M.R. Mele, E. Testa, C. Mansi, V. Savarino, R. Testa, Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis, Gut 52(8) (2003) 1200-5.
[99] S.S. Sami, D. Harman, K. Ragunath, D. Bohning, J. Parkes, I.N. Guha, Non-invasive tests for the detection of oesophageal varices in compensated cirrhosis: systematic review and meta-analysis, United European Gastroenterol J 6(6) (2018) 806-818.
[100] A. Colecchia, L. Montrone, E. Scaioli, M.L. Bacchi-Reggiani, A. Colli, G. Casazza, R. Schiumerini, L. Turco, A.R. Di Biase, G. Mazzella, L. Marzi, U. Arena, M. Pinzani, D. Festi, Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis, Gastroenterology 143(3) (2012) 646-654.
[101] M. Buechter, A. Kahraman, P. Manka, G. Gerken, C. Jochum, A. Canbay, A. Dechene, Spleen and Liver Stiffness Is Positively Correlated with the Risk of Esophageal Variceal Bleeding, Digestion 94(3) (2016) 138-144.
[102] A. Colecchia, A. Colli, G. Casazza, D. Mandolesi, R. Schiumerini, L.B. Reggiani, G. Marasco, M. Taddia, A. Lisotti, G. Mazzella, A.R. Di Biase, R. Golfieri, M. Pinzani, D. Festi, Spleen stiffness measurement can predict clinical complications in compensated HCV-related cirrhosis: a prospective study, J Hepatol 60(6) (2014) 1158-64.
[103] G.L. Wong, R. Kwok, H.L. Chan, S.P. Tang, E. Lee, T.C. Lam, T.W. Lau, T.M. Ma, B.C. Wong, V.W. Wong, Measuring spleen stiffness to predict varices in chronic hepatitis B cirrhotic patients with or without receiving non-selective beta-blockers, J Dig Dis 17(8) (2016) 538-546.
[104] J. Guo, C. Buning, E. Schott, T. Kroncke, J. Braun, I. Sack, C. Althoff, In vivo abdominal magnetic resonance elastography for the assessment of portal hypertension before and after transjugular intrahepatic portosystemic shunt implantation, Invest Radiol 50(5) (2015) 347-51.
[105] G.I. Nedredal, M. Yin, T. McKenzie, J. Lillegard, J. Luebke-Wheeler, J. Talwalkar, R. Ehman, S.L. Nyberg, Portal hypertension correlates with splenic stiffness as measured with MR elastography, J Magn Reson Imaging 34(1) (2011) 79-87.
[106] S.U. Shin, J.M. Lee, M.H. Yu, J.H. Yoon, J.K. Han, B.I. Choi, K.J. Glaser, R.L. Ehman, Prediction of esophageal varices in patients with cirrhosis: usefulness of three-dimensional MR elastography with echo-planar imaging technique, Radiology 272(1) (2014) 143-53.
[107] C. Levick, J. Phillips-Hughes, J. Collier, R. Banerjee, J.F. Cobbold, L.M. Wang, S.K. Piechnik, M.D. Robson, S. Neubauer, E. Barnes, M. Pavlides, Non-invasive assessment of portal hypertension by multi-parametric magnetic resonance imaging of the spleen: A proof of concept study, PLoS One 14(8) (2019) e0221066.
[108] S.K. White, D.M. Sado, M. Fontana, S.M. Banypersad, V. Maestrini, A.S. Flett, S.K. Piechnik, M.D. Robson, D.J. Hausenloy, A.M. Sheikh, P.N. Hawkins, J.C. Moon, T1 mapping for myocardial extracellular volume measurement by CMR: bolus only versus primed infusion technique, JACC Cardiovasc Imaging 6(9) (2013) 955-62.
[109] S. Bandula, S.M. Banypersad, D. Sado, A.S. Flett, S. Punwani, S.A. Taylor, P.N. Hawkins, J.C. Moon, Measurement of Tissue interstitial volume in healthy patients and those with amyloidosis with equilibrium contrast-enhanced MR imaging, Radiology 268(3) (2013) 858-64.
[110] S. Augustin, M. Pons, J.B. Maurice, C. Bureau, H. Stefanescu, M. Ney, H. Blasco, B. Procopet, E. Tsochatzis, R.H. Westbrook, J. Bosch, A. Berzigotti, J.G. Abraldes, J. Genesca, Expanding the Baveno VI criteria for the screening of varices in patients with compensated advanced chronic liver disease, Hepatology (2017).
[111] S. Petta, G. Sebastiani, E. Bugianesi, M. Vigano, V.W. Wong, A. Berzigotti, A.L. Fracanzani, Q.M. Anstee, F. Marra, M. Barbara, V. Calvaruso, C. Camma, V. Di Marco, A. Craxi, V. de Ledinghen, Non-invasive prediction of esophageal varices by stiffness and platelet in non-alcoholic fatty liver disease cirrhosis, J Hepatol 69(4) (2018) 878-885.
[112] P. Jangouk, L. Turco, A. De Oliveira, F. Schepis, E. Villa, G. Garcia-Tsao, Validating, deconstructing and refining Baveno criteria for ruling out high-risk varices in patients with compensated cirrhosis, Liver Int 37(8) (2017) 1177-1183.

