Novel biomarkers for predicting outcomes in patients with decompensated cirrhosis

Kohilan Gananandan

A Thesis submitted for Doctorate of Philosophy from the UCL Institute of Liver and Digestive Health, Royal Free Campus, University College London

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I, Kohilan Gananandan, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Decompensated cirrhosis is associated with a high morbidity and mortality. Whilst prognostic scoring systems have emerged over time, these often underperform, and are solely focussed on predicting mortality. Given the onset of decompensation drastically worsens prognosis, there is an urgent unmet need for new biomarkers to predict decompensation, with the aim of preventing this.

This thesis first emphasises this requirement by demonstrating the deleterious effects of the COVID-19 pandemic on the outcomes of patients with decompensated cirrhosis at a tertiary hepatology centre. Through a subsequent systematic review and meta-analysis, the current state of biomarker research in this field is evaluated, highlighting strengths but limitations thus far. The bulk of the thesis then goes on to evaluate a range of biomarkers based on the pathophysiological mechanisms underlying decompensation.

Metabolic dysfunction is first investigated, with low density lipoprotein demonstrating an ability to independently predict readmissions following acute decompensation, whilst fat mass is demonstrated to be a potential nutritional biomarker that can successfully be monitored remotely using bioimpedance analysis. A second digital biomarker, an app-base cognitive test called the CyberLiver-Animal Recognition Test, is then studied, demonstrating an ability to predict re-hospitalisation due to HE, as well as provide a signal for all-cause decompensation. Finally, the last chapter

evaluates the role of a novel scoring system composed of dimethylarginines which are implicated in portal hypertension and systemic inflammation. The new score exhibits an ability to independently predict acute-on-chronic liver failure development as well as readmissions.

In conclusion the studies in this thesis highlight the unmet need for improved prognostic biomarkers in decompensated cirrhosis. It then systematically investigates a number of novel biomarkers, all of which demonstrate a range of beneficial features as well as significant results and therefore should be considered for inclusion in future scoring systems.

Impact statement

Liver disease is recognised globally as a leading cause of morbidity and mortality.[1] In the UK it is the fifth highest cause of death, and it is estimated to cost the National Health Service (NHS) >£4.53 billion per year.[2,3] Both the mortality and costs are predominantly due to the development of liver-related complications such as ascites, hepatic encephalopathy and variceal bleeding which often requires urgent hospitalisation.[4] Indeed, even if discharged, these individuals remain at high risk of further complications requiring additional interventions and rehospitalisation.[5]

The COVID-19 pandemic had a huge impact on society and necessitated a redistribution of resources towards the virus, inevitably reducing resources available to patients with chronic liver disease. Whilst it was speculated that the pandemic would have a negative impact on the outcome of patients with decompensated cirrhosis, the study in this thesis was one of the first to highlight this and has now been published.[6,7] Given that the effects of the pandemic will be long lasting, hopefully this, as well as other studies can help justify re-distribution of resources and funding back to liver disease.

The systematic review and meta-analysis in this thesis is impactful in highlighting the current state of the biomarker field in decompensated cirrhosis and has also been published. Whilst there are many positives, this review highlighted important limitations in studies to date in terms of their heterogeneity and bias. Indeed, this has formed a basis for discussions with hepatology colleagues across Europe regarding the need for a potential

‘biomarker checklist’ to ensure future studies in this field are carried out with robust and reproducible study designs.

The focus of novel biomarkers in this thesis is based on the concept that cirrhosis care currently is reactive, responding to complications as they occur, as opposed to trying to be proactive in predicting and preventing them. With regards to blood-based biomarkers studied in this thesis, both low density lipoprotein as well as the novel dimethylarginine scored (DAS), demonstrated a positive predictive capacity for subsequent liver-related events. Both are linked to the underlying pathophysiology of decompensation, and with these significant findings further plans have been made for the next stage of validation. With regards to the digital biomarkers investigated in this thesis, both fat mass measured through bioimpedance analysis, and the CyberLiver-Animal Recognition Test, also demonstrated positive findings and the results have been presented at conferences and/or published.[8,9] This has significant potential, as digital healthcare could provide the key to an overburdened healthcare system with significant disparity in care currently existing.[10] Indeed, digitising care and incorporating technology is in keeping with governmental policy internationally.

In conclusion, this thesis has been impactful in highlighting the need to refocus attention on patients with decompensated cirrhosis as well as important steps to progress research in this field. The novel blood-based and digital biomarkers studied here should be considered for incorporation into future prognostic scoring systems.

Acknowledgments

I would first like to take this opportunity to thank my supervisor, Professor Rajeshwar Prosad Mookerjee for believing in me and taking me under his wing. He has taught me a great deal during my PhD, giving me the guidance I needed to stay on course whilst giving me the freedom required to develop my own scientific curiosity. His mentorship, enthusiasm and friendship has been greatly appreciated and I will be forever grateful. A special thanks must also go to the head of the Liver Failure Group, Professor Rajiv Jalan. His pastoral support throughout, as well as his passion and expertise for research has been inspirational and fuelled my own enthusiasm.

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Publications, posters and awards

PUBLICATIONS

1. Pose E…**Gananandan K**…Gines P. Simvastatin and rifaximin to prevent acute-on-chronic liver failure in patients with decompensated cirrhosis: A randomised, double-blind placebo-controlled trial – The LiverHope efficacy trial. *JAMA. (2025*) Mar 11;333(10):864-874.

2. **Gananandan K**, Kazankov K, Tapper EB, Mookerjee RP. The new digital era in decompensated cirrhosis. *Lancet Digital Health (2025)* Jan;7(1):e54-e63.

3. **Gananandan K,** Singh R, Mehta G. Systematic review and meta-analysis of biomarkers predicting decompensation in patients with compensated cirrhosis. *BMJ Open Gastroenterol (2024)* Aug 25;11(1).

4. **Gananandan K**, Wiese S, Møller S, Mookerjee RP. Cardiac dysfunction in patients with cirrhosis and acute decompensation. *Liver International* (2024) 2024 Aug;44(8):1832-1841.

5. Juanola A, Ma AT, de Wit K, **Gananandan K** et al. Novel prognostic biomarkers in decompensated cirrhosis: a systematic review and meta-analysis. *Gut (*2023) Dec 7;73(1):156-165.

6. **Gananandan K,** Thomas V, Woo WL et al. Fat mass: A novel digital biomarker for remote monitoring that may indicate risk for malnutrition and new complications in decompensated cirrhosis. *BMC Medical Informatics and Decision Making* (2023) Sep 13;23(1).

7. **Gananandan K,** Phillips A, Chikhlia et al. The negative impact of the pandemic on hospital admissions, morbidity and early mortality for acute cirrhosis decompensation. *BMJ Open Gastroenterology* (2023) Jan;10(1).

8. **Gananandan K** & Mookerjee RP. Letter to the Editor: Subjective and objective burden on providers from a multicenter app-based study of patients with cirrhosis and caregivers. *Hepatology Communications.* (2023) May 4;7(5).

9. **Gananandan, K,** Mookerjee RP, & Jalan R. Use of Non-selective Beta blockers in Decompensated Cirrhosis and ACLF. *Current Hepatology Reports*. (2022) June 21:29-36.

PUBLICATIONS IN SUBMISSION

1. Kumar A, **Gananandan K** et al. Limitations in real world telemonitoring applicability: a systematic review. *Submitted to Frontline Gastroenterology March 2025 after revisions.*

2. Greenham O*, **Gananandan K*** et al. A prospective multicentre randomised controlled trial to assess the clinical effectiveness of the novel CirrhoCare digital therapeutic management system: Study Protocol. Submitted and accepted to BMJ Open 2025.

PRESENTATIONS

Gananandan K et al. Low-density lipoprotein can predict hospital readmissions and outcomes following acute decompensation of cirrhosis. Poster presentation at the International Liver Conference (ILC) June 2024.

Gananandan K et al. CL-ART: A novel smartphone application that can help predict future hospitalisation secondary to cirrhosis acute decompensation**.** **Oral presentation** at American Association for the Study of Liver Disease (AASLD) conference November 2023 and British Association for the Study of Liver Disease (BASL) conference September 2023.

Rabiah S, **Gananandan K** and Mehta G. A systematic review and meta-analysis of biomarkers predicting decompensation in patients with compensated cirrhosis. Poster presentation at BASL September 2023.

Pose E…**Gananandan K**…Gines et al. Simvastatin plus Rifaximin to prevent ACLF in patients with decompensated cirrhosis. A randomised, double-blind, placebo-controlled, phase-3 trial. **Oral presentation** at the ILC June 2023.

Elshabrawi A, **Gananandan K** et al. Real-world evidence of reversibility in patients with acute-on-chronic-liver failure that survive hospitalization. Poster presentation at ILC June 2023.

Juanola A, Ma A, de Wit K, **Gananandan K** et al. Novel biomarkers for prognosis assessment of patients with decompensated cirrhosis: A systematic review and meta-analysis. Poster presentation at AASLD Conference November 2022.

Thomas V, **Gananandan K** and Mookerjee R. Fat mass: A novel digital biomarker for remote monitoring that may indicate risk for new complications in decompensated cirrhosis. Poster presentation at BASL Conference September 2022.

Phillips A, **Gananandan K** et al. MELDNa as a predictor of 6-week mortality for patients presenting with portal-hypertensive bleeding precipitating acute cirrhosis decompensation. Poster presentation at BASL conference September 2022.

Gananandan K et al. CyberLiver Animal Recognition Test (CL-ART): a novel remote monitoring tool to assess hepatic encephalopathy. Poster presentation at ILC July 2022.

Gananandan K et al. The negative impact of the pandemic on hospital admissions, morbidity and 30-day mortality for acute cirrhosis

decompensation: a tertiary-care perspective. Poster presentation at the ILC July 2022.

Alviri NK, Jessa F, Shah T, **Gananandan K**, Yu U, Patch D. Audit assessing initiation, monitoring, outcomes and impact of specialist pharmacist interventions on the use of low dose tissue plasminogen activator (Alteplase) thrombolysis for splanchnic vein thrombosis. Virtual presentation at the BASL conference September 2021.

AWARDS

1. **Wellcome Trust/ UCL Devices and Diagnostics Pilot Data Scheme 2023** – Awarded £10,000 for dimethylarginine study in final chapter.

2. **1st place prize at Royal Free London Annual Research Symposium 2023** – Technology category.

3. **EASL Young Investigator Full Bursary Awards 2024 and 2025** - Conference attendance, travel allowance and free membership for 1 year.

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   British Medical Journal

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KG was involved in the manuscript design, literature search, data interpretation, writing and figure development. KK was involved in the literature search, data interpretation and writing. EBT was involved in the literature search, data interpretation, writing, figure development and editing of the manuscript. RPM was involved in the article design, data interpretation, writing and editing of the manuscript.

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b) **Please include a link to or doi for the work**

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d) **Who published the work?** (e.g. OUP)

British Medical Journal

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Abbreviations

AASLD	American association for the study of liver disease
ACLF:	acute-on-chronic liver failure
AD:	acute decompensation
ADMA:	asymmetric dimethylarginine
A-FABP4:	Adipocyte-fatty acid binding protein
AIH:	autoimmune hepatitis
AKI:	acute kidney injury
ARFI:	acoustic radiation force impulse
ANT:	animal naming test
Apps:	applications
ArLD:	alcohol related liver disease
ALT:	alanine transaminase
AST:	aspartate aminotransferase
AUROC:	area under the receiver operating characteristic
AVP:	arginine vasopressin
BASL:	British association for the study of liver disease
BEST:	Biomarkers, EndpointS, and other Tools
BIA:	bioimpedance analysis
BMI:	body mass index
CFF:	critical flicker frequency
CFS:	clinical frailty score
CHE:	covert hepatic encephalopathy
CI:	confidence interval

CL-ART: CyberLiver-animal recognition test

CLIF-C AD: chronic liver failure-consortium acute decompensation

CO: cardiac output

CPS: Child-Pugh Score

CPG: clinical practice guidelines

CRP: c-reactive protein

CSPH: clinically significant portal hypertension

CT: computerised tomography

DAA: direct-acting antiviral

DAMPS: damage-associated molecular patterns

DEXA: dual energy x-ray absorptiometry

DILI: drug-induced liver injury

DL: deep learning

EASL: European association for the study of liver disease

EEG: electroencephalography

EF CLIF: European foundation for the study of chronic liver failure

EMR: electronic medical record

eNOS: endothelial nitric oxide synthetase

ePROMS: electronic patient reported outcome measures

FDA: food and drug administration

FFM: fat-free tissue mass

FM: fat mass

G-CSF: granulocyte colony stimulating factor

GI: gastrointestinal

HCC: hepatocellular carcinoma

HDL: high-density lipoprotein

HE: hepatic encephalopathy

HVPG: hepatic venous pressure gradient

HR: hazards ratio

HRS-AKI: hepatorenal syndrome – acute kidney injury

HRV: heart rate variability

HSCs: hepatic stellate cells

ICAM-1: intracellular adhesion molecule-1

IFNγ: interferon-gamma

IGF: insulin-like growth factor

IL: interleukin

INR: international normalised ratio

IQR: interquartile range

ITU: intensive treatment unit

IVR: interactive voice response

LCMS: liquid chromatography mass spectrometry

LFI: liver frailty index

KIM-1: kidney injury molecule-1

L-FABP1: liver fatty-acid binding protein 1

LBP: lipopolysaccharide binding protein

LDL: low-density lipoprotein

LPS: lipopolysaccharides

LSM: liver stiffness measurement

MASLD: metabolic dysfunction-associated steatotic liver disease

MCP-1: monocyte chemoattractant protein-1

MELD: model for end-stage liver disease

MELD Na: model for end-stage liver disease sodium

MHE: minimal hepatic encephalopathy

ML: machine learning

MR: mannose receptor

MRI: magnetic resonance imaging

5-MTHF: 5-methyltetrahydrofolate

NAD: non-acute decompensation

NF- *κB* : nuclear factor kappa B

NGAL: neutrophil gelatinase-associated lipocalin

NHS: national health service

NIAAA: national institute on alcohol abuse and alcoholism

NIH: National Institutes of Health

NO: nitric oxide

OHE: overt hepatic encephalopathy

OR: odds ratio

PAMPS: pathogen-association molecular patterns

PCR: polymerase chain reaction

PEM: protein energy malnutrition

PIIINP: procollagen type III aminoterminal peptide

PHES: psychometric hepatic encephalopathy score

PRISMA: patient reporting items for systematic reviews and meta-analyses

PROs: patient reported outcomes

PROMS: patient reported outcome measures

PREsTo: primary sclerosing cholangitis risk estimation tool

PSC: primary sclerosing cholangitis

PT: prothrombin

QUIPS: quality in prognosis studies

RCT: randomised control trial

SBP: spontaneous bacterial peritonitis

sCD163: soluble CD163

SDC: stable decompensated cirrhosis

SDMA: symmetric dimethylarginine

SMI: skeletal mass index

SVR: systemic vascular resistance

suPAR: soluble urokinase plasminogen activator receptor

TE: transient elastography

TIMP-1: tissue inhibitor matrix metalloproteinase 1

TIPS: transjugular intrahepatic portosystemic shunt

TNFα: tumour necrosis factor alpha

UDC: unstable decompensated cirrhosis

UKELD: United Kingdom model for end-stage liver disease

US: United States

VCAM-1: vascular cell adhesion protein-1

VEGF: vascular endothelial growth factor

VITRO: Von Willebrand Factor Antigen/Thrombocyte Ratio

WBC: white blood cells

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Chapter 1 - Introduction

1.1 Background of cirrhosis

Liver cirrhosis is the final common pathological pathway caused by a variety of chronic liver diseases inducing chronic liver inflammation. This results in the degeneration and necrosis of hepatocytes, the replacement of normal liver parenchyma by fibrotic tissue and regenerative nodules, and the loss of liver function leading to liver failure.[11,12] Chronic liver inflammation does not progress to cirrhosis in all patients, however genetic and environmental factors, particularly from an ongoing hepatic insult from the underlying aetiological factor, are likely to drive progression.[12,13] This progression, if it occurs, does so over several decades as displayed in Figure 1-1.[14]

The initial, asymptomatic phase of cirrhosis is termed compensated cirrhosis and carries a good prognosis with mortality tending to be due to non-liver-related causes such as cardiovascular disease, renal disease and malignancy.[13] Once a patient develops liver-related complications, this signals the onset of decompensated cirrhosis with median survival decreasing from greater than 10 years down to 2 years (further details in subsequent section).[15] The Baveno guidelines have suggested that compensated cirrhosis can now be divided into two categories depending on the presence or absence of clinically significant portal hypertension (CSPH), which is defined as a hepatic venous pressure gradient (HVPG) ≥ 10 mm Hg.[4] Patients with CSPH are at much greater risk of decompensation and must be targeted to prevent this transition from occurring.