[113] J. Bae, D.H. Sinn, W. Kang, G.Y. Gwak, M.S. Choi, Y.H. Paik, J.H. Lee, K.C. Koh, S.W. Paik, Validation of the Baveno VI and the expanded Baveno VI criteria to identify patients who could avoid screening endoscopy, Liver Int 38(8) (2018) 1442-1448.
[114] P. Cales, F. Buisson, F. Ravaioli, A. Berger, C. Carboni, G. Marasco, D. Festi, How to clarify the Baveno VI criteria for ruling out varices needing treatment by non-invasive tests, Liver Int (2018).
[115] G.T. Everson, M.L. Shiffman, J.C. Hoefs, T.R. Morgan, R.K. Sterling, D.A. Wagner, S. Lauriski, T.M. Curto, A. Stoddard, E.C. Wright, H.-C.T. Group, Quantitative liver function tests improve the prediction of clinical outcomes in chronic hepatitis C: results from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis Trial, Hepatology 55(4) (2012) 1019-29.
[116] A. Lisotti, F. Azzaroli, A. Cucchetti, F. Buonfiglioli, P. Cecinato, C. Calvanese, P. Simoni, R. Arena, M. Montagnani, R. Golfieri, A. Colecchia, D. Festi, G. Mazzella, Relationship between Indocyanine Green Retention Test, decompensation and survival in patients with Child-Pugh A cirrhosis and portal hypertension, Liver Int (2016).
[117] A. Zipprich, O. Kuss, S. Rogowski, G. Kleber, E. Lotterer, T. Seufferlein, W.E. Fleig, M.M. Dollinger, Incorporating indocyanin green clearance into the Model for End Stage Liver Disease (MELD-ICG) improves prognostic accuracy in intermediate to advanced cirrhosis, Gut 59(7) (2010) 963-8.
[118] A. Lisotti, F. Azzaroli, F. Buonfiglioli, M. Montagnani, P. Cecinato, L. Turco, C. Calvanese, P. Simoni, M. Guardigli, R. Arena, A. Cucchetti, A. Colecchia, D. Festi, R. Golfieri, G. Mazzella, Indocyanine green retention test as a noninvasive marker of portal hypertension and esophageal varices in compensated liver cirrhosis, Hepatology 59(2) (2014) 643-50.
[119] G. Schuhmann-Giampieri, M. Mahler, G. Roll, R. Maibauer, S. Schmitz, Pharmacokinetics of the liver-specific contrast agent Gd-EOB-DTPA in relation to contrast-enhanced liver imaging in humans, J Clin Pharmacol 37(7) (1997) 587-96.
[120] S. Gschwend, W. Ebert, M. Schultze-Mosgau, J. Breuer, Pharmacokinetics and imaging properties of Gd-EOB-DTPA in patients with hepatic and renal impairment, Invest Radiol 46(9) (2011) 556-66.
[121] T. Katsube, M. Okada, S. Kumano, M. Hori, I. Imaoka, K. Ishii, M. Kudo, H. Kitagaki, T. Murakami, Estimation of liver function using T1 mapping on Gd-EOB-DTPA-enhanced magnetic resonance imaging, Invest Radiol 46(4) (2011) 277-83.
[122] M. Haimerl, N. Verloh, C. Fellner, F. Zeman, A. Teufel, S. Fichtner- Feigl, A.G. Schreyer, C. Stroszczynski, P. Wiggermann, MRI-based estimation of liver function: Gd-EOB-DTPA-enhanced T1 relaxometry of 3T vs. the MELD score, Sci Rep 4 (2014) 5621.
[123] Y. Ding, S.X. Rao, C. Chen, R. Li, M.S. Zeng, Assessing liver function in patients with HBV-related HCC: a comparison of T(1) mapping on Gd-EOB-DTPA-enhanced MR imaging with DWI, Eur Radiol 25(5) (2015) 1392-8.
[124] M. Haimerl, I. Fuhrmann, S. Poelsterl, C. Fellner, M.D. Nickel, K. Weigand, M.H. Dahlke, N. Verloh, C. Stroszczynski, P. Wiggermann, Gd-EOB-DTPA-enhanced T1 relaxometry for assessment of liver function determined by real-time (13)C-methacetin breath test, Eur Radiol (2018).
[125] M. Haimerl, M. Schlabeck, N. Verloh, F. Zeman, C. Fellner, D. Nickel, A.P. Barreiros, M. Loss, C. Stroszczynski, P. Wiggermann, Volume-assisted estimation of liver function based on Gd-EOB-DTPA-enhanced MR relaxometry, Eur Radiol (2015).
[126] J.L. Ulloa, S. Stahl, J. Yates, N. Woodhouse, J.G. Kenna, H.B. Jones, J.C. Waterton, P.D. Hockings, Assessment of gadoxetate DCE-MRI as a biomarker of hepatobiliary transporter inhibition, NMR Biomed 26(10) (2013) 1258-70.
[127] M.D. Robson, S.K. Piechnik, E.M. Tunnicliffe, S. Neubauer, T1 measurements in the human myocardium: the effects of magnetization transfer on the SASHA and MOLLI sequences, Magn Reson Med 70(3) (2013) 664-70.
[128] F.E. Mozes, E.M. Tunnicliffe, M. Pavlides, M.D. Robson, Influence of fat on liver T1 measurements using modified Look-Locker inversion recovery (MOLLI) methods at 3T, J Magn Reson Imaging 44(1) (2016) 105-11.
[129] P.J. Eddowes, N. McDonald, N. Davies, S.I.K. Semple, T.J. Kendall, J. Hodson, P.N. Newsome, R.B. Flintham, R. Wesolowski, L. Blake, R.V. Duarte, C.J. Kelly, A.H. Herlihy, M.D. Kelly, S.P. Olliff,

S.G. Hubscher, J.A. Fallowfield, G.M. Hirschfield, Utility and cost evaluation of multiparametric magnetic resonance imaging for the assessment of non-alcoholic fatty liver disease, Aliment Pharmacol Ther 47(5) (2018) 631-644.
[130] F.E. Mozes, E.M. Tunnicliffe, A. Moolla, T. Marjot, C.K. Levick, M. Pavlides, M.D. Robson, Mapping tissue water T1 in the liver using the MOLLI T1 method in the presence of fat, iron and B0 inhomogeneity, NMR Biomed 32(2) (2019) e4030.
[131] M. Pavlides, Multi-Parametric Magnetic Resonance for the Non-Invasive Assessment of Patients with Liver Disease, Radcliffe Department of Medicine, University of Oxford, United Kingdom, 2015.
[132] M.A. Richards, J.A. Webb, S.E. Jewell, W.M. Gregory, R.H. Reznek, In-vivo measurement of spin lattice relaxation time (T1) of liver in healthy volunteers: the effects of age, sex and oral contraceptive usage, Br J Radiol 61(721) (1988) 34-7.
[133] Y.X.J. Wang, M. Deng, J. Lin, A.W.L. Kwok, E.K.W. Liu, W. Chen, Age- and Gender-Associated Liver Physiological T1rho Dynamics Demonstrated with a Clinically Applicable Single-Breathhold Acquisition, SLAS Technol 23(2) (2018) 179-187.
[134] M. Koopmann, K. Hong, E.G. Kholmovski, E.C. Huang, N. Hu, J. Ying, R. Levenson, S. Vijayakumar, D.J. Dosdall, R. Ranjan, D. Kim, Post-contrast myocardial T(1) and ECV disagree in a longitudinal canine study, NMR Biomed 27(8) (2014) 988-95.
[135] P. Kellman, J.R. Wilson, H. Xue, M. Ugander, A.E. Arai, Extracellular volume fraction mapping in the myocardium, part 1: evaluation of an automated method, J Cardiovasc Magn Reson 14 (2012) 63.
[136] C. de Meester de Ravenstein, C. Bouzin, S. Lazam, J. Boulif, M. Amzulescu, J. Melchior, A. Pasquet, D. Vancraeynest, A.C. Pouleur, J.L. Vanoverschelde, B.L. Gerber, Histological Validation of measurement of diffuse interstitial myocardial fibrosis by myocardial extravascular volume fraction from Modified Look-Locker imaging (MOLLI) T1 mapping at 3 T, J Cardiovasc Magn Reson 17 (2015) 48.
[137] S. Bandula, S. Punwani, W.M. Rosenberg, R. Jalan, A.R. Hall, A. Dhillon, J.C. Moon, S.A. Taylor, Equilibrium contrast-enhanced CT imaging to evaluate hepatic fibrosis: initial validation by comparison with histopathologic sampling, Radiology 275(1) (2015) 136-43.
[138] M. Ziol, A. Handra-Luca, A. Kettaneh, C. Christidis, F. Mal, F. Kazemi, V. de Ledinghen, P. Marcellin, D. Dhumeaux, J.C. Trinchet, M. Beaugrand, Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C, Hepatology 41(1) (2005) 48-54.
[139] S.K. Piechnik, V.M. Ferreira, E. Dall'Armellina, L.E. Cochlin, A. Greiser, S. Neubauer, M.D. Robson, Shortened Modified Look-Locker Inversion recovery (ShMOLLI) for clinical myocardial T1-mapping at 1.5 and 3 T within a 9 heartbeat breathhold, Journal of Cardiovascular Magnetic Resonance 12(1) (2010) 69.
[140] J.C. Moon, D.R. Messroghli, P. Kellman, S.K. Piechnik, M.D. Robson, M. Ugander, P.D. Gatehouse, A.E. Arai, M.G. Friedrich, S. Neubauer, J. Schulz-Menger, E.B. Schelbert, I. Society for Cardiovascular Magnetic Resonance, C. Cardiovascular Magnetic Resonance Working Group of the European Society of, Myocardial T1 mapping and extracellular volume quantification: a Society for Cardiovascular Magnetic Resonance (SCMR) and CMR Working Group of the European Society of Cardiology consensus statement, J Cardiovasc Magn Reson 15 (2013) 92.
[141] S. Rosmini, H. Bulluck, G. Captur, T.A. Treibel, A. Abdel-Gadir, A.N. Bhuva, V. Culotta, A. Merghani, M. Fontana, V. Maestrini, A.S. Herrey, K. Chow, R.B. Thompson, S.K. Piechnik, P. Kellman, C. Manisty, J.C. Moon, Myocardial native T1 and extracellular volume with healthy ageing and gender, Eur Heart J Cardiovasc Imaging 19(6) (2018) 615-621.
[142] R. Stricker, R. Eberhart, M.C. Chevailler, F.A. Quinn, P. Bischof, R. Stricker, Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer, Clin Chem Lab Med 44(7) (2006) 883-7.
[143] C.P. White, C.L. Hitchcock, Y.M. Vigna, J.C. Prior, Fluid Retention over the Menstrual Cycle: 1-Year Data from the Prospective Ovulation Cohort, Obstet Gynecol Int 2011 (2011) 138451.

[144] N.S. Stachenfeld, H.S. Taylor, Effects of estrogen and progesterone administration on extracellular fluid, J Appl Physiol (1985) 96(3) (2004) 1011-8.
[145] C.S. Hicks, C.N. McLester, T.A. Esmat, J.R. McLester, A Comparison of Body Composition Across Two Phases of the Menstrual Cycle Utilizing Dual-Energy X-Ray Absorptiometry, Air Displacement Plethysmography, and Bioelectrical Impedance Analysis, Int J Exerc Sci 10(8) (2017) 1235-1249.
[146] N.S. Stachenfeld, Sex hormone effects on body fluid regulation, Exerc Sport Sci Rev 36(3) (2008) 152-9.
[147] Y.X.J. Wang, M. Deng, G.G. Lo, D. Liang, J. Yuan, W. Chen, Breath-hold black-blood T1rho mapping improves liver T1rho quantification in healthy volunteers, Acta Radiol 59(3) (2018) 257-265.
[148] M. Pavlides, C. Levick, J. Phillips-Hughes, J. Collier, R. Banerjee, L.M. Wang, J. Cobbold, M. Robson, S. Neubauer, E. Barnes, Multi-Parametric Magnetic Resonance Imaging of the Spleen: An Accurate and Non-Invasive Biomarker of Portal Pressure, Journal of Hepatology 64(2) (2016) S277.
[149] S.F. Keevil, G. Dolke, A.P. Brooks, P. Armstrong, M.J. Farthing, E.M. Alstead, M.A. Smith, Proton NMR relaxation times in the normal human liver at 0.08 T, Clin Radiol 45(5) (1992) 302-6.
[150] A. Sawai, Y. Tochigi, N. Kavaliova, A. Zaboronok, Y. Warashina, B.J. Mathis, N. Mesaki, H. Shiraki, K. Watanabe, MRI reveals menstrually-related muscle edema that negatively affects athletic agility in young women, PLoS One 13(1) (2018) e0191022.
[151] A. Marot, E. Trepo, C. Doerig, A. Schoepfer, C. Moreno, P. Deltenre, Liver stiffness and platelet count for identifying patients with compensated liver disease at low risk of variceal bleeding, Liver Int 37(5) (2017) 707-716.
[152] B.K. Kim, K.H. Han, J.Y. Park, S.H. Ahn, J.K. Kim, Y.H. Paik, K.S. Lee, C.Y. Chon, D.Y. Kim, A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis, Am J Gastroenterol 105(6) (2010) 1382-90.
[153] M. Abu Rajab, L. Guerin, P. Lee, K.E. Brown, Iron overload secondary to cirrhosis: a mimic of hereditary haemochromatosis?, Histopathology 65(4) (2014) 561-9.
[154] D.Y. Yoon, B.I. Choi, J.K. Han, M.C. Han, M.O. Park, S.J. Suh, MR findings of secondary hemochromatosis: transfusional vs erythropoietic, J Comput Assist Tomogr 18(3) (1994) 416-9.
[155] H.Y. Sun, J.M. Lee, J.K. Han, B.I. Choi, Usefulness of MR elastography for predicting esophageal varices in cirrhotic patients, J Magn Reson Imaging 39(3) (2014) 559-66.
[156] S. Singh, J.E. Eaton, M.H. Murad, H. Tanaka, H. Iijima, J.A. Talwalkar, Accuracy of spleen stiffness measurement in detection of esophageal varices in patients with chronic liver disease: systematic review and meta-analysis, Clin Gastroenterol Hepatol 12(6) (2014) 935-45 e4.
[157] A. Colecchia, F. Ravaioli, G. Marasco, A. Colli, E. Dajti, A.R. Di Biase, M.L. Bacchi Reggiani, A. Berzigotti, M. Pinzani, D. Festi, A combined model based on spleen stiffness measurement and Baveno VI criteria to rule out high-risk varices in advanced chronic liver disease, J Hepatol 69(2) (2018) 308-317.
[158] E.J. Mullin, M.S. Metcalfe, G.J. Maddern, How much liver resection is too much?, Am J Surg 190(1) (2005) 87-97.
[159] M. Haimerl, N. Verloh, F. Zeman, C. Fellner, R. Muller-Wille, A.G. Schreyer, C. Stroszczynski, P. Wiggermann, Assessment of clinical signs of liver cirrhosis using T1 mapping on Gd-EOB-DTPA-enhanced 3T MRI, PLoS One 8(12) (2013) e85658.
[160] B.E. Van Beers, C.M. Pastor, H.K. Hussain, Primovist, Eovist: what to expect?, J Hepatol 57(2) (2012) 421-9.
[161] Y.H. Chuang, H.Y. Ou, M.Z. Lazo, C.L. Chen, M.H. Chen, C.C. Weng, Y.F. Cheng, Predicting posthepatectomy liver failure by combined volumetric, functional MR image and laboratory analysis, Liver Int (2017).
[162] H.C. Yu, H. You, H. Lee, Z.W. Jin, J.I. Moon, B.H. Cho, Estimation of standard liver volume for liver transplantation in the Korean population, Liver Transpl 10(6) (2004) 779-83.