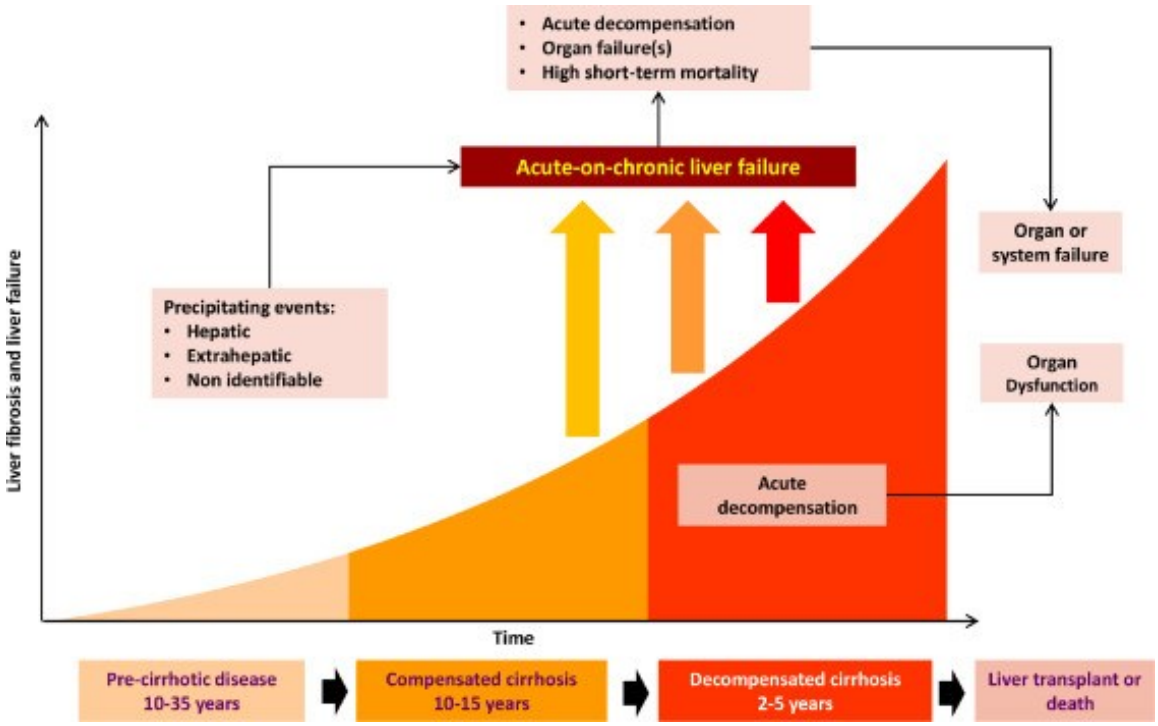

Figure 1-1: Progression of liver disease over time

Cirrhosis is a leading cause of liver-related death and accounts for 2-3% of deaths globally and in Europe is the second leading cause of years of working life lost.[1,16] In the United Kingdom it is the 3rd most common cause of premature death and has been increasing at a more rapid rate than the 4 most commonly diagnosed cancers; lung, breast, bowel and prostate.[17,18] The British Liver Trust indicates deaths from liver disease have increased by 400% since 1970, with 62,000 years of working life lost per year, and NHS care costs of over £4.53 billion per year.[3] There are a variety of causes but the most common worldwide are alcohol-related liver disease (ArLD) , metabolic dysfunction-associated steatotic liver disease (MASLD) and chronic viral hepatitis B and C with the burden of both ArLD and MASLD predicted to continue increasing.[13]

1.2 Defining decompensated cirrhosis

The most recent Baveno guidelines have defined first decompensation by the development of overt ascites (or pleural effusion with increased serum ascites albumin gradient [>1.1g/dl]), overt hepatic encephalopathy (West Haven grade ≥II) or variceal bleeding. Further decompensation has been separately defined as the development of a second portal hypertensive-driven event as this is associated with an increased mortality compared to first decompensation. This includes the development of recurrent variceal bleeding, recurrent ascites (requiring x≥3 large-volume paracentesis within 1 year), recurrent HE, spontaneous bacterial peritonitis (SBP), hepatorenal syndrome-acute kidney injury (HRS-AKI) and/or jaundice.[4]

The last European Association for the Study of Liver Disease (EASL) clinical practice guidelines (CPG) included the development of jaundice as a decompensating event.[19] However, jaundice has not been included in the more recent Baveno guidance for decompensation which is contentious. The authors suggest that whilst jaundice alone may be a presentation of cirrhosis in a small group of patients with non-cholestatic aetiologies, it is currently not clear whether this reflects true decompensation or superimposed liver injury/ acute-on-chronic liver failure (ACLF) in individuals with compensated cirrhosis.[4] Furthermore, large prospective European studies such as the PREDICT and CANONIC studies have included infection as a decompensating event as part of their inclusion criteria.[20,21] However, with the exception of SBP which is disease specific, some view infection as an important precipitant which can result in decompensation as opposed to being a direct consequence of this disease process. Although it is clear that

once bacterial infections occur, they can alter the course of compensated cirrhosis by increasing the risk of decompensation and death.[22]

Patients with acute decompensation (AD) of cirrhosis are at high risk of hospitalisation, and even when discharged, despite optimal management, have short-term re-admission rates between 30-50%, with 3-month mortality rates in the sickest cohort reported over 50%.[20,23,24] Moreover, there is a significant impact on quality of life with a substantial burden on patients and carers, with data suggesting that early readmissions are associated with a reduced chance of independent living at one year.[25,26]

Whilst decompensated cirrhosis has historically been classified as one group, in the last decade it has increasingly been recognised that this is a very heterogeneous group. The CANONIC study identified a subgroup of patients with AD at the most severe end of the spectrum, with severe systemic inflammation, organ failures and high short-term mortality (>15% at 28 days), termed acute-on-chronic liver failure (ACLF). [21] This distinct syndrome affects almost one-third of patients with AD, and approximately 40% of patients with cirrhosis within 10 years.[21,27]

Recently D'Amico et al have suggested 2 distinct pathways to decompensation: acute onset and non-acute onset decompensation (NAD). [28] Acute onset is characterised by first or recurrent grade 2 ascites within less than 2 weeks, first or recurrent acute HE in patients with previous normal consciousness, or acute gastrointestinal bleeding. This group are more likely to be hospitalised and the majority (almost 80%) are likely to have had prior

decompensation. In contrast, non-acute decompensation is characterised by slow ascites formation, mild grade 1 or grade 2 HE, or progressive jaundice in non-cholestatic cirrhosis. It most often presents with a single decompensating event (58 -72%) and does not tend to require hospitalisation. A summary of the proposed criteria can be seen in Figure 1-2. A recent multicentre study prospectively followed outpatients with cirrhosis with no previous decompensation. Of those who decompensated during follow-up, 44.8% developed NAD and 55.2% developed AD, and 42% of those with NAD developed a further episode of AD. In a multivariable analysis, both AD and NAD were significant predictors of mortality (HR 21.07 and 7.13 respectively).[29] However, controversy remains over whether this distinction is required, with both ascites and HE presenting acutely and chronically. Furthermore, evidence is currently lacking to show who develops which form of decompensation and what the underlying pathophysiological mechanisms are. Finally, one of the predominant criticisms remains over the need for hospitalisation which discriminates AD and NAD. There is significant variability in hospitalisation depending on patient factors, physician factors and geographical location with significant centre variability.[30]

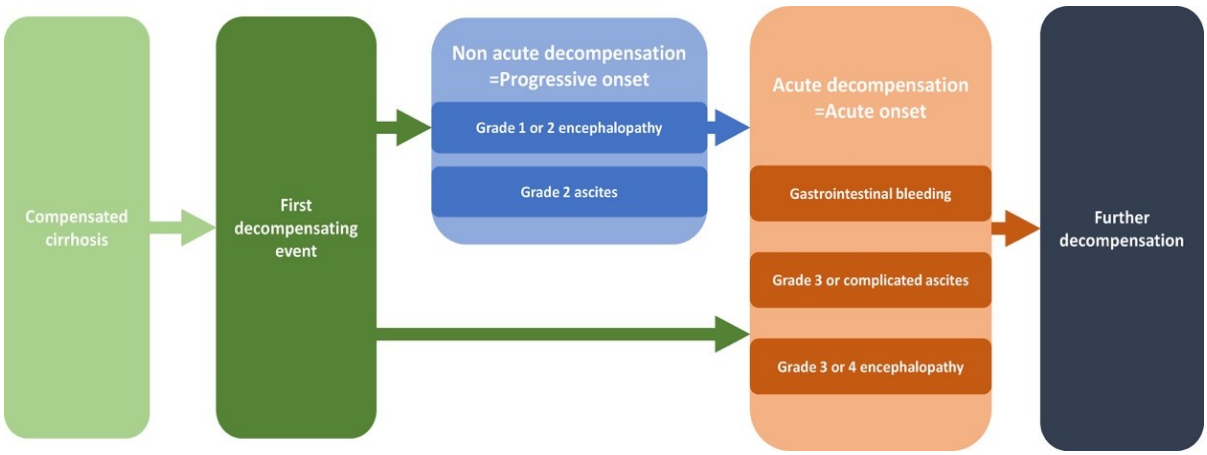

Figure 1-2: Novel proposition of decompensated model

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Further sub-groups of decompensation have been proposed based on the number and type of decompensation events. It has been shown that ascites is associated with increased mortality compared to bleeding alone, but the worst outcomes are seen in combination.[31,32] This was supported by a study by D'Amico et al that demonstrated a 5-year mortality risk for decompensation with bleeding alone at 20%, any non-bleeding decompensation which was mostly ascites being 30%, and a combination of ≥2 events leading to 88% mortality.[33] These findings remained true even when adjusted for comorbidities, Child-Pugh Scores (CPS) and Model For End-Stage Liver Disease (MELD) scores. However, it is worth noting that the aetiology of liver disease in the majority of these patients was hepatitis C and patients were untreated, and therefore their outcomes may have been different in the current day with the emergence of direct antiviral agents (DAAs).

Following acute decompensation, the PREDICT study suggested that individuals could be divided into 3 further categories depending on their

pathophysiology and outcomes: stable decompensated cirrhosis (SDC), unstable decompensated cirrhosis (UDC) and pre-ACLF.[20] The SDC cohort did not die, have re-admissions or develop ACLF within the 3-month follow-up and had the best outcomes, with 1-year mortality rates of 10%. The UDC cohort was either re-admitted or died within 3 months and had 3-month and 1-year mortality rates of 21% and 36% respectively. The worst outcomes were observed in the pre-ACLF group who developed ACLF within 3 months and had 3-month and 1-year mortality rates of 54% and 67%. A different pathophysiological process was proposed for each category, and this will be explored further in the subsequent section. Similar findings to PREDICT have been replicated in other studies.[34]

Whatever definition of decompensation is used, there is consensus that the event should be a direct consequence of the disease, associated with a worse prognosis and must be easy to diagnose. The onset of decompensation, in whatever form, heralds an important transition in a patient's disease trajectory and it requires prompt and effective intervention.

1.3 Pathophysiology of decompensation

Portal hypertension and systemic haemodynamics

Portal hypertension is the most common haemodynamic abnormality caused by liver cirrhosis and is the main cause of decompensation in terms of ascites, variceal bleeding and encephalopathy.[4] Clinically this is most accurately measured using HVPG, of which a gradient >5mmHg indicates sinusoidal portal hypertension and ≥10 mmHg signifies CSPH. Thresholds of

>12mmHg have been associated with an increased risk of bleeding in those with gastro-oesophageal varices and >20mmHg has been associated with treatment failure and mortality.[35–37]

Portal hypertension is based on Ohms law as demonstrated in Figure 1-3. Increased portal pressures are due to increased portal blood flow, increased vascular resistance, or a combination of both. 90% of cases of portal hypertension are due to liver cirrhosis.[38] It was traditionally thought that increased hepatic resistance was purely due to architectural disruption caused by hepatic stellate cells (HSCs) producing extracellular matrix and collagen resulting in fibrosis, as well as vascular occlusion with microthrombi.[39] However, it is now known that up to 30% of intrahepatic resistance is dynamic and due to endothelial dysfunction.[40] This imbalance is due to decreased endogenous vasodilators, mainly nitric oxide (NO), and increased endogenous vasoconstrictors, such as noradrenaline, angiotensin-2, endothelin and thromboxane A2.[40]

This increase in intrahepatic resistance leads to circulatory disturbances with the most important being splanchnic vasodilatation leading to increased portal inflow.[41] This vasodilatation is due to increased NO levels from endothelial NO synthetase (eNOS), which is in contrast to the deficiency noted in the intrahepatic microenvironment.[42] This increased NO is initially due to shear stress but subsequently bacterial translocation associated with systemic inflammation which will be explored further in a subsequent section. Other vasodilators such as carbon monoxide, glucagon and endocannabinoids have also been implicated in this process. [40] Splanchnic

vasodilation is also exacerbated by vascular endothelial growth factor (VEGF) driven angiogenesis and portosystemic collateral formation which initiates increased portal blood flow. It has been suggested that over 90% of portal blood flow in decompensated cirrhosis may be collateralised. [38] The most relevant portosystemic collaterals are varices with increased size being associated with an increased risk of haemorrhage. Portosystemic shunting together with liver dysfunction also results in HE through impaired clearance of gut-derived ammonia.[13]

The splanchnic circulation compromises 25% of the total systemic vascular resistance (SVR), therefore splanchnic vasodilatation leads to a decrease in effective circulating arterial volume. This leads to the activation of the compensatory vasoconstrictor sympathetic nervous system, as well as neurohumoral activation of the renin-angiotensin-aldosterone system and arginine-vasopressin axis, leading to sodium and water retention.[43] This results in increased plasma volume, part of which leaks into the peritoneal space as portal hypertension increases, leading to the formation of ascites.[13] With progressive cirrhosis, despite maximal vasoconstrictor action, systemic hypotension results, leading to decreased organ perfusion. Decreased renal perfusion leads to significant renal arterial vasoconstriction with subsequent development of HRS-AKI.[44]

Profound circulatory dysfunction has been acknowledged as a hallmark of decompensated cirrhosis since the 1950s.[45] The pronounced arterial vasodilatation, low systemic vascular resistance, and low effective central blood volume is followed by compensatory activation of potent neurohumoral

systems leading to increased cardiac output (CO) and a hyperdynamic circulation. Whilst increased CO can strain the heart leading to heart failure, this is masked in cirrhosis due to reduced afterload through reduced SVR and increased arterial compliance. This deficit may only become apparent when the heart is put under stress with these abnormalities now termed 'cirrhotic cardiomyopathy'. This has most recently been defined by the Cirrhotic Cardiomyopathy Consortium in 2019 which has taken into account new imaging modalities that can identify subclinical systolic and diastolic dysfunction.[46] It has been estimated to affect up to 60% of the cirrhotic population and significantly contributes to morbidity and mortality in decompensated cirrhosis.[47,48]

The close relationship between portal hypertension and decompensation has been clearly demonstrated with an 11% increased risk of decompensation for every 1mmHg increase in the portal pressure gradient. [49] Furthermore, a recent randomised controlled trial (RCT) demonstrated that beta blockers, which are the only validated therapeutic in portal hypertension management, led to a significant reduction in decompensation and death.[50]

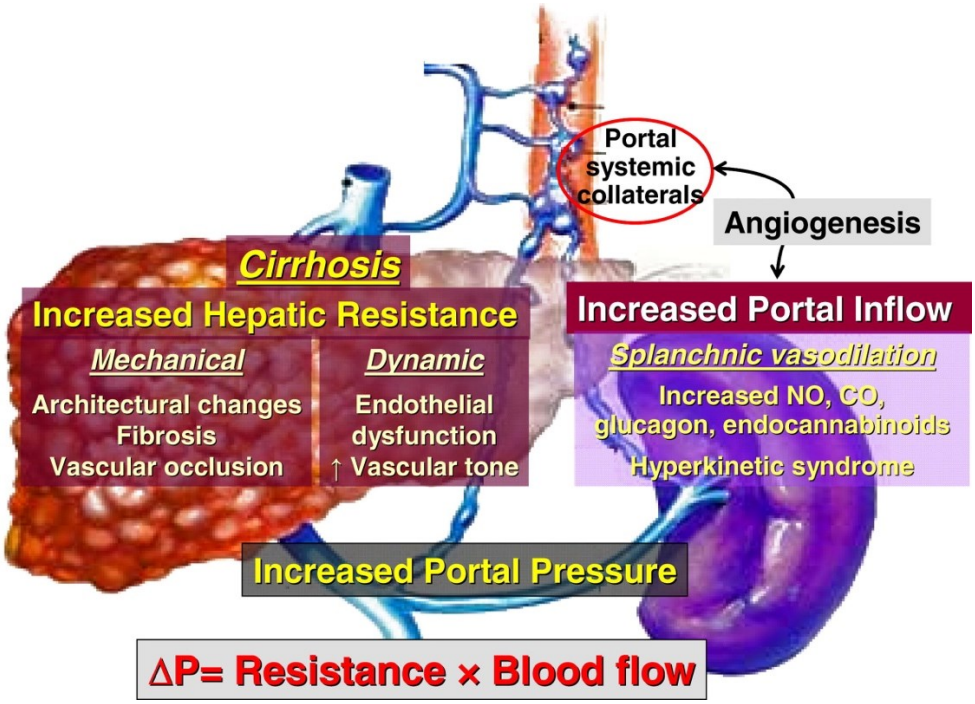

Figure 1-3: Mechanisms underlying portal hypertension

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Systemic inflammation

Systemic inflammation has been proposed as a key mechanism for the progression from compensated to decompensated cirrhosis for which there are two main triggers. The first mechanism involves bacterial translocation, which is the passage of bacteria or bacterial by-products, termed pathogen-associated molecular patterns (PAMPs) from the gut mucosa to the systemic circulation.[51] Bacterial overgrowth, slow gut transit and increased gut permeability through portal hypertension are thought to play a key role in translocation.[52] In addition, alterations in gut microbiota with reduced diversity and loss of function have been demonstrated to correlate with cirrhosis progression.[53] It has been suggested that there an increase in pathogenic species may lead to increased endotoxaemia, exacerbating both systemic inflammation and bacterial translocation.[54] The second mechanism for systemic inflammation involves injury to the hepatocytes themselves resulting

in cell death and the release of damage-associated molecular proteins (DAMPs).[55] The persistent influx of PAMPS and DAMPs from the leaky gut and underlying liver disease drive the sustained inflammatory response.

The PREDICT study which recruited over 1200 patients, demonstrated that only 0.24% of patients with AD demonstrated no systemic inflammation.[20] Only 40 patients (3.3%) of patients did not have an elevated plasma Interleukin-6 (IL), of which 37 showed elevated levels of 2 or more other biomarkers of systemic inflammation (Tumour necrosis factor α [TNF α], IL-8, IL-10, IL-1RA and C-reactive protein [CRP]). Pre-ACLF, UDC and SDC were shown to have distinct inflammatory profiles. The pre-ACLF group has significantly higher grades of inflammation at baseline which increased during follow-up in association with the development of ACLF. In contrast, the degree of inflammation decreased rapidly in those with SDC during follow-up, whereas no significant change was noted in the UDC cohort. In fact, the UDC cohort demonstrated complications related more to portal hypertension (i.e. circulatory dysfunction, increased gastrointestinal haemorrhage and transjugular intrahepatic portosystemic shunt [TIPS] placement).