[163] C. Besa, O. Bane, G. Jajamovich, J. Marchione, B. Taouli, 3D T1 relaxometry pre and post gadoxetic acid injection for the assessment of liver cirrhosis and liver function, Magn Reson Imaging 33(9) (2015) 1075-82.
[164] T. Kamisako, Y. Kobayashi, K. Takeuchi, T. Ishihara, K. Higuchi, Y. Tanaka, E.C. Gabazza, Y. Adachi, Recent advances in bilirubin metabolism research: the molecular mechanism of hepatocyte bilirubin transport and its clinical relevance, J Gastroenterol 35(9) (2000) 659-64.
[165] N. Verloh, M. Haimerl, F. Zeman, A. Teufel, S. Lang, C. Stroszczynski, C. Fellner, P. Wiggermann, Multivariable analysis of clinical influence factors on liver enhancement of Gd-EOB-DTPA-enhanced 3T MRI, Rofo 187(1) (2015) 29-35.
[166] Y. Ding, S.X. Rao, T. Zhu, C.Z. Chen, R.C. Li, M.S. Zeng, Liver fibrosis staging using T1 mapping on gadoxetic acid-enhanced MRI compared with DW imaging, Clin Radiol 70(10) (2015) 1096-103.
[167] U. Asenbaum, A. Ba-Ssalamah, M. Mandorfer, R. Nolz, J. Furtner, T. Reiberger, A. Ferlitsch, K. Kaczirek, M. Trauner, M. Peck-Radosavljevic, A.G. Wibmer, Effects of Portal Hypertension on Gadoxetic Acid-Enhanced Liver Magnetic Resonance: Diagnostic and Prognostic Implications, Invest Radiol 52(8) (2017) 462-469.
[168] T. Yoneyama, Y. Fukukura, K. Kamimura, K. Takumi, A. Umanodan, S. Ueno, M. Nakajo, Efficacy of liver parenchymal enhancement and liver volume to standard liver volume ratio on Gd-EOB-DTPA-enhanced MRI for estimation of liver function, Eur Radiol 24(4) (2014) 857-65.
[169] Y.Y. Wang, X.H. Zhao, L. Ma, J.Z. Ye, F.X. Wu, J. Tang, X.M. You, B.D. Xiang, L.Q. Li, Comparison of the ability of Child-Pugh score, MELD score, and ICG-R15 to assess preoperative hepatic functional reserve in patients with hepatocellular carcinoma, J Surg Oncol 118(3) (2018) 440-445.
[170] N. Kato, T. Yokawa, A. Tamura, A. Heshiki, W. Ebert, H.J. Weinmann, Gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid interaction with clinical drugs in rats, Invest Radiol 37(12) (2002) 680-4.
[171] A. Huppertz, J. Breuer, L.M. Fels, M. Schultze-Mosgau, G. Sutter, S. Klein, B. Frericks, B. Hamm, M. Wagner, Evaluation of possible drug-drug interaction between gadoxetic acid and erythromycin as an inhibitor of organic anion transporting peptides (OATP), J Magn Reson Imaging 33(2) (2011) 409-16.
[172] H. Nilsson, L. Blomqvist, L. Douglas, A. Nordell, H. Jacobsson, K. Hagen, A. Bergquist, E. Jonas, Dynamic gadoxetate-enhanced MRI for the assessment of total and segmental liver function and volume in primary sclerosing cholangitis, J Magn Reson Imaging 39(4) (2014) 879-86.
[173] H. Nilsson, L. Blomqvist, L. Douglas, A. Nordell, I. Janczewska, E. Naslund, E. Jonas, Gd-EOB-DTPA-enhanced MRI for the assessment of liver function and volume in liver cirrhosis, Br J Radiol 86(1026) (2013) 20120653.
[174] G. D'Amico, L. Pasta, A. Morabito, M. D'Amico, M. Caltagirone, G. Malizia, F. Tine, G. Giannuoli, M. Traina, G. Vizzini, F. Politi, A. Luca, R. Virdone, A. Licata, L. Pagliaro, Competing risks and prognostic stages of cirrhosis: a 25-year inception cohort study of 494 patients, Aliment Pharmacol Ther 39(10) (2014) 1180-93.
[175] V. Calvaruso, V. Di Marco, M.G. Bavetta, D. Cabibi, E. Conte, F. Bronte, F. Simone, A.K. Burroughs, A. Craxi, Quantification of fibrosis by collagen proportionate area predicts hepatic decompensation in hepatitis C cirrhosis, Aliment Pharmacol Ther 41(5) (2015) 477-86.
[176] K. Nielsen, J.O. Clemmesen, E. Vassiliadis, B. Vainer, Liver collagen in cirrhosis correlates with portal hypertension and liver dysfunction, APMIS 122(12) (2014) 1213-22.
[177] C. Stasi, E.A. Tsochatzis, A. Hall, W. Rosenberg, S. Milani, A.P. Dhillon, M. Pinzani, Comparison and correlation of fibrosis stage assessment by collagen proportionate area (CPA) and the ELF panel in patients with chronic liver disease, Dig Liver Dis (2018).
[178] G. Isgro, V. Calvaruso, L. Andreana, T.V. Luong, M. Garcovich, P. Manousou, A. Alibrandi, S. Maimone, L. Marelli, N. Davies, D. Patch, A.P. Dhillon, A.K. Burroughs, The relationship between transient elastography and histological collagen proportionate area for assessing fibrosis in chronic viral hepatitis, J Gastroenterol 48(8) (2013) 921-9.
[179] S.H. Chen, C.Y. Peng, H.C. Lai, I.P. Chang, C.J. Lee, W.P. Su, C.H. Lin, J.T. Kao, P.H. Chuang, Head-to-Head Comparison between Collagen Proportionate Area and Acoustic Radiation Force

Impulse Elastography in Liver Fibrosis Quantification in Chronic Hepatitis C, PLoS One 10(10) (2015) e0140554.
[180] H. Ding, J.J. Ma, W.P. Wang, W.J. Zeng, T. Jiang, B.J. Huang, S.Y. Chen, Assessment of liver fibrosis: the relationship between point shear wave elastography and quantitative histological analysis, J Gastroenterol Hepatol 30(3) (2015) 553-8.
[181] J. Vergniol, J. Foucher, E. Terrebonne, P.H. Bernard, B. le Bail, W. Merrouche, P. Couzigou, V. de Ledinghen, Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C, Gastroenterology 140(7) (2011) 1970-9, 1979 e1-3.
[182] T.Y. Kim, J.G. Lee, J.H. Sohn, J.Y. Kim, S.M. Kim, J. Kim, W.K. Jeong, Hepatic Venous Pressure Gradient Predicts Long-Term Mortality in Patients with Decompensated Cirrhosis, Yonsei Med J 57(1) (2016) 138-45.
[183] A. Blasco, X. Forns, J.A. Carrion, J.C. Garcia-Pagan, R. Gilabert, A. Rimola, R. Miquel, M. Bruguera, J.C. Garcia-Valdecasas, J. Bosch, M. Navasa, Hepatic venous pressure gradient identifies patients at risk of severe hepatitis C recurrence after liver transplantation, Hepatology 43(3) (2006) 492-9.
[184] Y. Takuma, Y. Morimoto, H. Takabatake, N. Toshikuni, J. Tomokuni, A. Sahara, K. Matsueda, H. Yamamoto, Measurement of Spleen Stiffness With Acoustic Radiation Force Impulse Imaging Predicts Mortality and Hepatic Decompensation in Patients With Liver Cirrhosis, Clin Gastroenterol Hepatol 15(11) (2017) 1782-1790 e4.
[185] X.P. Cheng, J. Zhao, Y. Chen, F.K. Meng, B. Xu, H.W. Yu, Q.H. Meng, Y.M. Liu, S.B. Zhang, S. Meng, J.Y. Zhang, J.Y. Zhang, Z.P. Duan, S.J. Zheng, Comparison of the ability of the PDD-ICG clearance test, CTP, MELD, and MELD-Na to predict short-term and medium-term mortality in patients with decompensated hepatitis B cirrhosis, Eur J Gastroenterol Hepatol 28(4) (2016) 444-8.
[186] K. Sandrasegaran, E. Cui, R. Elkady, P. Gasparis, G. Borthakur, M. Tann, S. Liangpunsakul, Can functional parameters from hepatobiliary phase of gadoxetate MRI predict clinical outcomes in patients with cirrhosis?, Eur Radiol 28(10) (2018) 4215-4224.
[187] A. Jayaswal, M. Pavlides, R. Banerjee, M. Kelly, E. Barnes, S. Neubauer, Liver cT1 predicts clinical outcomes in patients with chronic liver disease, equivalent to biopsy, Conference abstract, Wiley, 2018, pp. 607A-608A.
[188] A. Unalp-Arida, C.E. Ruhl, Liver fibrosis scores predict liver disease mortality in the United States population, Hepatology 66(1) (2017) 84-95.
[189] S.W. Biggins, H.J. Rodriguez, P. Bacchetti, N.M. Bass, J.P. Roberts, N.A. Terrault, Serum sodium predicts mortality in patients listed for liver transplantation, Hepatology 41(1) (2005) 32-9.
[190] J.B. Saunders, J.R. Walters, A.P. Davies, A. Paton, A 20-year prospective study of cirrhosis, Br Med J (Clin Res Ed) 282(6260) (1981) 263-6.
[191] G. Borroni, A. Maggi, A. Sangiovanni, M. Cazzaniga, F. Salerno, Clinical relevance of hyponatraemia for the hospital outcome of cirrhotic patients, Dig Liver Dis 32(7) (2000) 605-10.
[192] S. John, P.J. Thuluvath, Hyponatremia in cirrhosis: pathophysiology and management, World J Gastroenterol 21(11) (2015) 3197-205.
[193] L. Ma, S. Zhao, Risk factors for mortality in patients undergoing hemodialysis: A systematic review and meta-analysis, Int J Cardiol 238 (2017) 151-158.
[194] S. Cabrerizo, D. Cuadras, F. Gomez-Busto, I. Artaza-Artabe, F. Marin-Ciancas, V. Malafarina, Serum albumin and health in older people: Review and meta analysis, Maturitas 81(1) (2015) 17-27.
[195] S.H. Kang, W.K. Jeong, S.K. Baik, S.H. Cha, M.Y. Kim, Impact of sarcopenia on prognostic value of cirrhosis: going beyond the hepatic venous pressure gradient and MELD score, J Cachexia Sarcopenia Muscle 9(5) (2018) 860-870.
[196] N. McDonald, D.M.L. Lilburn, N.J. Lachlan, G. Macnaught, D. Patel, A.N.A. Jayaswal, P.C. Hayes, S.I. Semple, J.A. Fallowfield, Assessment of Haemodynamic Response to Nonselective Beta-Blockers in Portal Hypertension by Phase-Contrast Magnetic Resonance Angiography, Biomed Res Int 2017 (2017) 9281450.