In Europe, bacterial infections and alcohol related hepatitis are the two most common precipitants for decompensation. In patients with identifiable precipitants, either alone or in combination with other precipitants, they account for over 90% of decompensations.[56] However, it is worth noting that a precipitant is not identified in around 50 % of patients with AD.[20,21] It has been suggested that there is likely a burst of bacterial translocation with a surge of PAMPs and DAMPs triggering an increase in systemic inflammation

in AD. Whilst the type of precipitant does not seem to impact the inflammatory profile, the severity of inflammation (leucocytes, neutrophils and CRP) has been shown to increase with the number of precipitants suggesting an additive effect.[56]

Metabolic dysfunction

A leading metabolic derangement in decompensated cirrhosis is significant proteolysis and lipolysis leading to a large release of amino acids and fatty acids.[57,58] Proteolysis leads to loss of muscle mass which is termed sarcopaenia. The combination of sarcopaenia and fat wasting is termed protein-energy malnutrition (PEM).[59] Sarcopaenia is a surrogate marker for severe malnutrition and a dominant component of frailty, affecting between 30-70% of patients with decompensated cirrhosis.[60,61] As well as muscle depletion, loss of fat mass is likely to be important, with evidence showing it may be protective against sarcopaenia as an alternative essential energy source.[62] Indeed, there is evidence to suggest that in earlier stages of cirrhosis, it is predominantly fat wasting that occurs, which may drive the muscle depletion in more advanced stages of the disease.[63] Whilst not currently considered a decompensating event in itself, PEM has been associated with increased mortality and as well as increased incidence of ascites, gastrointestinal bleeding, and HE in patients with liver cirrhosis.[64–66]. The liver plays a crucial role in fat and lipid homeostasis with pathological alterations in lipid and lipoprotein synthesis, secretion and catabolism in cirrhosis.[67] Therefore, targeting metabolic dysfunction is crucial in aiming to improve the outcomes of this population.

It is known that decompensated cirrhosis is associated with systemic inflammation as previously stated and that this progresses with liver disease severity.[20,21]. It is also known that this hyperinflammatory state is an energetically expensive process.[68] This higher level of catabolism with increasing liver disease severity is associated with increased mobilisation and oxidation of fat substrates and higher levels of PEM.[69,70] Similar findings have been demonstrated in other processes with high levels of systemic inflammation such as sepsis. This results in skeletal muscle proteolysis by glucocorticoid release from the hypothalamic-pituitary-adrenal axis which is activated by inflammatory cytokines. Lipolysis also results from sympathetic nervous system activation as part of a stress response. This catabolic state can utilise 10,000 calories per day in a septic patient.[71,72]

Whilst much focus has been placed on sarcopenia in cirrhosis, there is an increasing understanding of the importance of lipid metabolism in its pathogenesis. Whilst lipids have primarily been regarded as an energy source, increasing evidence shows their crucial role in cell survival, inter-organ communication and modulating immune response. Altered lipid composition is associated with immune dysfunction and uncontrolled inflammation.[72,73] Indeed, deficiencies in sphingolipids have been demonstrated to correspond with increased liver disease severity, risk of decompensation and mortality.[74,75] Furthermore, there is a clear pathomechanistic role of fatty acids, with an imbalance of pro and anti-inflammatory lipid mediators.[57,72] Systemic inflammation also leads to mitochondrial dysfunction, which under healthy conditions is responsible for a

significant proportion of a cell's energy, partly through impaired translocation of fatty acids which are a crucial metabolic substrate.[71]

In summary, the understanding of the pathophysiology of decompensated cirrhosis has greatly evolved over recent decades. Portal hypertension with alterations in systemic haemodynamics plays a crucial role in the progression from compensated to decompensated cirrhosis. In more advanced stages, particularly after exposure to precipitants such as bacterial infections and alcohol related hepatitis, compensatory mechanisms may fail due to overwhelming systemic inflammation which exacerbates metabolic dysfunction. Portal hypertension, systemic inflammation and metabolic dysfunction are not opposing theories, but instead complementary. They are intrinsically linked with a complex interplay and act synergistically in driving liver-related complications. Further research is required to investigate this relationship, as well as the pathophysiology of decompensation when no precipitant is found. Additionally, understanding the mechanisms underlying regeneration and recompensation is likely to be integral in developing new therapeutic strategies.

1.4 Current liver disease severity scores

The prognosis of patients with liver cirrhosis has traditionally been assessed by liver function tests and derived scores. The CP score was the first score developed to predict mortality in cirrhosis patients. It was developed in 1964 to guide the selection of patients who would benefit from elective surgery for portal decompression.[76] It consists of five components: encephalopathy,

ascites, bilirubin, albumin, and the international normalised ratio (INR) with a range of 5-15 points. Whilst historically used in liver transplantation allocation, it has been replaced in the modern day due to its limitations. These limitations include the lack of a parameter assessing renal function, the subjective assessment of ascites and HE, and a lack of ability to accurately distinguish liver disease severity.[77] An individual will score maximum points in each category if their bilirubin is >51 μmmol/L or INR >2.3, but it does not differentiate beyond this.

The MELD score was initially described in 2000 to predict survival in patients undergoing TIPS procedure but was slightly modified in 2001 to predict mortality in cirrhosis patients.[78] The MELD score has been widely used for liver allocation in the transplant setting. Its advantages over the CP score are its statistical validation and its use of objective and readily available blood-based parameters (bilirubin, creatinine and INR). Various limitations have been suggested with the model and it has been modified over time. The MELD-Na score was developed following the demonstration that the inclusion of sodium into the score improved its prognostic ability.[79] Most recently, the MELD 3.0 score has been proposed as a further improvement with the inclusion of female gender and albumin.[80] This was based on the concern that creatinine overestimated renal function in women and therefore underestimated mortality.[81] However, despite these modifications, concerns still remain as patients with low scores are still at high risk of liver-related death and it seems to underestimate mortality in the sickest cohort of patients with ACLF.[82,83]

Most recently, the Chronic Live Failure-Consortium Acute Decompensation score (CLIF-C AD) was developed and suggested to be superior to the CPS, MELD and MELD-Na in predicting mortality up to 1 year in patients hospitalised due to acute decompensation.[84] The score is composed of age, serum sodium, white-blood-cell count (WBC), creatinine and INR as the best predictors of mortality. However, concerns remain across all scores due to differences in laboratory methodologies for INR, creatinine and sodium measurement which can lead to significant differences in scores.[77] Furthermore, the use of creatinine as a marker of renal function remains controversial in this population. It is heavily influenced by muscle mass and therefore overestimates renal function in the decompensated cirrhosis population who are profoundly sarcopaenia.[85]

In summary, whilst scoring systems have been modified and improved over time, they have limitations and often underperform in contexts other than those in which they were initially developed. Furthermore, whilst these models have been generated to predict mortality, they do not predict the development of future decompensation events.[86] Therefore there is currently an unmet need to develop better tools to predict complications and prognosis. Improvement in these areas could lead to earlier interventions to avoid the development of complications and improve organ allocation.

1.5 Defining biomarkers and the need for novel biomarkers

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[87]

Before any new biomarker is introduced into clinical practice it must exhibit the qualities displayed in Figure 1-4. It must be biologically plausible, targeting the pathophysiological mechanisms detailed earlier. It should be minimally invasive to collect to ensure feasibility and acceptability amongst patients and clinicians. Along with this, it should be resistant to degradation allowing for delays between acquiring and processing the samples with acceptable storage conditions. A high sensitivity and specificity are crucial to correctly identify the population at risk. Furthermore, the biomarker should be generalisable, being informative across sociodemographic, geographic and ethnic differences. The biomarker needs to be analysed using an assay that is validated within and across laboratories. Ideally, the measurement will be inexpensive so it can be incorporated in all centres and does not discriminate based on financial capabilities. Finally, the biomarker should be easy to measure using technologies and equipment that are readily available all over the world.

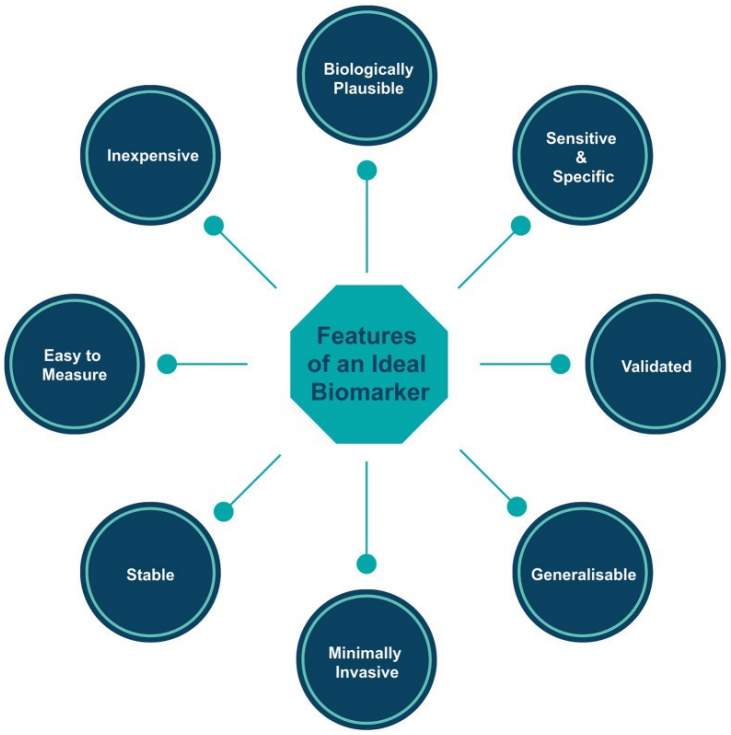

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Figure 1-4: Features of an ideal biomarker

There has historically been much heterogeneity in the use of the term biomarker, which is problematic given its frequent use as well as utility as endpoints in clinical trials. In an attempt to harmonise terminology, the Food and Drug Administration (FDA) and the National Institutes of Health (NIH) developed the BEST (Biomarkers, EndpointS, and other Tools) Resource.[89] They defined the following biomarkers: diagnostic, monitoring, response, predictive, prognostic and susceptibility/risk. The focus of this thesis is on prognostic biomarkers, which are used to identify the likelihood of a clinical event, disease recurrence or progression in patients who already have the disease, which in this case is decompensated cirrhosis. Prognostic biomarkers and predictive biomarkers can often be difficult to distinguish, with the latter generally requiring a comparison of a treatment to a control in patients with and without the biomarker. They are also distinct from

susceptibility/risk biomarkers in which individuals do not yet have the medical condition or disease of interest.

There have been many prognostic biomarkers that have emerged over recent years; however, few have been incorporated into clinical practice. This is likely due to a lack of clarity over which biomarkers are superior, which would work best in particular scenarios and whether they truly outperform scoring systems that already exist. It is unlikely that a single biomarker will be satisfactory, but more likely a composite score will be required to predict outcomes. There is an urgent need to develop novel biomarkers that could be incorporated into future models to accurately predict and help prevent liver-associated morbidity and mortality and provide potential therapeutic targets.

1.6 Current novel biomarkers in decompensated cirrhosis

The focus of this thesis will be on searching for novel biomarkers that can predict survival and further decompensation events in individuals with decompensated cirrhosis. Therefore, it is imperative to conduct a literature review at the outset to assess the current progress that has been made in this field. Novel biomarkers are defined as those not used in clinical practice. By this, we mean parameters/ tests that are not measured routinely and have not been incorporated into standard clinical practice.

Historically many of the studies in this area have studied a very diverse population, including patients from a spectrum of advanced fibrosis to

compensated cirrhosis to decompensated cirrhosis. As our knowledge has developed in cirrhosis there is an increasing understanding that there are distinct subpopulations with varying pathophysiology and different outcomes, as has already been stated. Therefore, this section will focus on biomarkers in clinical studies (not experimental studies) assessing patients with decompensated cirrhosis only. Furthermore, it will address publications that have follow-up to detect subsequent liver-related events and therefore exclude cross-sectional studies.

Mortality

Mortality is the most well-studied outcome in decompensated cirrhosis. This is logical given it is the most severe endpoint, is one of the most important factors to both clinicians and patients, and it is easy to record. Studies have varied looking at a range from inpatient mortality at the most short-term end to studies that have followed-up patients for years. Whilst short-term studies are easier to conduct and are less costly, there is a substantial benefit in conducting longer-term studies to gain insight into the progression of decompensated cirrhosis.

Inpatient mortality

As described in the previous pathophysiology section, systemic inflammation plays a crucial role in decompensated cirrhosis and outcomes. This is particularly true for acute decompensation of cirrhosis which is associated with hospitalisation and high levels of systemic inflammation. Therefore, it follows that markers of inflammation are likely to play a significant role in short-term mortality, such as prostaglandin E2 which is pro-inflammatory and

is a known regulator of the immune response and has demonstrated an ability to independently predict inpatient mortality.[90] Another crucial pathway to potentially target is the microbiome. There is an increasing understanding that decompensated cirrhosis is associated with dysbiosis with decreased microbial diversity. This is caused by decreased intestinal motility as well as increased gut permeability secondary to portal hypertension and systemic inflammation. This is a vicious cycle in which dysbiosis alters the integrity of the gut barrier leading to increased bacterial translocation and transfer of PAMPs into the systemic circulation, which further upregulates inflammation.[91,92] Bajaj et al demonstrated that an altered stool microbiome was associated with inpatient as well as 30-day mortality. They demonstrated that survival was associated with different patterns of serum metabolites related to microbial metabolism, in terms of bile acid, amino acid and lipid breakdown.[92]

28 day/ 1-month mortality

The majority of biomarker research in mortality analysis has targeted various components of systemic inflammation including the activation, differentiation, proliferation, migration, and adhesion of immune cells. A range of pro-inflammatory and anti-inflammatory mediators that compromise both the innate and adaptive immune systems have been studied. The biomarkers that have demonstrated an ability to predict 1-month mortality include; Interleukin-1 receptor antagonist protein (IL-1RA), IL-6, IL-8, IL-18, IL-22, Kidney Injury Molecule-1 (KIM-1), Lipopolysaccharide-Binding Protein (LBP), Monocyte Chemoattractant Protein-1 (MCP-1), soluble mannose receptor (MR), urinary and plasma neutrophil gelatinase-associated lipocalin (NGAL),

soluble CD163 (sCD163), TNFα, soluble urokinase plasminogen activator receptor (suPAR), chimerin and pro adrenomedullin.[93–104]

Other biomarkers that have demonstrated an ability to predict 1-month mortality include markers of cell death such as caspase-cleaved keratin 18 which results in the release of DAMPs which activate the inflammasome.[105] Markers of oxidative stress such as human non-mercaptalbumin-2 (HNA2) which reduce the binding capacity of circulating albumin have also been studied.[93,98] Given the understanding of the hyperdynamic circulation in cirrhosis, it is also logical that copeptin as a marker reflective of the endogenous vasoconstrictor system and systemic haemodynamics could also play a role.[106] On a similar theme, markers of endothelial dysfunction such as intercellular adhesion molecule 1 (ICAM1) and von Willebrand factor have been shown to have prognostic potential.[107]

Metabolic abnormalities are known to be crucial in the pathogenesis of decompensation, as detailed earlier, and have also demonstrated a predictive capacity in mortality. Adipocyte-fatty acid binding protein (A-FABP4) and Liver fatty-acid binding protein 1 (L-FABP1) play an integral role in regulating lipid metabolism and inflammatory response.[108] Similarly, Insulin-like growth factor-binding protein 3, which is predominantly produced in the liver and is the major binding protein and regulator of insulin-like growth factor (IGF), which has an important role in the metabolism of proteins, carbohydrates and lipids.[109]

90-day mortality

This is the most well-studied mortality outcome in the literature. This is potentially because the MELD score, which is the most renowned prognostic liver disease severity score, was initially used to predict 90-day survival post-TIPS insertion and then validated in predicting 90-day survival in patients in end-stage liver disease.[78] The vast majority of the biomarkers that have demonstrated efficacy at 1-month, also demonstrate prognostic ability for 3-month mortality, although to varying degrees. Other biomarkers which have only demonstrated benefit at 90 days include other markers that modulate the immune system, such as granulocyte colony-stimulating factor (G-CSF), Interferon-gamma (IFNγ), IL-1β, ascitic IL-6, macrophage migration inhibitory factor, human neutrophil peptides and siglec-7.[110–114]

Metabolic factors that have been implicated include urinary L-FABP, IGF-1 and cystatin C which is a protease inhibitor involved in the catabolism of proteins.[115–117] Indeed, metabolomic analysis has revealed different patterns of lipids, amino acids, phosphocholines and lactate between survivors and non-survivors.[118] Markers of endothelial dysfunction and angiogenesis that have been studied include vascular cellular adhesion molecule-1 (VCAM-1), VEGF and the VITRO score (Von Willebrand Factor Antigen/Thrombocyte Ratio).[111,119] Microbiome involvement in the pathogenesis of deterioration is supported by increased mortality being demonstrated in individuals colonised with drug-resistant bacteria.[120] Finally, physiological markers such as reduced heart rate variability (HRV) as a surrogate for inflammation have demonstrated significant differences.[121]

≥ 1-year mortality

The markers that demonstrate 1-year mortality that have been shown in the previous section to correlate with short-term mortality are; copeptin, cystatin C, IL-1RA, IL-6, LBP, urinary NGAL, suPAR and wWF.[122–128] Other biomarkers that have shown a signal at 1 year are cortisol, which is stimulated as a stress response following activation of the hypothalamic-pituitary-adrenal axis, and microfibrillar-associated protein 4, which is an extracellular matrix protein linked to hepatic neoangiogenesis and fibrogenesis.[129,130]

With regards to longer-term survival, gut dysbiosis has been shown to predict 4-year mortality, collagen type IV can predict 5-year mortality and Magnetic Resonance Elastography (MRE) has been shown to correlate with 10-year outcomes.[131–133]

Predicting future decompensation

Fewer studies have assessed the role of prognostic biomarkers in predicting cirrhosis decompensation events. HE is a heterogenous condition with various triggers making it difficult to predict. Furthermore, the diagnosis is based on a subjective clinical assessment using the West Haven criteria which makes its use as an endpoint in trials challenging. Early ascites formation can be subtle and insidious, whereas variceal bleeding can present acutely with little warning, each generating their own difficulties in developing prognostic biomarkers. Development of future HE, ascites and variceal bleeding have all been demonstrated with baseline differences in copeptin, soluble MR, MCP-1, sCD136 and vWF.[100,134–136] Given the importance of the microbiome, the MICROB-PREDICT study is an ongoing European multi-

centre project looking to discover microbiome-based biomarkers to develop personalised care.