[197] S. Roujol, S. Weingartner, M. Foppa, K. Chow, K. Kawaji, L.H. Ngo, P. Kellman, W.J. Manning, R.B. Thompson, R. Nezafat, Accuracy, precision, and reproducibility of four T1 mapping sequences: a head-to-head comparison of MOLLI, ShMOLLI, SASHA, and SAPPHIRE, Radiology 272(3) (2014) 683-9.
[198] C. Hutton, M.L. Gyngell, M. Milanesi, A. Bagur, M. Brady, Validation of a standardized MRI method for liver fat and T2* quantification, PLoS One 13(9) (2018) e0204175.
[199] M.F. Frances, Z. Dujic, J.K. Shoemaker, Splenic constriction during isometric handgrip exercise in humans, Appl Physiol Nutr Metab 33(5) (2008) 990-6.
[200] M.X. Richardson, A. Lodin, J. Reimers, E. Schagatay, Short-term effects of normobaric hypoxia on the human spleen, Eur J Appl Physiol 104(2) (2008) 395-9.
[201] e.e.e. European Association for the Study of the Liver. Electronic address, L. European Association for the Study of the, EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma, J Hepatol 69(1) (2018) 182-236.
[202] J.A. Luetkens, S. Klein, F. Traeber, F.C. Schmeel, A.M. Sprinkart, D.L.R. Kuetting, W. Block, K. Hittatiya, F.E. Uschner, R. Schierwagen, J. Gieseke, H.H. Schild, J. Trebicka, G.M. Kukuk, Quantitative liver MRI including extracellular volume fraction for non-invasive quantification of liver fibrosis: a prospective proof-of-concept study, Gut 67(3) (2018) 593-594.
[203] C. Caussy, M.H. Alquiraish, P. Nguyen, C. Hernandez, S. Cepin, L.E. Fortney, V. Ajmera, R. Bettencourt, S. Collier, J. Hooker, E. Sy, E. Rizo, L. Richards, C.B. Sirlin, R. Loomba, Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis, Hepatology 67(4) (2018) 1348-1359.
[204] G.L. Wong, L.Y. Liang, R. Kwok, A.J. Hui, Y.K. Tse, H.L. Chan, V.W. Wong, Low Risk of Variceal Bleeding in Patients With Cirrhosis After Variceal Screening Stratified by Liver/Spleen Stiffness, Hepatology (2019).
[205] M. Ng, T. Fleming, M. Robinson, B. Thomson, N. Graetz, C. Margono, E.C. Mullany, S. Biryukov, C. Abbafati, S.F. Abera, J.P. Abraham, N.M. Abu-Rmeileh, T. Achoki, F.S. AlBuhairan, Z.A. Alemu, R. Alfonso, M.K. Ali, R. Ali, N.A. Guzman, W. Ammar, P. Anwari, A. Banerjee, S. Barquera, S. Basu, D.A. Bennett, Z. Bhutta, J. Blore, N. Cabral, I.C. Nonato, J.C. Chang, R. Chowdhury, K.J. Courville, M.H. Criqui, D.K. Cundiff, K.C. Dabhadkar, L. Dandona, A. Davis, A. Dayama, S.D. Dharmaratne, E.L. Ding, A.M. Durrani, A. Esteghamati, F. Farzadfar, D.F. Fay, V.L. Feigin, A. Flaxman, M.H. Forouzanfar, A. Goto, M.A. Green, R. Gupta, N. Hafezi-Nejad, G.J. Hankey, H.C. Harewood, R. Havmoeller, S. Hay, L. Hernandez, A. Husseini, B.T. Idrisov, N. Ikeda, F. Islami, E. Jahangir, S.K. Jassal, S.H. Jee, M. Jeffreys, J.B. Jonas, E.K. Kabagambe, S.E. Khalifa, A.P. Kengne, Y.S. Khader, Y.H. Khang, D. Kim, R.W. Kimokoti, J.M. Kinge, Y. Kokubo, S. Kosen, G. Kwan, T. Lai, M. Leinsalu, Y. Li, X. Liang, S. Liu, G. Logroscino, P.A. Lotufo, Y. Lu, J. Ma, N.K. Mainoo, G.A. Mensah, T.R. Merriman, A.H. Mokdad, J. Moschandreas, M. Naghavi, A. Naheed, D. Nand, K.M. Narayan, E.L. Nelson, M.L. Neuhouser, M.I. Nisar, T. Ohkubo, S.O. Oti, A. Pedroza, D. Prabhakaran, N. Roy, U. Sampson, H. Seo, S.G. Sepanlou, K. Shibuya, R. Shiri, I. Shiue, G.M. Singh, J.A. Singh, V. Skirbekk, N.J. Stapelberg, L. Sturua, B.L. Sykes, M. Tobias, B.X. Tran, L. Trasande, H. Toyoshima, S. van de Vijver, T.J. Vasankari, J.L. Veerman, G. Velasquez-Melendez, V.V. Vlassov, S.E. Vollset, T. Vos, C. Wang, X. Wang, E. Weiderpass, A. Werdecker, J.L. Wright, Y.C. Yang, H. Yatsuya, J. Yoon, S.J. Yoon, Y. Zhao, M. Zhou, S. Zhu, A.D. Lopez, C.J. Murray, E. Gakidou, Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013, Lancet 384(9945) (2014) 766-81.
[206] S.K. Asrani, J.A. Talwalkar, P.S. Kamath, V.H. Shah, G. Saracino, L. Jennings, J.B. Gross, S. Venkatesh, R.L. Ehman, Role of magnetic resonance elastography in compensated and decompensated liver disease, J Hepatol 60(5) (2014) 934-9.
[207] R. Rustogi, J. Horowitz, C. Harmath, Y. Wang, H. Chalian, D.R. Ganger, Z.E. Chen, B.D. Bolster, Jr., S. Shah, F.H. Miller, Accuracy of MR elastography and anatomic MR imaging features in the diagnosis of severe hepatic fibrosis and cirrhosis, J Magn Reson Imaging 35(6) (2012) 1356-64.

[208] M. Wagner, I. Corcuera-Solano, G. Lo, S. Esses, J. Liao, C. Besa, N. Chen, G. Abraham, M. Fung, J.S. Babb, R.L. Ehman, B. Taouli, Technical Failure of MR Elastography Examinations of the Liver: Experience from a Large Single-Center Study, Radiology 284(2) (2017) 401-412.
[209] K. Dantendorfer, D. Wimberger, H. Katschnig, H. Imhoff, Claustrophobia in MRI scanners, Lancet 338(8769) (1991) 761-2.
[210] V. Bachtiar, M.D. Kelly, H.R. Wilman, J. Jacobs, R. Newbould, C.J. Kelly, M.L. Gyngell, K.E. Groves, A. McKay, A.H. Herlihy, C.C. Fernandes, M. Halberstadt, M. Maguire, N. Jayaratne, S. Linden, S. Neubauer, R. Banerjee, Repeatability and reproducibility of multiparametric magnetic resonance imaging of the liver, PLoS One 14(4) (2019) e0214921.
[211] S. Ray, N.N. Mehta, A. Golhar, S. Nundy, Post hepatectomy liver failure - A comprehensive review of current concepts and controversies, Ann Med Surg (Lond) 34 (2018) 4-10.

9 Appendix

9.1 Appendix A. Letter to the research ethics committee regarding the safety of gadoxetic acid in the MAPLE study

PETER MEDAWAR BUILDING
South Parks Road, Oxford, OX1 3SY
Tel: 01865 271199
Fax: 01865 281890
Email: ellie.barnes@ndm.ox.ac.uk

OXFORD CENTRE FOR CLINICAL MAGNETIC RESONANCE RESEARCH
University of Oxford, Level 0, John Radcliffe Hospital, Oxford, OX3 9DU
Email: stefan.neubauer@cardiov.ox.ac.uk, matthew.robson@cardiov.ox.ac.uk, christina.levick@ndm.ox.ac.uk



Oxford A Regional Ethics Committee
Health Research Authority
Bristol HRA Centre
Level 3, Block B, Whitefriars
Lewins Mead, Bristol
BS1 2NT

12th September 2017

Dear Oxford A REC,

Re: The use of gadoxetic acid (Primovist; Gd-DTPA-EOB) in the MAPLE study (ethics 16/SC/0110)

We are writing to seek support to maintain the use of gadoxetic acid (Primovist; Gd-DTPA-EOB) in the MAPLE study and restart the study in its existing observational design, by substantial amendment. The MAPLE study is fully funded by the NIHR, has recruited 104 volunteers to date (52% complete) and is due to run for a further 12 months and recruit a further 96 patients.

The MAPLE study uses a liver-specific gadolinium-based contrast agent (GCBA) called gadoxetic acid in order to assess the two primary endpoints of the study – liver function and portal hypertension. Most participants receive a single weight-based dose of gadoxetic acid, whilst a subgroup of 5 healthy volunteers and 20 liver disease patients receive 2 doses at least 1 week apart. Gadoxetic acid has been used in clinical practice since 2004. GCBAs can be subdivided by molecular structure into either macrocyclic or linear agents, of which gadoxetic acid is a subtype of linear agent.

The MAPLE study was halted by the chief investigator on 07/07/2017 after a report emerged following a safety review of linear GCBAs by the Pharmacovigilance Risk Assessment Committee (PRAC) [1].

The review found evidence of dose-related gadolinium deposition in the brain with linear GCBAs, but no clinical consequences of this. PRAC recommended that linear gadolinium-based contrast agents, excluding gadoxetic acid, should no longer be used, whilst macrocyclic gadolinium-based contrast agents could be used as an alternative. Importantly, gadoxetic acid could still be used because it is liver-specific, with no alternatives. These recommendations were then adopted by the European Medicines Agency (EMA), allowing gadoxetic acid to still be used in clinical care. In time, EMA will pass their recommendations to the European Commission, who will then pass them as European Union law.

The EMA has recommended that the product information of gadoxetic acid be changed to: "<Product name> should be used only when diagnostic information is essential and not available with unenhanced magnetic resonance imaging (MRI) and when delayed phase imaging is required."

It is unclear how these recommendations affect the use of gadoxetic acid in the MAPLE study, so the EMA, sponsor, ethics committee and MHRA were consulted. The EMA suggested that the Medicines and Healthcare Products Regulatory Agency (MHRA) should provide guidance on how gadoxetic acid could be used in the MAPLE study, but the MHRA do not consider the MAPLE study to be a clinical trial and so will not provide such guidance. In light of this information, the Oxford A regional ethics committee concluded it is the sponsor's responsibility to decide how to proceed based on the EMA guidance.

We have now explored various options as to how best to proceed with MAPLE. Conversion into a clinical trial would enable it to become under the regulation of the MHRA. However, the investigators recognise that the MAPLE study is not a clinical trial of gadoxetic acid and so regulation by MHRA is not appropriate. The idea of a clinical trial was therefore abandoned.

Another option would be changing the contrast agent in the study to gadobutrol, a macrocyclic GBCA that has already been used in the study. This would enable continuation as an observational study, but would lose one of the primary aims of the study (liver function assessment).

The final option is to continue the study with gadoxetic acid in its current format, which the sponsor and the investigators believe is the best option. As safety is the greatest concern, we have reviewed the evidence for gadolinium retention in the brain caused by gadoxetic acid. To the best of our knowledge, this includes all the studies included in the PRAC review and those published following the review. The highest quality method to detect gadolinium retention in the brain is by autopsy evidence, but whilst alive, an increase in tissue signal intensity on magnetic resonance imaging (MRI) is used as a surrogate marker. MRI is exquisitely sensitive to even tiny amounts of gadolinium. There are 4 studies - 2 studies with evidence of gadolinium retention in the brain of subjects who had received 10-37 administrations of gadoxetic acid and 2 studies showing no evidence of gadolinium retention in the brain with 1-18 administrations.

Murata et al., 2016 [2] provides the only autopsy study in which 1 patient had gadoxetic acid (10 administrations), 1 had gadobenate (linear GCBA) and 2 had gadobutrol (macrocyclic GCBA) and 5 had gadoteridol (macrocyclic GCBA). Gadolinium was found with all contrast agents in all brain areas sampled, particularly the globus pallidus and dentate nucleus.