In terms of HE specifically, IL-6 has been shown to able to predict future episodes of HE.[137] Furthermore, alterations in the gut microbiome with an increase in certain species are associated with future HE, likely through modifications in brain function through the gut-liver-brain axis.[138] With regards to variceal bleeding, IGFB-3 as well as a combined index score of MELD and US doppler (assessing left gastric vein blood flow direction and velocity) have shown efficacy.[109,139] Figure 1-5 is from a recent review and represents the main biomarkers that predict outcomes in decompensated cirrhosis.[140]

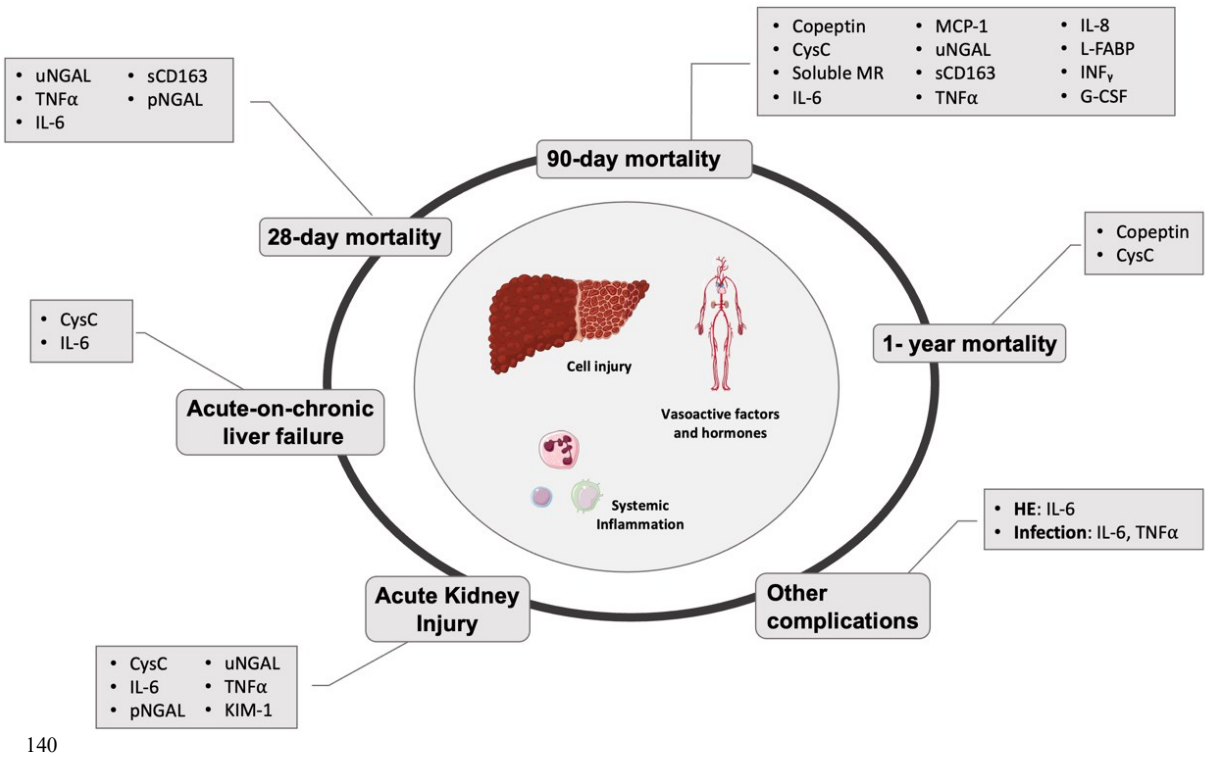

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Figure 1-5: Representation of main biomarkers that have consistently predicted outcomes, supported by 3 or more studies.

Discussion

As can be seen, there is a range of prognostic biomarkers that have emerged over recent years. Based on the findings of *Juanola et al*, IL-6, TNFα, sCD163, urinary NGAL and copeptin have been proposed as the leading biomarkers in predicting outcomes and in particular mortality in decompensated cirrhosis.[140] IL-6 and TNFα are pro-inflammatory cytokines that initiate the production of acute-phase proteins which stimulate the immune response as part of host defence.[141] Similarly, sCD163 is associated with inflammation as a marker of macrophage activation.[93] NGAL plays a role in the innate immune system, in particular through its production by neutrophils modulating oxidative stress. It is also produced in renal tubular cells and is an important marker of tubular injury.[101] In contrast, copeptin is considered a surrogate marker of arginine vasopressin (AVP), which is a mediator of the stress response and vasoconstrictor responses seen in advanced cirrhosis.[106] These biomarkers therefore reflect the inflammatory and circulatory status of patients with decompensated cirrhosis, which as stated in the pathophysiology section are integral in causing disease progression. This is why their performance may be superior to traditional scoring systems such as the MELD which do not take these mechanisms into account.

However, there are some important limitations of these markers that have been studied. Firstly, the recent systematic review and meta-analysis that has been previously referenced, highlighted substantial heterogeneity in the

reporting of these biomarker studies, with significant differences or lack of clarity over definitions of outcomes and measures of association.[140] Furthermore, whilst suggestive of a signal, many of these markers are non-specific and show significant differences in a wide range of medical conditions that are associated with systemic inflammation and circulatory dysfunction. Furthermore, focussing specifically on mortality, different biomarkers seem to be superior at different time points (1 month vs. 3 months vs. 1 year), suggesting that the ideal markers still remain elusive. Finally, there are limited studies looking at the prediction of HE, ascites and variceal bleeding, either independently or in combination. In addition, out of the studies that have been published, the significant heterogeneity between them has prevented them from being meta-analysed recently.

This review of the literature highlights the need for new biomarkers to be studied that are not only sensitive, but specific, and target underlying disease mechanisms in decompensated cirrhosis. Furthermore, in future studies, researchers in the field must strive to ensure robust study designs that are consistent and can be replicated. Only through these rigorous methods may we eventually find the 'holy grail' of novel biomarkers which can either complement existing,

or create new scoring systems.

1.7 The emerging role of digital healthcare

Many cirrhosis-related hospitalisations could be preventable through effective outpatient management with early detection of deterioration and proactive

changes in pharmacotherapy. However, timely follow-up is often not possible in an already overburdened healthcare system with an increasing incidence of cirrhosis, compounded by the legacy of the COVID-19 pandemic.[142] Furthermore, there is geographical disparity with increased mortality demonstrated in those who live in more rural and deprived areas with limited access to specialist care.[10] Innovative solutions are required to ensure timely and effective care, with access for all, and in this regard, digital healthcare could be the key. Digital healthcare is a heterogenous term with a vast expansion in research and clinical use in recent years. Indeed, digitising care and incorporating technology is in keeping with governmental policy in the UK with the NHS Transformation Directorate, Digital Health Applications in Germany, and the Department of Health and Human Services in the US. In what is now deemed routine practice, the process started with the transition from paper notes to electronic records, to virtual consultations, which have become common practice during and post the pandemic. The increasing complexity in innovations to support clinical workflow will be explored further in this section. This transformative approach is bringing a new meaning to patient-centred, personalised care resulting in a more collaborative approach between patient and care-provider. Moreover, the potential environmental benefits and improved quality of life through reduced hospital commutes and better utilisation of personal time and resources, cannot be understated.

This section will review the literature and focus on the opportunities and advancements in digital healthcare in patients with decompensated cirrhosis (search strategy detailed in Figure 1-6). I will explore the different therapeutic

options studied, as well as the potential benefits and limitations, and finally, suggest what maybe next in this fast-moving and dynamic field.

References for this review were identified through searches of PubMed and Embase with the search terms :“telemedicine”, “digital therapeutics”, “mobile applications”, “smartphone”, “mobile health”, “telecommunications”, “remote monitoring”, “artificial intelligence”, “machine learning”, “deep learning”, “decompensated liver cirrhosis”, “chronic liver disease” and “liver failure” from inception until December 2023. Articles were also identified through searches of the authors’ own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this review.

Figure 1-6: Search terms for review of digital healthcare literature in decompensated cirrhosis

Categories of digital healthcare

The are many definitions and terms within digital healthcare which are used interchangeably and can generate confusion for all parties involved. Digital health is an all-encompassing term, which broadly refers to use of any digital technologies to address health-related needs.[143] The World Health Organisation defines telemedicine as the delivery of healthcare services from a distance using telecommunications and virtual technology to provide health care outside of traditional-healthcare facilities.[144] Telehealth is often used interchangeably with telemedicine but can also be considered an umbrella term to include telemedicine as well as education, research, health surveillance and public health promotion.[145]

The following technology types have all been explored in liver disease:[146] A summary of these studies in cirrhosis can be seen in Table 1-1.

1. Telemedicine – providing clinical care remotely via two-way communication between the patient and healthcare provider.
2. Mobile applications (apps) –software applications designed to run and perform specific tasks on mobile devices.
3. Wearables (devices worn on the body that collect health related information) or biosensors (receptor-transducer devices that are applied externally or internally and provide clinical information).
4. Remote monitoring – technology to enable monitoring of patients outside of conventional clinical settings. This often incorporates wearables.
5. Risk prediction modelling – the ability of computers to perform tasks usually done by humans, which includes both machine learning (ML) and deep learning (DL). ML involves the use of algorithms to build mathematical models from sample data. DL is a subset of ML that uses artificial neural networks to mimic the learning process of the human brain.

Type of telehealth	**Study**	**Summary of study**
Telemedicine	*Su et al*[147]	SCAN-ECHO program implemented which involved videoconferencing between specialist teams and primary care providers to discuss cases. The study demonstrated a decreased risk of death compared to the control arm (HR 0.54, p=0.003)
	Thomson et al[148]	Patients received weekly interactive voice response calls for 3 months. They would hear pre-recorded messages and respond to queries using their touch-tone phone and receive tailored pre-recorded responses. Weakness and weight change ≥ 5

		pounds in a week were associated with increased rate of hospitalisation (HR 2.1, p=0.048 and HR 2.5, p=0.045, respectively).
	Konjeti et al[149]	SCAN-ECHO program was used to triage referrals for liver transplantation. The authors suggest that they could reduce futile transplantation evaluation by approximately 60%.
	John et al[150]	Patients referred for initial transplant assessment either received a telehealth visit mediated by a telehealth nurse technician, or a traditional in-person visit. The authors demonstrate telehealth resulted in significantly faster referral to evaluation, faster initial evaluation, and reduced time to listing compared to standard care.
Mobile Apps	*Bajaj et al*[151–153]	The EncephalApp-Stroop test is a point of care test which has demonstrated an AUROC for diagnosing minimal hepatic encephalopathy of 0.84-0.91. It has also demonstrated an ability predict future OHE and related admissions.
	Gananandan et al[9]	The CL-ART test demonstrated a strong ability to predict future HE-related admissions (AUROC 0.85). It remained an independent predictor in multivariable analysis with strong useability feedback (Chapter 5 of thesis).
	Bloom et al[154]	Assessed feasibility of a smartphone app linked to Bluetooth connected scales to facilitate outpatient ascites management. Weight data was successfully transmitted for 71.2% of the study period with interventions instigated for weight alerts (change of ≥5 lbs in 1 week) in the form of titrating diuretics or scheduling clinic visits or blood tests.
Remote monitoring	*Ganapathy et al*[155]	The Patient Buddy App was used for daily monitoring of medication adherence, sodium intake, weight and cognition. The App would send automatic alerts regarding adherence and critical values and advice/ management could be provided. The authors conclude they prevented eight HE related admissions.

	Kazankov et al[156]	Patients in the intervention arm would take daily readings including heart rate, blood pressure and cognitive function via devices linked to the CirrhoCare App. Inputs were monitored daily with interventions by the clinical team if indicated. The authors demonstrated 38% fewer admissions compared to controls. In addition, there was a reduction in unplanned paracentesis as well as improvement in liver disease severity scores.
	Kungar et al[157]	Devices to monitor blood pressure, heart rate, weight and medication administration were provided to patients, with clinical intervention instigated if results were outside of accepted parameters. The authors demonstrated that 0% of admissions were due to 'preventable' causes, compared to 33.8% in the control arm.
Risk prediction modelling	*Kanwal et al*[158]	Machine learning methods used to develop the Cirrhosis Mortality Model which demonstrated an increased ability to predict 1 year mortality compared to the MELD-Na score (AUROC 0.78 vs 0.67).
	Zou et al[159]	Deep learning used to develop a model including MELD, total muscle area index and subcutaneous fat density which was superior to MELD in predicting mortality (C statistic 0.71 vs 0.66).
	Choudhury et al[160]	XBG-CV model demonstrated superiority to Child Pugh Score and MELD-Na in predicting 90-day mortality by 16% and 15% respectively.
	Eaton et al[161]	PREsTo model showed increased ability to predict decompensation in patients with PSC (C statistic 0.90) compared to MELD and Mayo PSC risk score (C statistic 0.72 and 0.85 respectively)
Patient related outcome measures	*Orman et al*[162]	Addition of functional status and quality of life variables can lead to a slight improvement in predicting 30-day readmissions compared to clinical variables alone (AUROC 0.75 versus 0.72).
	Oram et al[163]	A 12-week interactive video telehealth exercise program (FIT) demonstrated no benefit in liver

		frailty index (LFI), measures of physical and mental health or quality of life measures.
	Thuluvath et al[164]	The LIFT intervention involved an individualised home exercise prescription, home exercise equipment and exercise tracking using an App. Whilst some benefit was demonstrated in in LFI and 4-meter gait speed, compliance was an issue (31% adherent).
	Gananandan et al[8]	In the CirrhoCare Pilot study, fat mass measured through bioimpedance analysis demonstrated a signal with markers of systemic inflammation (white blood cells) and liver disease severity (CLIF-C AD scores).

Table 1-1: Summary of digital health studies in decompensated cirrhosis

The relative merits and limitations of the different digital healthcare approaches in cirrhosis are outlined in Figure 1-7.

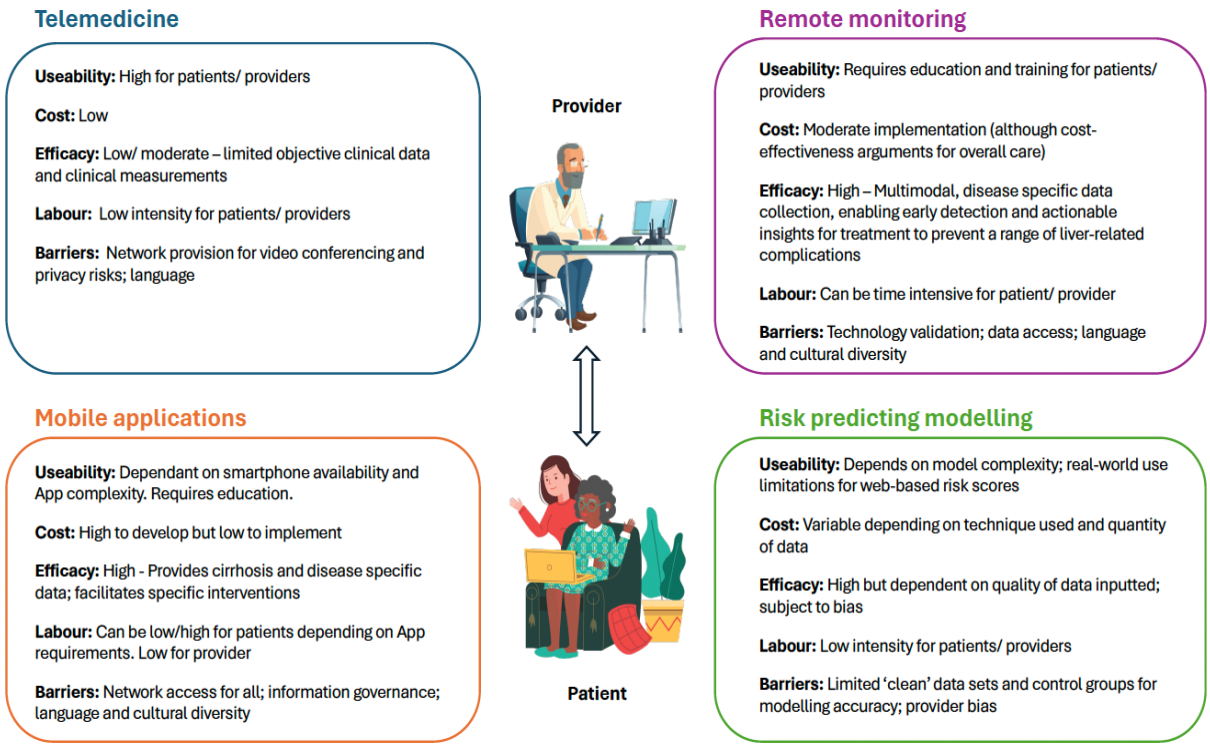

Figure 1-7: A summary of the advantages and disadvantages of different digital health technologies

Telemedicine

Chronic liver disease

The most well-known example of this concept is the SCAN-ECHO model (Specialty Care Access Network-Extension of Community Healthcare Outcome) developed by the Veterans Health Administration. The program involves patient cases being discussed through videoconferencing between specialist teams and primary care providers in real-time, with didactic learning for front-line providers. 513 patients with chronic liver disease had a virtual SCAN-ECHO visit during the study period out of the sampled 62,237. When compared to individuals with no visit, matched individuals who had a SCAN-ECHO visit were associated with decreased risk of death (hazard ratio 0.54, 95% confidence intervals 0.36-0.81 (CI), p=0.003), independent of baseline sociodemographic and clinical data as well as stage of fibrosis. They hypothesise this is due to a higher rate of variceal and hepatocellular carcinoma surveillance in the SCAN-ECHO cohort.[147] A limitation of the study was the lack of randomisation and only a limited range of patient factors were considered, with no provider-level factors included.

Decompensated cirrhosis

Thomson et al evaluated the use of interactive voice response (IVR) calls in the management of decompensated cirrhosis. 100 patients received weekly IVR telephone calls for 3 months. Patients would hear pre-recorded

messages and respond to queries using their touch-tone phone and receive tailored self-management education. Patients were asked to report on a variety of parameters including cirrhosis-related symptoms such as jaundice, confusion, fluid overload, paracentesis requirement and medication changes. Patients were also asked to weigh themselves and input this data weekly. 70% of patients completed >80% of their IVR calls. Weakness was associated with an increased risk of first hospitalisation (HR 2.14, 95% CI 1.13-4.05, p=0.02) and hospitalisation rate (HR 2.1, 95% CI 1.0-4.3, p=0.048). Weight change of ≥ 5 pounds in a week was also associated with increased rate of hospitalisation (HR 2.5, 95% CI 1.0-7.1, p=0.045). [148] Limitations of this study include that it is a single academic medical centre and may not be generalisable to other institutions, but it is nonetheless promising.

Transplant Evaluation

Telemedicine has also demonstrated a role in those with advanced cirrhosis being considered for liver transplantation. Konjeti et al assessed the use of SCAN-ECHO model to triage referrals for transplantation before completing a full work-up or travelling long distances to the referral centre. The authors demonstrate that patients triaged through SCAN-ECHO were more likely to be deemed non-candidates for transplantation at the time of initial referral and were also less likely to be found non-candidates at the time of completion of transplantation work-up. They conclude that the program could reduce futile transplantation evaluation by approximately 60%. [149]

John et al retrospectively analysed patients with advanced cirrhosis being referred for transplant assessment, 232 of which received telehealth and 233 of which received standard care. The patients in the telehealth arm attended their local clinic to conduct a virtual appointment with a transplant hepatologist, facilitated by a telehealth licensed nurse technician on-site. In the control group, the patients had a traditional in-person visit. Telehealth resulted in faster referral to evaluation (21.7 vs 79.5 days, $p<0.01$), faster initial evaluation (22 vs 54 days, $p<0.001$), and reduced time to listing (139 vs 249 days, $p<0.01$) compared to standard care. [150] A limitation of this study is that all patients before 2011 were evaluated in-person and all referred after 2011 who met inclusion criteria were evaluated by telehealth, therefore, some improvements in the telehealth arm may have been due to advances in care.

Mobile Applications

With the increased use of smartphones, there has been an explosion in the number of health-related apps. Within cirrhosis, one of the most validated is the EncephalApp-Stroop Test, which has demonstrated a role in the diagnosis of minimal hepatic encephalopathy (MHE) with an area under the receiver operating characteristic (AUROC) of 0.84-0.91.[151,152] Users are required to complete a series of ‘Off’ and ‘On’ runs. The ‘Off’ state involves a neutral stimulus (###) being presented in either red, blue, or green and the patient must select the correct colour. In the ‘On’ state, incongruent stimuli are presented, and patients must continue to select the correct colour as opposed to colour associated with the word, i.e., the word “RED” is displayed

in a blue colour and the correct response is blue. As well as a diagnostic role, some recent evidence suggests that it can predict the risk of overt HE and related hospitalisations over a 7-month period.[153] However, the test can be time-consuming, taking 10 minutes or more to complete, and patients need significant education in advance to use the test. As a result, a shortened form of the test ("QuickStroop") has been developed.[165]

More recently another test called the CyberLiver- Animal Recognition Test (CL-ART) has been developed as a tool to detect and predict OHE. The test involves participants being presented with a series of pictures of animals with animal names superimposed, which may either be the same as the picture or different. Participants must correctly identify the animal in the picture i.e., a picture of a dog is presented with the word cat over it, the correct response would be dog. The test takes less than 30 seconds to complete. As part of my thesis I have validated the predictive role of CL-ART and this will be explored further in Chapter 5.[9]

Bloom at al assessed the feasibility of using a smartphone App in facilitating outpatient ascites management. 25 patients were provided with a Bluetooth-connected scale which transmitted weight data to a smartphone app and then onwards to an electronic medical record (EMR). Providers responded to 84% of weight alerts (weight change of ≥5 lbs in 1 week) and intervened in 57%. An intervention could involve contacting the patient, titrating diuretic doses, scheduling blood tests or clinic/ paracentesis appointments. Interestingly, 60% of patients chose to extend beyond the 30-day study period. [154] A limitation of this study is a lack of control arm and patients did

report some form of technology issue on 16.5% of days enrolled. However, a subsequent cost-analysis study by the same group using a decision-analytic model and simulated patients suggested that the cost of standard of care for 100 patients with ascites over 6 months is $167,500 more expensive than telemonitoring.[166] These are important considerations for increasingly resource-limited healthcare services.

Bloom et al also demonstrated the potential role of speech as a possible biomarker for HE. The authors demonstrated significantly slower speech and longer word duration in individuals with lower neuropsychiatric scores as well as those with a history of HE.[167] This technology has also been evaluated using a smartphone App, showing good concordance of speech recorded by the patients at home with the recordings in a clinical setting, and a close correlation between speech enunciation and psychometric tests for HE.[168]

A smartphone, image-capture and app has also been trialled in patients with decompensated cirrhosis, where an algorithm to determine scleral colour value was shown to correlate closely with blood measurement for bilirubin.[169] It follows that such a tool could be used remotely to determine jaundice severity and potentially serve as an indicator of new cirrhosis decompensation.

These studies highlight the different modalities that could play a role in detecting specific cirrhosis complications early and potential avenues to expand digital healthcare applications.

Remote monitoring

Patients suffering from chronic disease spend very little time with health providers leaving much of their disease management burden falling upon the patients and their carers. Remote monitoring provides a way of performing regular assessments and obtaining useful information in the home environment, whilst empowering patients to participate in the management of their own disease (Figure 1-8).

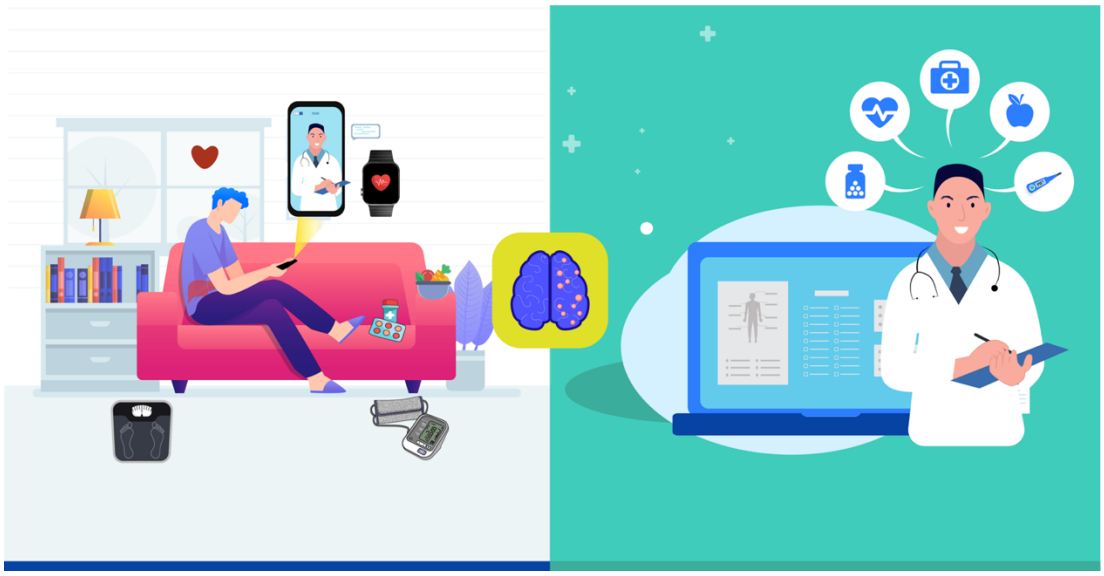

Figure 1-8: Representation of a remote monitoring programme in decompensated cirrhosis

Lactulose is first-line therapy for HE. However individual requirements with respect to the dose and frequency of lactulose are highly variable which can result in both over- and underdose. In a recent randomised trial of lactulose, Tapper et al used SMS-based text messaging to monitor bowel habits and stool consistency and titrate therapy accordingly.[170] Text messages are

therefore a promising, simple method, to ensure both safety and effectiveness of lactulose therapy.[171]

In another study, Ganapathy et al performed a prospective study of the Patient Buddy App, enabling patients/ caregivers to stay in close contact with the clinical team, with a particular focus on medication adherence, sodium intake, weight and cognition (via the EncephalApp-Stroop Test). 40 patients and 40 caregivers were monitored daily through the Patient Buddy App for 30 days post-hospital discharge. The App sent automatic alerts between patients and the clinical team regarding medication adherence and critical values, and the clinical team could provide outpatient management/ advice as required. Useability and feedback were largely positive with the authors concluding they prevented eight HE-related admissions. [155] It is worth noting that there was no change to overall readmission rate (42.5%), despite the intensive follow-up (3 study visits and 2 telephone calls). Nevertheless, this study demonstrates the feasibility and potential role of home monitoring in this population.

Further HE studies include a pilot study evaluating speech patterns among 43 subjects. Speech rate and precision distinguished overt from minimal from no HE. Furthermore, there were improvements in speech patterns with improvement of overt HE. As speech can be recorded and analysed remotely it may be a promising biomarker of treatment effectiveness.[172] Using a wearable fitness tracker called “Whoop,” Buckholz et al studied the sleep patterns of 25 patients. In this study, a model that included rapid eye

movement sleep, sleep disturbance, and sleep consistency offered a c-statistic for covert HE of 0.79.[173]

Kazankov et al recently published a novel integrated-approach aimed at early detection and management of the full range of cirrhosis decompensating events, termed CirrhoCare. Compared to previous studies, predominantly limited to 30 days, follow-up was up to 12 weeks which is important, given that the readmission rate remains high within 90 days of AD.[174] 20 cirrhotic patients were discharged home with monitoring devices blue-toothed to the CirrhoCare platform and App, following admission with AD, compared with 20 matched controls. CirrhoCare- managed patients took daily readings for heart rate, blood pressure, weight and cognitive function (using the CL-ART). The clinical team would monitor the patients via the CyberLiver platform, and the App had a 2-way, closed-loop, secure, patient-physician communication. System alerts when digital biomarkers were beyond mean values over the preceding week or/and baseline for a given patient, prompted further evaluation and clinician-directed intervention. 85% of patients showed good engagement with very positive useability feedback. Compared to the control group, the CirrhoCare cohort had 38% fewer admissions with shorter lengths of stay. Furthermore, they had significantly fewer unplanned paracentesis requirements and a greater improvement in liver disease severity scores over follow-up. [156] A limitation of this study is the lack of randomisation and small sample size, however, the CirrhoCare RCT is now actively recruiting in the UK, to build upon these findings.

Like the CirrhoCare study, Kungar et al provided 19 patients with 4G tablets and wireless devices to monitor blood pressure, heart rate, weight and medication administration. Telehealth nurses and clinicians intervened to prevent admissions if readings went out of accepted parameters. Whilst in the remote monitoring arm, 0% of readmissions were due to what the authors deemed preventable causes (fluid overload and HE); 33.8% of the controls experienced such admissions (p=0.02). [157] These studies highlight the potential role of remote monitoring in achieving sustainable healthcare delivery, reducing carbon footprint through reduced hospital visits, and improving patient outcomes.

Risk prediction modelling

ML methods have been used to develop scoring systems with improved performance for predicting outcomes compared to traditional prognostic models. Kanwal at al performed a retrospective analysis of 107,939 patients with potential predictors including demographic characteristics, liver disease aetiology, complications, use of health care resources, comorbidities as well as laboratory and medication data. The final cirrhosis mortality model demonstrated an increased ability to predict 1-year mortality compared to the MELD Na score (AUROC 0.78 vs 0.67). [158]

Tapper et al have used ML-methods to derive risk models for the prediction of both HE and falls. These models were derived from a cohort of 300 patients with cirrhosis and portal hypertension. In both cases, patient-reported outcomes (PROs) were found to predict the outcome of interest.

The resulting MASQ-HE (c-statistic 0.82 for HE at 1-year) and FallSSS scores (c-statistic for injurious falls at 1-year) integrated measures of disease severity, PROs such as health-related quality of life (measured using the Short Form-8), and a visual analogue scale for the impact of cirrhosis on daily activity.[175,176]

Zou et al developed a DL (DeepLabv3+) algorithm to predict mortality based on body composition measured from CT scans. The algorithm was developed from 12,067 patients and then prospectively validated in 238 patients. The authors developed a model including MELD, total muscle area index and subcutaneous fat density and showed this was superior to MELD alone in predicting mortality (C statistic 0.71, 95% CI 0.61-0.82 vs 0.66, 95% CI 0.55-0.78).[159] An alternative model, named XBG-CV, derived by Choudhury et al is composed of 43 variables and performed better than CPS and MELD Na in predicting 90-day mortality by 16% and 15% respectively.[160]

Eaton et al used ML in a derivation and validation cohort to generate a model to predict hepatic decompensation in individuals with primary sclerosing cholangitis (PSC). Gradient boosting, an ML technique, was used to create the PSC risk estimate tool (PREsTo) consisting of biochemical and haematological variables, patient age, and number of years since PSC was diagnosed. The authors demonstrated that PResTo was superior in predicting decompensation (C-statistic 0.90, 95% CI 0.77-.92) compared to the MELD score (C-statistic 0.72, 95% CI 0.57-0.84) and Mayo PSC risk score (C-statistic 0.85, 95% CI 0.77-0.92). [161]

The problem with all models determined through ML and DL is that the quality of the output is determined by the quality of the data input. Therefore, if there is bias in the data sets through missing variables or criteria selection, this will be reflected in the model, as highlighted by the study by Garcia et al.[177] In addition, concerns also remain over the transparency in the development and testing of such algorithms.

Patient related outcome measures and other additional directions for digital healthcare in cirrhosis

Patient-reported outcome measures (PROMs) are important in the assessment of a patient's general well-being, as well as specific symptomatology. PROMs are provided by the patient directly without interpretation by healthcare professional, thus reflecting the impact and burden of their disease from the patient's perspective. A number of PROMs are highly relevant for patients with cirrhosis, and a recent study by Orman et al showed an association between PROMs and the risk of readmission in decompensated cirrhosis. [26,162] PROMs can be easily obtained using a smartphone and present an obvious feature to be included in digital healthcare solutions as electronic PROMs (ePROMs). ePROMs have been evaluated in diseases such as ulcerative colitis and would be appropriate to investigate in patients with cirrhosis.[178] Additionally, patient-initiated follow-up could be a promising avenue, although not fully explored in this patient group. Whilst other specialities have demonstrated potential benefit in

reducing unnecessary appointments, clear cost-effectiveness and the impact on primary care/community services needs to be investigated.[179,180]

Patients with cirrhosis have cognitive impairment in terms of HE, but also markedly reduced physical function, malnutrition and sarcopenia, leading to increased risk of falls and serious injury.[176] In this regard, the assessment of frailty and its improvement is essential for the care of patients with cirrhosis, to guide nutritional therapy, physical exercise and optimisation of HE management.[181] Current technological offerings can assist clinicians in targeting HE as discussed above, as well as delivering personalised exercise programs to patients at home. A video telehealth-based exercise program has recently been tested in a pilot study of patients with cirrhosis and frailty, without clear benefit.[163] In contrast, a more comprehensive program of individualized home exercise with exercise tracking using a smartphone application, and reminder prompts to exercise, showed good feasibility and efficacy in liver transplant candidates. This was despite some difficulties with home exercise equipment impacting on patient compliance.[164] Similarly, telemedicine may allow easier nutritional guidance for patients, enabling frequent feedback from the patients to assess their dietary adherence, and monitoring of their physical parameters (fat mass and weight) using portable technology to measure bioimpedance as explored in Chapter 4 of this thesis.[8]

In patients with cirrhosis, medication adherence is vital; unfortunately, there is data to suggest that medication compliance in decompensated cirrhosis is poor, ranging from 21-37%.[182] In addition, patients may also take medication

such as non-steroidal anti-inflammatory agents, which are contraindicated, whilst others are potentially harmful.[183] A digital solution may help ameliorate this issue, by deploying a smartphone app to reinforce patients' awareness of their medication list, and provide an opportunity for patients to communicate any concern or medication query to their clinician.

Thus, these essential aspects of care for patients with cirrhosis seem well-suited for digital therapeutics, however, it is clear that well-designed and large studies are warranted.

Acceptability of digital healthcare to patients and providers

Whilst there is much interest in digital transformation, the success of this change in practice will require the engagement of all stakeholders. A multicentre prospective study of patients with decompensated cirrhosis demonstrated that 71.5% of patients had a smartphone. Smartphone users tended to be younger, married, employed, living in areas with high incomes and have a non-alcoholic aetiology of cirrhosis.[184] Another small study showed that 78% of patients and 80% of carers owned a smartphone. 85% of patients were interested in a smartphone app that could communicate with their physician, 79% would like an app to educate them about their liver disease, 85% of those with ascites would be willing to transmit weight data, and 67% would be willing to perform tests/ games to assess for cognitive decline.[185]

A prospective study by Acharya et al demonstrated that the reasons for declining to participate in a study using a smartphone app were caregiver reluctance (43%) and perceived burden (31%). It is therefore imperative that any digital intervention has good useability and acceptance by both patients and their care providers.[186]

Louissaint et al carried out a survey to determine predictors of acceptance and utilisation of a smartphone App for cirrhosis management.[187] They demonstrated that acceptance of technology correlated with patient perception of its usefulness (r=0.77, 95% CI 0.67-0.84), ease of use (r=0.65, 95% CI 0.52-0.75), as well as computer anxiety (r= -0.54, 95% CI -0.66 to -0.38). Whilst the study demonstrated that nearly 70% of patients perceived benefit of the App, only 32% used it.[187] This suggests that to ensure success, digital interventions must be simple to use and require regular training/ education, as well as demonstrating clinical effectiveness.

It is also crucial that digital healthcare is acceptable to care providers. The Patient Buddy App detailed previously in the remote monitoring section, is now being assessed in an RCT. Whilst feedback from a small group of providers was largely positive, 25% of individuals had issues with initial patient entry, 67% felt they did not have sufficient technical support, and only 42% felt it contributed significantly to patient care.[188] A limitation of this study is that the authors used a survey instrument which has not been validated, and perhaps future studies should gather healthcare provider opinions through directed interviews or workshops.[189]

The barriers to overcome and the future of digital health research

Whilst digital healthcare is revolutionising the way we view healthcare, there are still some important barriers. Concerns do exist amongst some given the limited ability to perform physical examination, the lack of data if patients are uncontactable or do not complete monitoring measurements, as well as concerns over establishing patient-physician trust in a remote environment.[144] It is also imperative to close the digital divide that exists, with pre-existing racial and socioeconomic disparities that exist in telehealth, due to factors such as lack of internet access, being exacerbated during the pandemic.[190] For the success of digital healthcare, it is imperative to ensure that all patients have access to devices, internet connectivity and crucially, technology education and support to ensure digital literacy and willingness to engage. Health systems need to ensure inclusivity by working with communities, particularly isolated and racially diverse populations, overcoming language and cultural barriers, to build programmes that work for consumers and meet their needs. This will need to be supported by governmental policy in order to implement real change with mechanisms in place to ensure the protection and correct use of patient data. The key barriers and facilitators to digital healthcare implementation are highlighted in Figure 1-9.