The other positive study was by Kahn et al. 2017 [3] who acquired T1-weighted brain MRIs of 91 patients prospectively. All patients had gadoxetic acid administration at least 60 days previously, with no more than 2 previous doses of macrocyclic GCBAs. The patient groups were subdivided into those having 1-4 ($n = 32$), 5-10 ($n = 27$) or >10 ($n = 32$, range 11-37) gadoxetic acid doses. A separate group of 51 patients referred for brain MRI with no previous exposure to any GCBAs were used as controls. Signal intensity ratios of the dentate nucleus to middle cerebellar peduncle (DN/MCP), DN to pons (DN/pons), globus pallidus to thalamus (GP/thalamus) and DN to cerebrospinal fluid (DN/CSF) were calculated. The number of gadoxetic acid administrations had significant correlations with the DN/MCP ($r = 0.51$, $p < 0.0001$) and DN/pons ratios ($r = 0.41$, $p = 0.0001$), but not with DN/CSF. In subgroup analysis, only patients receiving >10 doses of gadoxetic acid had greater signal intensity ratios than controls (DN/MCP, $p < 0.0001$ and DN/pons, $p = 0.002$). The dentate nucleus signal intensity increase in gadoxetic acid-exposed patients was approximately 2%, which is lower than that reported for other linear GCBAs in the literature. Although other linear GCBAs had been associated with gadolinium deposition in the globus pallidus, the GP/thalamus in Kahn's study did

not show significant correlation with the number of gadoxetic acid administrations ($r = 0.13$, $p = 0.23$) and the difference in ratios between gadoxetic acid-exposed patients and controls could be explained by differences in age.

In contrast to Kahn et al.'s findings, Ichikawa et al., 2017 [4] found no correlation between the number of gadoxetic acid administrations (range 1-16) and DN/pons, and no difference in DN/pons between either single ($n = 33$) or ≥5 gadoxetic acid dose ($n = 33$) groups and controls ($n= 33$) ($p = 1.00$ and $p = 0.39$, respectively). In a separate group of 33 patients, gadodiamide (linear GCBA with quadruple dose and weaker molecular binding of the gadolinium than gadoxetic acid) administrations did correlate with DN/pons, which was significantly higher with ≥5 gadodiamide doses than in controls ($p < 0.0001$), thus supporting PRAC's review of other linear GCBAs.

Finally, Conte et al., 2017 [5] assessed DN/pons and GP/thalamus by MRI in an intra-individual, retrospective study of 18 melanoma patients before and after ≥2 gadoxetic acid administrations (range 2-18) and no other GCBAs. These patients had been part of a prospective, stage III clinical trial of Ipilimumab. DN/pons and GP/thalamus did not change following gadoxetic acid administrations ($p = 0.21$ and $p = 0.09$, respectively) and did not correlate with the number of gadoxetic acid administrations ($r = 0.153$, $p = 0.542$ and $r = -0.165$, $p = 0.512$, respectively).

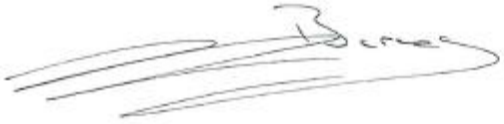
In combination these studies provide some evidence of gadolinium accumulation in the dentate nucleus and globus pallidus of the brain associated with ≥10 doses gadoxetic acid. The lack of accumulation at fewer administrations supports the safety of EMA's recommendation to continue using Primovist in clinical practice.

Importantly, no studies have reported adverse effects from gadolinium retention in the brain from any GCBA.

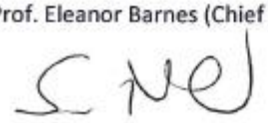
We therefore believe it would be safe to continue using gadoxetic acid in the MAPLE study. In the MAPLE study ≤2 doses of gadoxetic acid are administered to participants, giving scope for a further 8 doses before detectable gadolinium accumulation in the brain may occur. It is extremely unlikely that so many doses would be given in these patients' clinical care throughout their lifetime. The gadolinium accumulation with gadoxetic acid is likely less than other GCBAs because gadoxetic acid is used in quarter the dose, has stronger molecular binding of gadolinium than other linear GCBAs and has hepatic as well as renal excretion. Patients with significant renal impairment are already excluded from the MAPLE study, minimising risk.

Having reviewed the evidence, we conclude that gadoxetic acid remains a very low risk contrast agent that is irreplaceable in the MAPLE study, just as it is for clinical care. The value of gadoxetic acid's potential abilities for liver function and portal hypertension assessment outweigh the minimal risk in its application. We therefore propose to continue the study as an observational study, adding additional safety information to the protocol and participant information sheets to enable informed consent. We have the full support of the University of Oxford as our sponsor and we are asking that REC support these conclusions too so that the MAPLE study may continue.

Yours sincerely,



Prof. Eleanor Barnes (Chief Investigator)



Prof. Stefan Neubauer (Investigator)



Prof Matthew Robson (Investigator)



Dr Christina Levick (Investigator and DPhil candidate)

References

1. Agency, E.M. *Gadolinium-containing contrast agents*. 2017; Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Gadolinium-containing_contrast_agents/human_referral_prac_000056.jsp&mid=WC0b01ac05805c516f.
2. Murata, N., et al., *Macrocyclic and Other Non-Group 1 Gadolinium Contrast Agents Deposit Low Levels of Gadolinium in Brain and Bone Tissue: Preliminary Results From 9 Patients With Normal Renal Function.* Invest Radiol, 2016. **51**(7): p. 447-53.
3. Kahn, J., et al., *Is There Long-term Signal Intensity Increase in the Central Nervous System on T1-weighted Images after MR Imaging with the Hepatospecific Contrast Agent Gadoxetic Acid? A Cross-sectional Study in 91 Patients.* Radiology, 2017. **282**(3): p. 708-716.
4. Ichikawa, S., et al., *Contrast Agent-Induced High Signal Intensity in Dentate Nucleus on Unenhanced T1-Weighted Images: Comparison of Gadodiamide and Gadoxetic Acid.* Invest Radiol, 2017. **52**(7): p. 389-395.
5. Conte, G., et al., *Signal intensity change on unenhanced T1-weighted images in dentate nucleus and globus pallidus after multiple administrations of gadoxetate disodium: an intraindividual comparative study.* Eur Radiol, 2017.