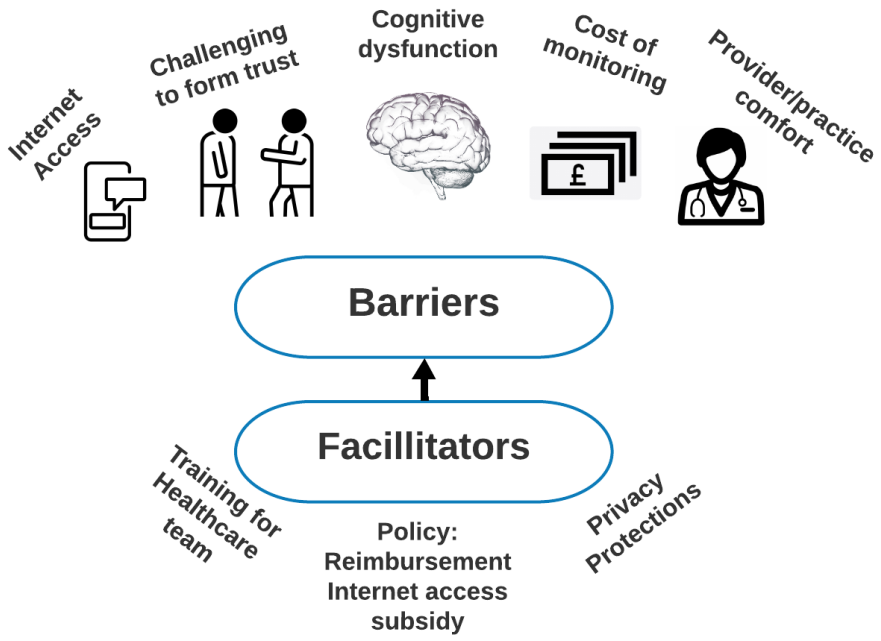

Figure 1-9: Barriers and facilitators to digital health applications in cirrhosis management

Whilst there are many promising studies, most are single-arm interventions and those with controls often lack randomisation or use historical controls. Multicentre randomised controlled trials including some of the most vulnerable patients are required to determine the true benefits of digital interventions, and it is hugely promising that some of these are now underway. In addition, in order for healthcare systems to utilise digital health on a large scale, studies clearly assessing cost-effectiveness, which are currently lacking, will be mandatory, as well as ways of incorporating data collected into established clinical platforms. Furthermore, efforts should be made to standardise digital tools for utilisation across healthcare settings, and ideally with generalisable components that can be used for other medical conditions to facilitate uptake into wider healthcare systems. At a fundamental level, the most important step for the success of digital care

delivery is a buy-in from all stakeholders. Further studies identifying factors preventing patients and carers from engaging with digital healthcare as well as potential solutions to tackle these issues, are needed. In addition, concerns from care providers both in terms of their own digital literacy as well as concerns about potential increased work burden, must be addressed. Whilst AI is an exciting field, researchers must ensure transparency in reported outputs. In addition, lawmakers must ensure patient's digital rights are safeguarded to ensure trust in intelligence systems and enable everyone to reap potential benefits.

Conclusions

We have already entered the digital era of decompensated cirrhosis management, with the transformation well underway. For clinicians, the future will involve digital platforms being part of routine clinical practice, with a wealth of data available and interventions being possible in real-time. For patients, apps, devices and monitors will become part of routine home-life. A more collaborative approach will become the norm with greater possibilities for proactive as opposed to reactive care delivery. Personalised medicine will take on a new meaning as care will be tailored to an individual's 'digital fingerprint' through digital biomarkers. The digital future in cirrhosis is truly exciting, and it is our collective responsibility to ensure that no-one is left behind.

1.8 Rationale and aims of thesis

Rationale for thesis and overview

In summary, throughout this introduction I have described how decompensated cirrhosis is a complex and evolving concept. Regardless of how it is defined, it is clear that it is associated with a significant morbidity and mortality with an increasing disease burden. Whilst prognostic scoring systems have been developed, they have significant limitations and often underperform, and whilst other novel biomarkers have been studied, none have made it into routine clinical practice. This has set the premise for this thesis to try and address this urgent unmet need.

I will first attempt to provide a contemporary perspective of the management and outcomes of patients with decompensated cirrhosis, particularly given the impact of the COVID-19 pandemic. Whilst it has been speculated that this would have a detrimental impact, this has not been fully explored. Prior to assessing any new markers, I have conducted a systematic review and meta-analysis of biomarkers that have already been investigated to predict decompensation. Whilst this thesis focusses on patients with decompensated cirrhosis, it is imperative to take a step back to look at prediction of decompensation in patients who are compensated, as once decompensation occurs, this significantly alters the trajectory of a patient and puts them at risk of further events. This review will partly enable determination of which markers have the most evidence for prediction, but also enable exploration of limitations and flaws of previous studies to help guide future research.

I then systematically explore the role of a range of novel blood-based and digital biomarkers to determine whether they can predict liver-related

outcomes. All biomarkers have been carefully selected based on mechanistic links to the pathophysiology driving decompensation, as well as exhibiting desirable qualities of a biomarker as detailed earlier on the introduction.

Aims and objective

i. To determine whether there is a difference in the characteristics and clinical outcomes of patients following hospitalisation with AD admitted during the pandemic, compared to patients admitted prior to the emergence of COVID-19 in a tertiary hepatology and transplantation centre.

ii. To systematically review and meta-analyse biomarkers that predict decompensation, as defined by Baveno criteria, in patients with compensated cirrhosis.[4]

iii. To determine the ability of lipoproteins to predict liver-related outcomes following AD, including ACLF development, readmissions and mortality.

iv. To investigate whether fat mass can be monitored reliably remotely and whether it correlates with markers of systemic inflammation and liver disease severity.

v. To evaluate whether CL-ART can predict future hospitalisation due to decompensation, particularly secondary to HE, comparing its performance to established cognitive tests.

vi. To determine whether a novel dimethylarginine scoring system termed DAS can predict liver-related outcomes following AD, including ACLF development, readmissions and mortality.

Chapter 2 - The negative impact of the pandemic on hospital admissions, morbidity and early mortality for acute cirrhosis decompensation.

2.1 Introduction

In the United Kingdom liver cirrhosis is the 3rd most common cause of premature death and has been increasing at a more rapid rate than the 4 most commonly diagnosed cancers; lung, breast, bowel and prostate.[17,18] The British Liver Trust indicates deaths from liver disease have increased by 400% since 1970 with 62,000 years of working life lost per year.[3]

As cirrhosis progresses to decompensation, the median survival decreases sharply from greater than 10 years to 2 years.[15] At the most severe end, the distinct entity of ACLF has also been identified which is an acute deterioration of pre-existing chronic liver disease associated with extrahepatic organ failure and a short-term mortality of over 30% at 28 days.[21,191]

Patients with decompensated cirrhosis usually require a regular clinical assessment, within weeks of hospital discharge, and even despite optimal management have re-admission rates in excess of 30% at 30 days[192]. Moreover, data suggests that early readmissions are associated with reduced chance of independent living at one year.[25]

The COVID-19 pandemic has necessitated an unusual allocation of healthcare resources to acute respiratory presentations, which inevitably negatively impacts on resources available to care for patients with chronic diseases including liver disease. Such was the concern of the potential impact of the pandemic on cirrhosis care that the EASL-ESCMID position paper was produced to try and reduce predicted increased morbidity and mortality.[6] Moreover, there are an increasing numbers of publications addressing outcomes of those with chronic liver disease who develop COVID-19 infection .[5,193] Whilst it has been speculated that the pandemic would have a negative impact on decompensated cirrhosis admissions and outcomes, there is very little data in the literature highlighting the real-world impact on this patient group. [194,195]

The aim of this study was to address whether there was a difference in the clinical course, characteristics and outcomes of decompensated liver cirrhosis patients admitted during the pandemic, when compared to cirrhosis patients admitted prior to the emergence of COVID-19, in a tertiary UK hepatology and transplantation centre.

2.2 Methods

Setting and study design

We conducted a single-centre retrospective cohort study evaluating admissions to the Royal Free Hospital London with acute decompensation of liver cirrhosis, from October 2018 to February 2021.

Admissions with decompensated cirrhosis were identified from inpatient records including ward lists, electronic patient records and hospital endoscopy reporting software (Unisoft). Data was collected using medical notes, laboratory, radiology and histology reports, clinic letters, discharge summaries and endoscopy reports. Data collected included patient demographics; aetiology of liver disease; precipitant of decompensation event; type of decompensation event; prior decompensation history; length of hospital stay; blood test results (admission, including admission to the Intensive Treatment Unit (ITU) if applicable and discharge) and presence of infection and/or SBP and COVID-19 status, which was determined by a polymerase chain rection (PCR) test on admission. In addition, physiological parameters and observations such as blood pressure, oxygen saturation by pulse oximetry (%), and fraction of inspired oxygen at admission were also recorded. For admissions involving stays in intensive care, data was collected on use of mechanical ventilation, inotropic support and haemofiltration. Supplementary data was collected on patients requiring interventional procedures including abdominal paracentesis, endoscopy, liver biopsy and TIPS. For patients requiring paracentesis between admissions, the frequency of paracentesis was recorded in intervals of weeks. Dates of death and/or liver transplantation were recorded for patients meeting these outcomes within the study period. Data was collected for all included patients throughout the study period with 6-month follow-up data obtained. Attempts were made to reduce the following sets of bias: information and selection bias by using multiple record systems to maximise data capture and minimise missing data, and confounding bias through multivariable analysis.

The following severity scores were calculated; United Kingdom Model for End-Stage Liver Disease (UKELD), MELD Na and CPS. Definitions from the European Foundation for the Study of Chronic Liver Failure (EF CLIF) were used to calculate CLIF-C organ failure (OF) scores and to establish the presence of ACLF. For patients without ACLF, CLIF-C AD scores on admission and discharge were recorded.[21]

Participants and ethical approval

Our cohort consisted of 388 patients, with a total of 591 admissions with decompensated cirrhosis. Patients were included if they had an admission with a decompensating event within the study period. Patients referred and transferred from external referral sites were identified and included in the cohort. Admissions were excluded if they lasted less than 24 hours, were planned elective admissions, or if they occurred post liver transplant.

All data collected contained no personal health identifiers. Formal local audit approval was sought and received for data acquisition from hospital records (Registration number – RFHBU_180621).

Definitions

Decompensated cirrhosis was defined as the presence of ascites, HE, portal hypertension-related bleeding, infection, or a combination of these, on a background of radiologically or histologically confirmed cirrhosis. Precipitants of decompensation were categorised as harmful drinking of alcohol, infection, gastrointestinal (GI) haemorrhage, hepatitis B reactivation, new portal vein

thrombus, autoimmune hepatitis (AIH) flare, drug induced liver injury (DILI), new/progression of hepatocellular carcinoma (HCC) or as unknown.

Alcohol related hepatitis was also recorded and defined using the National Institute on Alcohol Abuse and Alcoholism (NIAAA) definition: history of heavy alcohol consumption (≥ 4 drinks per day), serum bilirubin >3mg/dl (51.3μmol/l), AST 50-400U/L and AST: ALT >1.5.[196]

Confirmed infection was determined by blood, sputum and/or urine culture positivity and/or radiological evidence of chest infection on chest x-ray, or evidence of microbial growth from another source. Suspected infection was classified as cases where antimicrobials were prescribed based on clinical suspicion of infection, in the absence of positive culture results.

Statistical Analysis

Summary statistics were performed on patient demographics, aetiology of disease, precipitant of decompensation, disease severity scores, symptoms of decompensation, interventions performed during admission, length of admission, intensive care admission, liver transplantation and mortality outcomes. A non-parametric assumption was used for all statistical tests. Any missing data was excluded from analysis. Data was grouped for analysis defined by admission date, with admission between October 2018 and February 2020 defined as pre-COVID and admissions from March 2020 to February 2021 as the COVID period. The COVID time-period was defined by when healthcare systems at the Royal Free Hospital London were impacted

by the COVID pandemic, with all patients having mandatory COVID-19 PCR testing on admission. A Pearson's chi-squared test was used to test for statistically significant differences in nominal or ordinal data between pre-COVID and COVID groups, in addition to local and transferred patient groups. A Mann-Whitney U test was used to test for statistical significance in variables of continuous data between pre-COVID and COVID cohorts, and local and transferred patient groups. Survival analysis for mortality and transplant-free survival outcomes were performed using Kaplan-Meier procedure and log rank test with censoring to 30 or 90 days. In addition, a multivariable analysis was performed for mortality using odds ratios (OR) with 95% CIs provided.

2.3 Results

Summary demographics and characteristics of population

Data collected on patient admissions over 29 months was assessed. There were 390 patients with 591 admissions to the Royal Free Hospital London, with acute cirrhosis decompensation. The summary of demographics, co-morbidities, aetiology of liver disease, precipitants of admission as well as liver disease severity scores for the entire cohort can be seen in Table 2-1. The admissions were split between the pre-COVID (October 2018 to February 2020) time-period with 247 patients having 351 admissions, and the COVID period (March 2020 to February 2021) with 167 patients having 240 admissions. 143 (86%) patients admitted during the COVID period had no prior admissions in the pre-COVID period. There was a median of 21 admissions per month over the total time (range 10-33) with some variations

noted across the non-COVID and COVID time periods in Figure 2-1. This included a relative reduction in winter cirrhosis admissions by 30% in the COVID period, from December 2020- February 2021, when compared with the 2 equivalent winter periods previously. In addition, other notable changes include times reflecting the UK national lockdown periods, and a notable spike in September 2020, after a major Governmental initiative to re-open the hospitality sector.

	Total	Pre-COVID	COVID	p value
Male	264 (67.7%)	161 (65.2%)	117 (70.1%)	0.186
Female	126 (32.3%)	86 (34.8%)	50 (29.9%)	
Age (median, IQR)	58 (16)	59 (17)	57 (16)	0.314
Ethnicity				
-White	247 (63.3%)	149 (60.3%)	113 (67.7%)	0.146
-Asian	37 (9.5%)	27 (10.9%)	11 (6.6%)	
-Black	17 (4.4%)	13 (5.3%)	5 (3.0%)	
-Other	81 (20.8%)	50 (20.2%)	38 (22.8%)	
-Mixed	1 (0.3%)	1 (0.4%)	0	
-Not stated	7 (1.8%)	7 (2.8%)	0	
Co-morbidities				
-Diabetes	87 (22.3%)	61 (24.7%)	32 (19.2%)	0.135
-Cardiac	48 (12.3%)	31 (12.6%)	21 (12.6%)	0.845
-Respiratory	52 (13.3%)	33 (13.4)	20 (12%)	0.980
-Chronic kidney disease	24 (6.2%)	16 (6.5%)	11 (6.6%)	0.724
-Neurological	26 (6.7%)	18 (7.3%)	13 (7.8%)	0.516
-Malignancy	33 (8.5%)	25 (10.1%)	9 (5.4%)	0.121
-Other	81 (20.8%)	61 (24.5%)	24 (14.4%)	0.030
Aetiology				
-Alcohol	246 (63.1%)	152 (61.5%)	110 (65.9%)	0.401
-MASLD	55 (14.1%)	40 (16.2%)	23 (13.8%)	

-Hepatitis C	49 (12.6%)	33 (13.4%)	18 (10.8%)	
-Hepatitis B	19 (4.9%)	13 (5.3%)	6 (3.6%)	
-Autoimmune hepatitis	25 (6.4%)	13 (5.3%)	12 (7.2%)	
-Primary biliary cholangitis	8 (2.1%)	7 (2.8%)	1 (0.6%)	
-Primary sclerosing cholangitis	11 (2.8%)	8 (3.2%)	3 (1.8%)	
-Cryptogenic cirrhosis	11 (2.8%)	9 (3.6%)	3 (1.8%)	
-Wilson's disease	3 (0.8%)	2 (0.8%)	2 (1.2%)	
-Other	9 (2.3%)	5 (2%)	4 (2.4%)	
Precipitant				
-Alcohol	150 (25.4%)	82 (23.5%)	59 (35.3%)	0.301
-Infection	131 (22.2%)	79 (22.6%)	31 (18.6%)	
-GI bleed -Varices	111 (18.8%) 75 (67.6%)	73 (20.9%) 47 (64.4%)	37 (15.4%) 28 (75.7%)	
-Hepatitis B reactivation	2 (0.3%)	2 (0.6%)	0	
-Portal vein thrombosis	1 (0.2%)	0	1 (0.6%)	
-HCC	5 (0.8%)	2 (0.6%)	3 (1.8%)	
-AIH flare	3 (0.5%)	1 (0.3%)	2 (1.2%)	
-DILI	2 (0.3)	2 (0.6%)	0	
-Unknown	185 (31.3%)	108 (30.9%)	47 (28.1%)	
Admission scores (median, IQR)				
-UKELD	56 (52-62)	56 (52-62)	57 (52-62)	0.392
-MELD Na	21 (16-26)	20 (16-25)	21 (16-27)	0.684
-CPS	10 (8-11)	9 (8-11)	10 (8-11)	0.820
-AD score	54 (48-61)	54 (48-61)	55 (49-62)	0.690

Table 2-1: Table showing demographics, aetiology, precipitant and disease severity scores of decompensated cirrhosis admissions. Data

has been shown for the total cohort, as well as a breakdown of the pre-COVID and COVID time periods

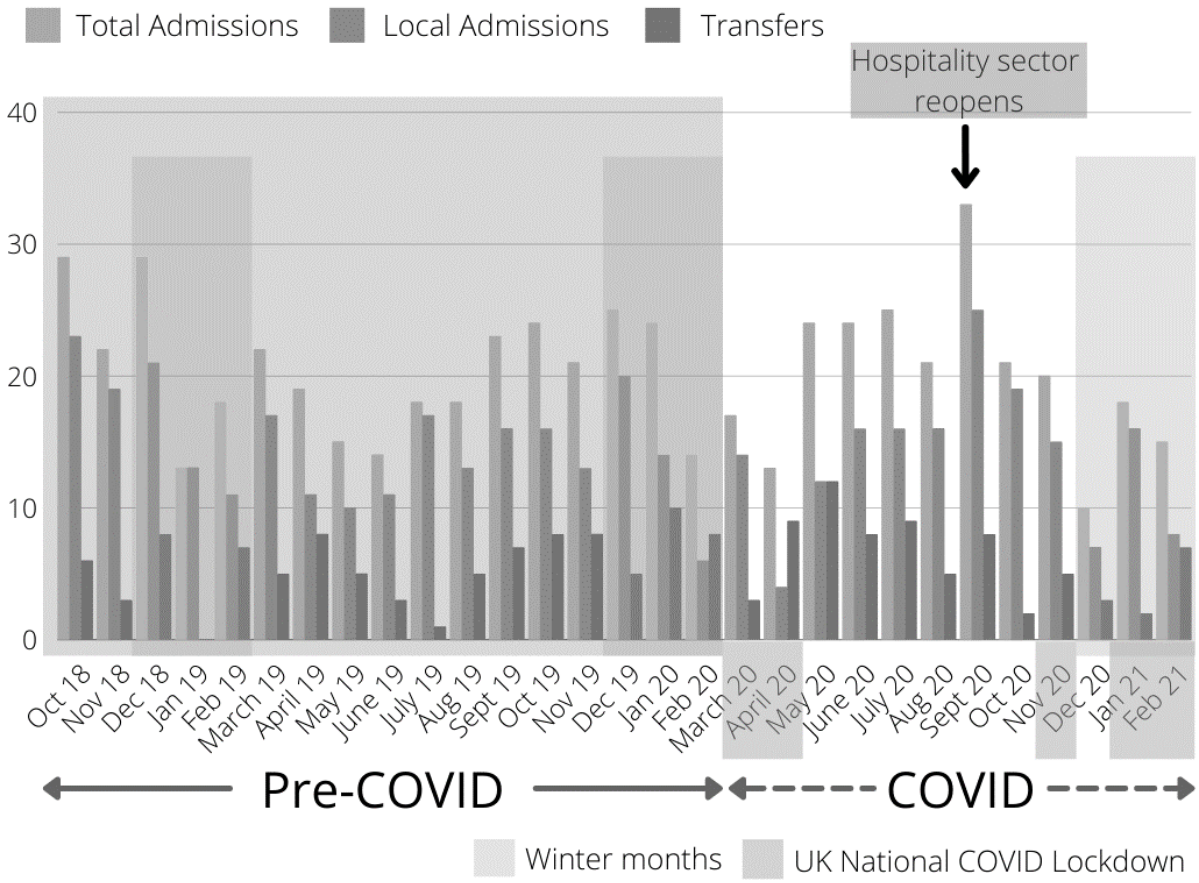

Figure 2-1: Graph showing admissions per month as a total number, as well as the split between local admissions and external referrals transferred to the unit from other hospitals

During the COVID period, 4 out of all patients tested at admission, were positive for COVID-19 on PCR (1.7% of admissions) of which 3 had symptoms consistent with infection, the other being asymptomatic. A further 3 patients had symptoms of COVID-19, however, had tested negative in hospital, and in 2 cases, COVID-19 was identified as the cause, or contributed to death.

Portal hypertension driven complications were the most predominant cause of presentation, with 432 (73.1%) admissions with ascites noted either at presentation or during the admission, whilst 233 (39.4%) had hepatic encephalopathy. Moreover 210 patients (35.5%) had gastrointestinal bleeding, of which 126 (60%) were found to have varices on endoscopic examination. Amongst 262 (44.3%) admissions with infection, 60 patients (22.9%) had suspected infection, whilst the remainder had confirmed infection with culture positivity or consolidation seen on chest x-ray examination, with 74 (12.5%) admissions requiring treatment for spontaneous bacterial peritonitis.

As anticipated, acute decompensation admissions often required interventions, including: 75 (12.7%) patients requiring liver biopsy, 59 (10%) requiring an emergency TIPS insertion, and 277 (46.8%) required inpatient paracentesis at least once. Of those requiring TIPS insertion, 39 (66.1%) had an indication of bleeding, 19 (32.2%) for ascites management and 1 for portal vein thrombus. The median length of stay per admission was 7 days (IQR: 11). Amongst the admissions, 102 (26.2%) of the index admissions during the recruitment period, required a further readmission, with a median number of admissions of 2 per patient. The median time from index admission to readmission was 40 days (IQR: 106.3).

Differences in patient characteristics between periods

Hospital admissions with cirrhosis AD were compared between the pre-COVID and COVID time periods. The average number of admissions per

month did not differ between the two cohort periods (20.6 versus 20.0). In addition, there was no significant difference in the ratio of presentations or development of ACLF compared to AD (17% pre-COVID and 16.6% during COVID). Of the 351 admissions during the pre-COVID period, 152 (61.5%) had alcohol listed as their primary aetiology compared to 110 (65.9%) during the COVID period (p=0.454). Patients presenting with alcohol as their precipitant for AD increased from 82 (23.5%) pre-COVID to 59 (35.3%) during the COVID period, although not reaching statistical significance (p=0.221). The number of admissions with alcohol related hepatitis according to NIAAA clinical criteria definition remained stable at 53 (15.1%) pre-COVID, and 39 (16.3%) during COVID. [196]

Those presenting with GI bleeding on admission non-significantly reduced from 73 (20.9%) pre-COVID to 37 (15.4%) during COVID (p=0.327), of which 47 (64.4%) and 28 (75.7%), respectively, were found to have varices. There was also a significant reduction in TIPS procedures performed during the COVID period, down from 45 (12.9%) to 14 (5.8%) (p=0.006), however the proportion of TIPS inserted for bleed indication remained at a comparable proportion (64% vs 69%). The number of admissions with documented infection remained similar between the pre-COVID and COVID periods (43.1% to 46.5%, p=0.376). However, the proportion of patients with spontaneous bacterial peritonitis increased significantly from 32 (9.1%) to 42 (17.4%) (p=0.005).

		Pre-COVID		COVID	
		Domestic (n=254)	**Transferred (n=97)**	**Domestic (n=167)**	**Transferred (n=73)**
Severity Scores Median (IQR)	MELD Na	21 (9)	18 (11)	21 (9)	22 (11.5)
	CPS	9 (3)	10 (3)	10 (3)	10 (2)
	AD score	55 (13)	52 (11)	55 (12)	55 (16)
Symptoms	Ascites	192 (75.6%)	60 (61.9%)	125 (74.9%)	55 (75.3%)
	HE	102 (40.2%)	32 (33%)	68 (40.7%)	32 (43.8%)
	Infection	107 (42.1%)	44 (45.4%)	69 (41.3%)	43 (58.9%)
	Gastrointestinal bleeding	32 (12.5%)	42 (43.3%)	25 (15%)	12 (16.4%)

Table 2-2: Table showing severity scores and symptoms comparing the pre-COVID and COVID periods with a breakdown of admissions between local admissions and external referrals

Of the 591 total admissions, 170 (28.8%) were external hospital tertiary referrals for specialist intervention with a breakdown seen in Table 2-2. The liver disease severity scores on admission of locally admitted patients had little variation between the pre-COVID and COVID period (Table 2-2). However, the liver disease severity scores of tertiary transferred patients during COVID, were consistently higher than pre-COVID transfers, with the CLIF-C AD and MELD-Na scores both showing statistically significant increases (p=0.032 and p=0.006). There was a significant decrease in the number of transferred patients presenting with a GI bleed (pre-covid 43.3% to

covid 16.4%, p<0.001) and a non-significant increase in presentations with new decompensation events including ascites (62 increasing to 75%), hepatic encephalopathy (33 to 44%) and infections (45 to 59%).

The length of stay per admission between pre-COVID and COVID time periods remained at a median of 7 days. However, there was a rising trend in readmission rates from 21.5% to 29.5%, p=0.067, from pre-COVID to COVID periods, although the median time to first readmission during the COVID period was 57 days, compared to 33 days pre-COVID (p=0.056).

ITU support requirements

During the COVID period, there was no significant change in the number of ITU admissions, length of stay, organ support requirements nor mortality. The proportion of patients with all grades of ACLF on admission to Intensive Care non-significantly increased from 63.8% pre-COVID to 73.9% during the COVID period (p=0.241). Importantly, of the patients admitted to Intensive Care without organ failures, the median AD score increased significantly from 48 (IQR: 12) pre-COVID to 58 (IQR: 18) during the COVID period, (p=0.009). The proportion of patients with an AD score over 60 increased from 7.4% to 50% in the COVID period (p=0.002), with an equal number of these being local versus transferred patients.

Outcomes and mortality data

The proportion of patients who received a liver transplant within 90 days of follow up during the COVID and pre-COVID periods was comparable (3.5%

versus 4.8%, p=0.535). However, the median time to transplantation non-significantly increased from 28 days pre-COVID to 70 days during COVID (p=0.328).

Overall mortality did not change throughout the study period with pre-COVID inpatient mortality at 12.2% compared to 14.8% during COVID (p=0.466). However, patients who died post hospital discharge during the COVID period (and without COVID infection), did so over a significantly shorter time with a median time to death of 35 days, compared to 62 days in pre-COVID times (p=0.005). Figure 2-2 shows the Kaplan-Meier survival analysis for time to early deaths post discharge after first admission, censored at 30 days, with those in the COVID period having a significantly shorter survival (p<0.001). When analysing the whole cohort, a multivariable analysis demonstrated that bilirubin, CRP and HE could independently predict 30-day mortality with a trend to significance with creatinine, INR and infection (Table 2-3).

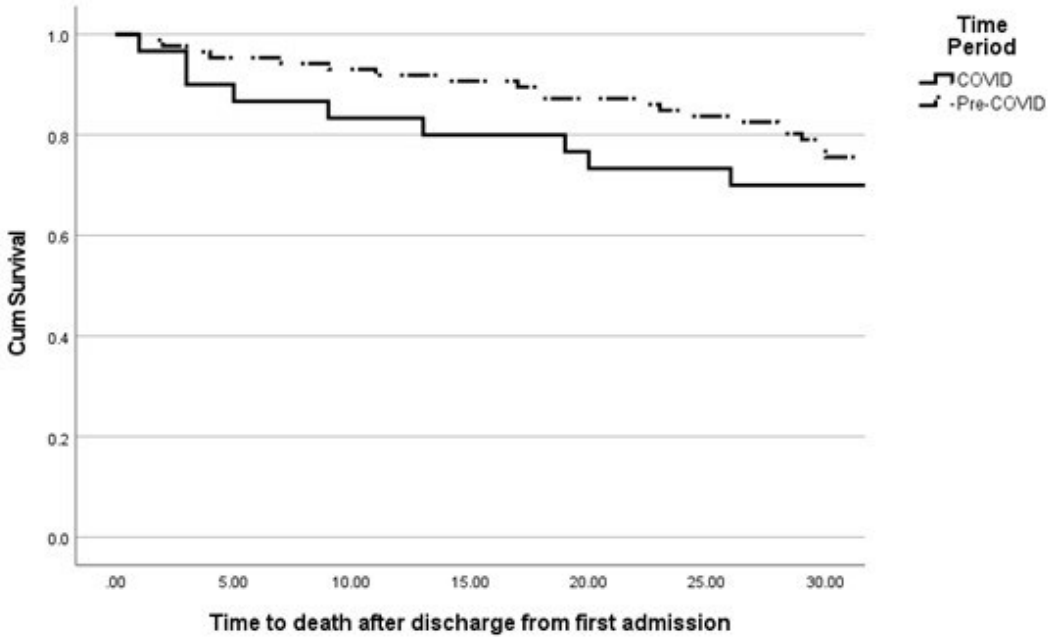

Figure 2-2: Figure 2: Kaplan-Meier survival curve for time to death post discharge after first admission censored to 30 days comparing the pre-COVID and COVID periods

Variables	Univariable Analysis			Multivariable Analysis	
	Mortality within 30 days (n=81) *Mean (SD)*	**Survival beyond 30 days (n=307)** *Mean (SD)*	**P value**	**Odds Ratio (95% CI)**	**P value**
Age	55.9 (12.5)	56.9 (13.6)	0.564		
Sex (Male) *N(%)*	58 (71.6)	204 (66.4)	0.379		
Sodium (mmol/L)	132 (9)	135 (7)	**0.001**	0.98 (0.94-1.01)	0.181
Creatinine (µmol/L)	143 (121)	98 (92)	**<0.001**	1.00 (1.00-1.00)	0.096
Bilirubin (µmol/L)	194 (180)	101 (135)	**<0.001**	**1.00 (1.00-1.00)**	**0.011**
ALT (U/L)	99 (173)	60 (109)	**0.013**	1.00 (1.00-1.01)	0.573
AST (U/L)	197 (305)	112 (166)	**<0.001**	1.00 (1.00-1.00)	0.465
Albumin (g/L)	28 (5)	30 (6)	**0.007**	0.96 (0.91-1.01)	0.076
INR	1.9 (1.0)	1.5 (0.6)	**<0.001**	1.38 (0.95-2.00)	0.095
Platelets (x10^9/L)	128 (81)	127 (72)	0.956		
WBC (x10^9/L)	12.4 (7.5)	8.8 (7.5)	**<0.001**	1.02 (0.99-1.04)	0.209
CRP (mg/L)	57 (57)	30 (39)	**<0.001**	**1.01 (1.00-1.01)**	**0.017**
Ascites *N(%)*	66 (81.5)	217 (70.7)	0.052		
HE *N(%)*	48 (59.3)	96 (31.3)	**<0.001**	**1.70 (1.00-2.87)**	**0.049**

GI Bleeding *N(%)*	36 (44.4)	111 (36.2)	0.172		
Infection *N(%)*	57 (70.4)	125 (40.7)	**0.007**	1.75 (0.97-3.15)	0.063
SBP *N(%)*	20 (24.7)	26 (8.5)	**<0.001**	1.14 (0.55-2.37)	0.725
First admission during COVID time period *N(%)*	30 (37.0)	112 (36.5)	0.927		
Transferred from another centre *N(%)*	35 (43.2)	110 (35.8)	0.193		

Table 2-3: A univariable and multivariable analysis of variables predicting 30-day mortality

2.4 Discussion

COVID-19 has clearly impacted on the ability of healthcare providers to deliver care to patients with cirrhosis but the actual consequences on patient morbidity, outcomes, hospital and ITU utilisation, in decompensated cirrhosis patients, not directly infected by the virus, remains unclear.

The key findings of our study included higher liver disease severity scores at presentation in patients externally transferred during COVID compared to pre-COVID, with notably higher CLIF-C AD scores in those not developing ACLF, when admitted to the ITU. A trend was also observed towards increased re-admission rates during the COVID period and increased early mortality (median <10 days post-discharge).

Whilst the average number of admissions did not differ between the two periods, monthly admissions did fall during national lockdown periods.

Consequently, the seasonal spike common for winter was not seen in 2020 but instead higher peaks in early Summer and Autumn 2020 were observed, between the first and second lockdowns in the UK. This is consistent with the literature with some studies showing reduced cirrhosis admissions during the pandemic and others demonstrating no significant difference.[197–199]

Looking at the overall data, alcohol was the most predominant aetiology with MASLD second, which is consistent with European data.[20,200] In terms of precipitants for decompensation, the proportion of patients presenting with infection and GI bleeding are consistent with larger observational studies such as the CANONIC study, from non-COVID times.[21] No significant difference was noted in alcohol as precipitant between the COVID and pre-COVID time periods, despite some of the highest levels of alcohol consumption in the word reported in the UK as well as Nordic countries.[201] We speculate that this perhaps was due to reluctance of patients to attend hospital due to concerns regarding COVID-19 infection, as well as a lack of power due to insufficient sample size.

Whilst the purpose of this study was not to address the direct impact of COVID-19 infection in patients with established cirrhosis, given all patients were tested upon arrival to the hospital during the COVID period, interestingly we show that only 4 admissions amongst this tested cohort were positive for the infection, of which 2 were symptomatic. This is consistent with large scale studies which demonstrate no significant increased risk of acquiring COVID-19 with chronic liver disease.[202]

We noted a non-significant reduction in GI bleeding during the COVID period, especially amongst tertiary referrals, which correlated with a significant reduction in TIPS insertions that were performed (p=0.006). This is likely due to reduced availability of bed resource, especially ventilated ITU beds in our tertiary bleeding referral center, for external transfers requiring airway protection prior to salvage TIPS consideration, and also in part, through overall reduced endoscopic services activity during the COVID period.

Interestingly there was a statistically significant increase in the rate of SBP during the COVID period. This is unexplained, though given that admissions during the COVID period had higher CLIF-C AD scores and more frequently met ACLF criteria, one might expect such patients to have higher risk for developing SBP, with higher portal pressures driving bacterial translocation. Another potential explanation for greater SBP during COVID could be sub-optimal ascites management and patient compliance with or access to appropriate antibiotic prophylaxis, albeit this is speculative, as our study design could not avail information on medication compliance or oversight of patients being managed at home.

Although not significant, an increasing trend in cirrhosis decompensation hospital re-admission rates (p=0.067) was noted in the COVID period. This is likely to have a multi-factorial basis which includes: lack of ease of access to early, post-discharge follow-up; diminished community/primary care access to support, and potential for expedited premature hospital discharges, reflecting pressure on healthcare systems consequent upon the pandemic. Of note, there was also, a trend towards increased time to first re-admission

during the COVID period, which may have been due to a reluctance of patients to attend hospital until they were more unwell, compounded by pressures on secondary-care beds leading to reduced access, which is supported by our data showing higher liver disease severity scores particularly in patients accepted as transfers in from secondary care sites.

Whilst there was no difference in overall survival between the time periods, the median time to death post index hospital discharge, was significantly shorter (35 vs. 62 days) in the COVID cohort compared to non-COVID times. This is at variance with some of the published literature, albeit in smaller cohorts, which have shown mortality increases of 52%.[203] Increased mortality has also been reported in other conditions such as respiratory, cancer and sepsis admissions.[204] This has been thought to be as a consequence of: (i) patients presenting late, in an attempt to avoid hospital presentation for fear of acquiring COVID-19; (ii) impact of redistribution of clinical resources to acute medical care and away from supporting standard hepatology care pathways, most likely to impact on secondary care units, faced with more general medical case loads (iii) increased numbers with more advanced disease (CLIF-AD score or ACLF progression) and (iv) reduced availability of liver transplantation.[203] The concept of patients presenting with more advanced disease during the pandemic is supported by the multivariable analysis showing that markers of liver disease severity such as bilirubin as well as presence of HE independently predicted 30-day mortality.

Consistent with the early mortality data, we show an increase in cirrhosis decompensation severity scores on admission to the ITU during the COVID

period, especially noted in externally transferred patients. This is again consistent with patients presenting with more advanced disease which has been suggested in the literature.[5] The fact that overall mortality was not significantly different during the COVID period compared to pre-COVID times, despite higher liver-disease severity scores, is a testament of the ability of units to continue to provide high-quality supportive hepatology care, despite the constraints of the pandemic.

Our data shows CLIF-C AD scores of those admitted to ITU without meeting organ failure criteria, were significantly higher during the COVID period, especially in patients with AD scores over 60, who have been shown to have a higher risk of mortality and are more likely to progress to ACLF and thereby, requirement for organ support.[20,191] This may be in part be attributable to patients being transferred in from secondary care, who had consistently higher AD disease severity scores during COVID period, compared with local patients, who had similar scores across both time periods. A possible explanation is likely to reflect the lack of specialist hepatology input within secondary care sites in the UK which has been described, and patients being referred to tertiary care centers when they were more advanced in their decompensation.[205]

The key limitations of this observational study include that it is a single centre study and is retrospective in nature. Factors influencing hospital readmissions rates and mortality in the community were also not easy to discern in the data accessible and warrant further investigation in prospective studies. Outcome data for secondary care transferred patients was not

always possible to verify once the patients returned to their local units for further follow-up and this may have introduced information bias. In addition, the cohort size may have limited the possibility to detect potential statistically significant differences in some of the outcomes measured.

In conclusion, this study provides useful insight into the effects that the COVID-19 pandemic has had on hospital admissions with decompensated cirrhosis, in a large UK tertiary liver center. In particular, this study, shows increased cirrhosis decompensation severity and early mortality, highlighting the need to focus on maintaining high-level specialist hepatology care, even after the patient is discharged from the hospital. Given that the effects of the pandemic will continue to impact hepatology service provision for years to come, this necessitates considerations for alternative-care pathways that mitigate reduced access for direct clinical review, especially early post hospital discharge, such as considering remote-monitoring in this vulnerable patient population.

Chapter 3 – A systematic review and meta-analysis of biomarkers predicting decompensation in patients with compensated cirrhosis.

3.1 Introduction

Cirrhosis is a leading cause of liver-related death, accounting for 2-3% of deaths globally, and in Europe is the second leading cause of years of working life lost.[1,16] Concerningly the epidemic of chronic liver disease is worsening, largely driven by the increasing prevalence of obesity and harmful alcohol consumption, with an associated unprecedented socioeconomic cost.[1,206]

The initial, asymptomatic phase of cirrhosis is termed compensated cirrhosis and carries a good prognosis with mortality tending to be due to non-liver-related causes such as cardiovascular disease, renal disease and malignancy.[13] However, once a patient develops liver-related complications, this signals the onset of decompensated cirrhosis with a drastic reduction in median survival from over 10 years to just 2 years.[15] The most recent Baveno guidelines have defined decompensation by the development of overt ascites, overt hepatic encephalopathy (West Haven grade ≥II) or variceal bleeding.[4] Patients with AD of cirrhosis are at high risk of hospitalisation, and even despite optimal management, have short-term re-admission rates between 30-50%, with 3-month mortality rates in the sickest cohort reported over 50%.[20,23,24] Moreover, there is a substantial impact on

quality of life with a significant reduction in independent living at one year, placing an extensive burden on patients and carers .[25,26]

Given that decompensation heralds a pivotal change in the disease trajectory of cirrhosis, there is an urgent unmet need to discover biomarkers that can predict its occurrence, in order to help prevent its onset. The ideal biomarker should demonstrate biological plausibility, high sensitivity and specificity, generalisability, undergo validation, be minimally invasive as well as easy to measure, demonstrate stability, and crucially, for healthcare services be affordable.[89] When performing biomarker research, it is imperative to use appropriate terminology, and whilst addressing prediction of decompensation, one is actually referring to prognostic biomarkers which identify the likelihood of a clinical event or disease progression.[89]

Whilst liver disease scoring systems have been developed over time, such as the CPS and MELD, they have generally focussed on predicting mortality as opposed to decompensation, and often underperform in contexts other than those in which they were initially developed.[76,78] Whilst a range of other prognostic biomarkers have emerged over recent years, few have been incorporated into clinical practice. This is likely due to a lack of clarity over which biomarkers are truly superior, whether they actually outperform existing scores and the high heterogenicity in published studies.

The aim of this systematic review and meta-analysis is to identify which biomarkers have the strongest evidence for determining future

decompensation in compensated cirrhosis, to help guide future research and highlight potential therapeutic targets.

3.2 Materials and Methods

Study design

To identify relevant studies, PubMed and EMBASE database searches were conducted from inception until February 2024. The bibliographies of relevant studies were also reviewed to ensure that no eligible publications were missed. Only full manuscripts with English versions were included. This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and registered on PROSPERO.[207] The inclusion and exclusion criteria for the study are detailed below and the full search terms used are detailed below in Figure 3-1.

("Compensated cirrho*" OR "compensated liver cirrhosis"[Mesh]) AND (“prognos*” OR “predict*” OR "biomarkers"[MeSH Terms] OR biomarkers[Text Word]) AND (("Acute Kidney Injury*"[Mesh] OR "Hepatorenal syndrome"[Mesh] OR "Acute Kidney Injury" OR "AKI" OR "Acute kidney disease" OR "AKD" "acute renal failure" OR "acute kidney failure" OR "hepatorenal syndrome" or HRS) OR ("Hepatic Encephalopathy"[Mesh] OR "hepatic encephalopathy" OR HE) OR ("Liver failure"[Mesh] OR "Acute-on-chronic liver failure"[Mesh] OR "Acute-on-chronic liver failure" OR "ACLF") OR ("Infection*"[Mesh] OR "peritonitis"[Mesh] OR "infection*" OR "bacteremi*" OR "bacteraemi*" OR "urinary tract infection*" OR "UTI" OR "pneumonia" OR "spontaneous bacterial peritonitis" OR "SBP" OR "sepsis" OR "cellulitis" OR "soft tissue infection*") OR ("gastrointestinal hemorrhage"[Mesh] OR "variceal bleed*" OR "gastrointestinal hemorrhage" OR "gastrointestinal haemorrhage" OR "gastrointestinal bleed*" OR "GI hemorrhage" OR "GI haemorrhage" OR "GI bleed*") OR ("ascites"[Mesh] OR "ascitic fluid"[Mesh] OR "edema"[Mesh] OR "ascit*") OR ("jaundice"[Mesh] OR "jaundice*" OR "icter*") OR ("clinical outcome" OR "disease course" OR "disease severity" or "complicat" or "decompensat*") OR ("COVID-19"[All Fields] OR "COVID-19"[MeSH Terms] OR "SARS-CoV-2"[All Fields] OR "sars-cov-2"[MeSH Terms] OR "Severe Acute Respiratory Syndrome Coronavirus 2"[All Fields] OR "NCOV"[All Fields] OR "2019 NCOV"[All Fields]) OR ("pneumonia"[MeSH Terms] OR pneumonia[Text Word])) NOT REVIEW [Publication Type] NOT CONGRESS [Publication Type]

Figure 3-1: The search used in PubMed and EMBASE databases.

Inclusion criteria

1. Adult patients (>18 years old).
2. Patients with compensated liver cirrhosis according to the Baveno VII guidelines.[4]
3. Studies in which the primary or secondary outcome is the prognostic or predictive role for cirrhosis decompensation events (variceal bleeding, ascites, or overt hepatic encephalopathy).
4. Cohort (prospective or retrospective), case-control, and control arm of RCTs.

Exclusion criteria

1. Experimental studies (i.e., animal studies, in vitro studies).
2. Cross-sectional studies, case series,

   case reports, letters, editorials, reviews, systematic reviews, and

   meta-analyses.
3. Studies performed only in patients with decompensated cirrhosis.
4. Studies performed only in patients with hepatocellular carcinoma.

Data extraction

The Covidence® system was used for managing references.[208] The initial searches and obtaining of references were conducted independently by two investigators with duplicates automatically removed. Initial screening of titles and abstracts was performed independently by the same two investigators, with studies only passing through to the next full-text phase if both investigators agreed. Any disagreements were resolved by a third reviewer. The same process was repeated at the full-text phase to generate the final studies for inclusion, as demonstrated in Figure 3-2.

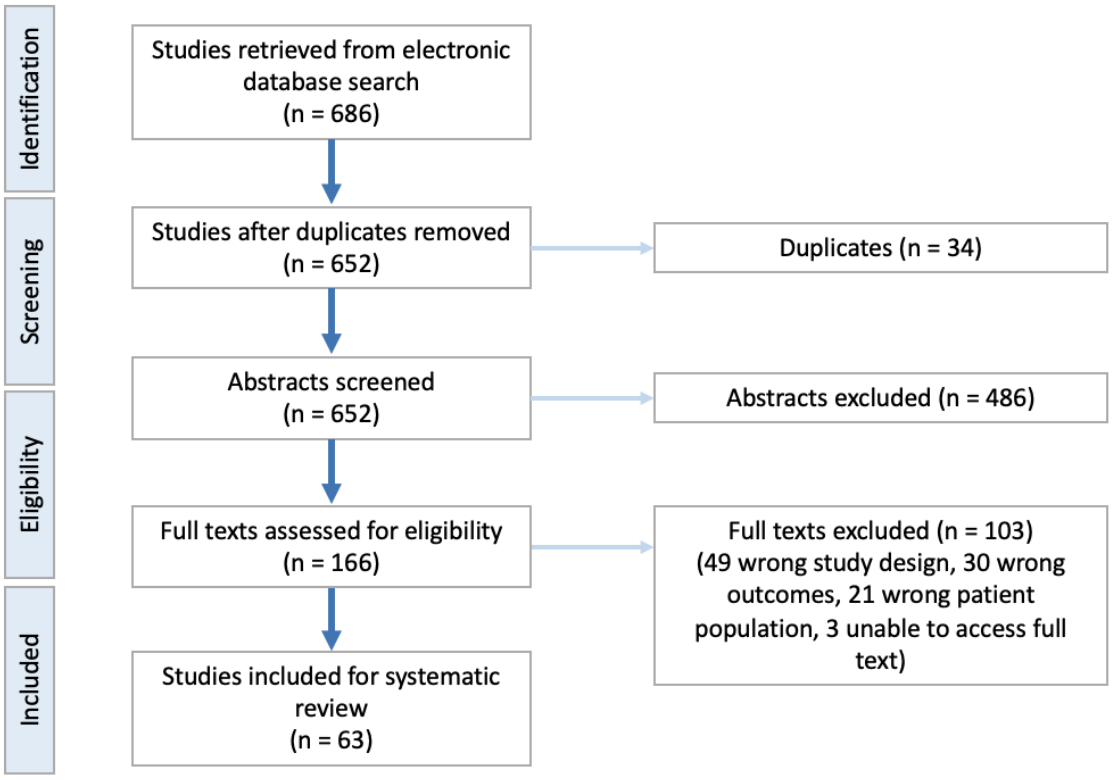

Figure 3-2: Flowchart showing the study selection process for the review

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Data extraction was performed using REDCap®.[209] The parameters recorded included number of patients, age, gender, etiologies of disease, study design, duration of follow-up, and liver disease severity scores. Biomarker data, as well as outcome data in terms of decompensation events, were recorded along with statistical tests utilised.

Studies were classified *a priori* into six biomarker categories: blood-based, HVPG, liver stiffness, physiological, imaging, and miscellaneous. If a study reported on two or more cohorts, for example, a derivation and validation group, then the study would be considered twice (once for each cohort). Furthermore, if multiple biomarkers were investigated in one study, then that

study was considered multiple times resulting in the variable *biomarker study* and *biomarker patients*.

Assessment of quality

To evaluate the quality and risk of bias of eligible studies, the Quality in Prognosis Studies (QUIPS) tool was utilised.[210] This assessed study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, statistical analysis and reporting. Each paper was graded as a low, moderate, or high risk of bias in each of the six domains, as well as overall.

Statistical methods

A descriptive analysis was performed reporting on the following measures of association where reported; mean ± standard deviation, median [P25-P75], OR, hazards ratio (HR), AUROC with respective 95% CI, as well as biomarker thresholds. The meta-analysis was performed using a random effects model with a log transformation undertaken due to skewed data. Studies with hazard ratios were included as this was the most commonly reported outcome measure. Statistical heterogeneity was assessed by the I^2 test. Finally, a funnel plot and Egger's regression test were performed to assess for bias. Statistical analyses were performed using STATA (StataCorp. 2019. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.).

3.3 Results

Summary

Of the 652 studies initially identified, 63 studies (n=31,438 patients) were included in the final review. The weaning of studies with reasons for final selection is demonstrated in Figure 3-2.

Out of the 63 studies, 25 (40%) were prospective and 3 were RCTs. Heterogeneity was evident with sample size varying between 35-5123 patients, and mean/median age and follow-up ranging from 40-67 years and 12-455 months respectively. The majority of studies looked at all-cause decompensation (57 [90%]), with only 4 looking at variceal bleeding alone and 2 addressing ascites. In total 49 biomarkers were assessed, and a summary of all studies and characteristics can be seen in Table 3-1, subclassified by biomarker category.

Study	**Publication year**	**Number of patients**	**Number of patients with biomarker and outcome**	**Biomarker(s)** Significant predictors of decompensation or not significant predictors	**Design**	**Male** %	**Age (y)** mean or median	**MELD** mean or median	**Follow-up time (months)** mean or median
BLOOD MARKERS									
Allen, A.M. et al [211]	2022	5123	5123	Albumin Bilirubin Platelets INR Creatinine	Prospective cohort	47	52*	n/a	76.9*
Are, V.S. et al [212]	2021	162	162	ELF score (TIMP-1, PIINP and hyaluronic acid)	Phase 2 RCT	n/a	n/a	n/a	12.0
Bajaj, J.S. et al [213]	2023	157	72	Bacterial composition	Prospective cohort	n/a	59	8.9	14.3
Calzadilla-Bertot, L. et al [214]	2021	543	543	ABIDE score Albumin Bilirubin Platelets AST/ALT INR	Retrospective cohort	46	n/a	8.2	67.2*
Chen, Q. et al[215]	2023	688	688	Laminin Collagen IV Gamma GT Platelets	Retrospective cohort	60	52*	7*	22.0*

Cordova, C. et al [216]	1986	41	41	Prekallikrein	Prospective cohort	66	n/a	n/a	n/a
Fujiwara, N. et al [217]	2022	122	122	PLSec (VCAM-1, IGFBP-7, gp130, matrilysin, IL-6, CCL-21, angiogenin and protein S)	Prospective cohort	66	51*	n/a	66.0*
Garcia Garcia de Paredes, A. et al [218]	2021	105	105	Serum miR-181b-5p	RCT	60	65*	6.0*	36.0*
Gatselis, N.K. et al [219]	2020	632	632	Golgi protein-73	Retrospective cohort	60	57*	n/a	50.0*
Guéchot, J. et al [220]	2000	91	91	Serum hyaluronan Albumin Bilirubin Platelets ALP INR CPS	Retrospective cohort	70	56	n/a	38.0
Guha, I.N. et al [221]	2019	379	379	ALBI score MELD score	Prospective cohort	66	61*	7.50*	455.1*
Hartl, L. et al [222]	2021	663	307	Renin ProBNP Copeptin	Retrospective cohort	68	57	11.0	26.2*
Hsu, C.Y. et al [223]	2021	3722	3722	ALBI-FIB4 score MELD score CPS	Retrospective cohort	55	59	9.6	17.2
Innes, H. et al [224]	2022	1196	907	ALBI-FIB4 score MELD score	Prospective cohort	73	56	9.8	26.4
Merchante, N. et al [225]	2018	282	282	Bacterial translocation CP score	Retrospective analysis of two prospective cohorts	81	45*	8.0*	51.0*
Navadurong, H. et al[226]	2023	123	123	ALBI	Retrospective cohort	59	64	8.7*	36.0*
Qamar, A.A. et al [227]	2009	213	213	Hematologic indices	Retrospective cohort nested in RCT	n/a	n/a	n/a	54.9*
Saeki, C. et al[228]	2023	148	148	Insulin-like growth factor 1 Albumin	Retrospective cohort	65	69*	8.0*	57.1*
Schneider, A.R.P. et al [229]	2022	6049	6049	EPOD score Albumin Bilirubin Platelets	Retrospective cohort	55	61*	9.0*	60.0*
Schwarzer, R. et al [230]	2020	194	194	VITRO score (von Willebrand, platelets) MELD score	Prospective cohort	56	56*	9.0*	45.0*
Tornai, D. et al [231]	2021	244	101	Serum ferritin	Retrospective cohort	47	54*	9.0*	24.0
Wong, Y.J. et al [232]	2022	633	633	CHESS-ALARM score	Retrospective cohort	69	53	7.0	39.0*
Yuan, L. et al [233]	2019	164	83	ALT	Retrospective cohort	87	40	n/a	n/a
HVPG									
Colecchia, A. et al [234]	2014	122	92	HVPG Spleen stiffness	Prospective cohort	69	57*	9.0*	24.0

				Platelets MELD score AST/ALT					
Jindal, A. et al [235]	2020	741	741	HVPG	Retrospective cohort	71	n/a	10.4	19.2
Joly, J.G. et al [236]	1971	38	38	HVPG	Prospective cohort	90	46	n/a	n/a
Pérez-Latorre, L. et al [237]	2014	60	60	HVPG Liver stiffness	Retrospective cohort	72	46*	n/a	42.0*
Rincón, D. et al [238]	2013	145	145	HVPG Albumin Platelets MELD score	Retrospective cohort	77	51*	9.0*	27.0*
Ripoll, C. et al [239]	2007	213	213	HVPG Albumin Platelets AST/ALT MELD score CPS	Retrospective cohort nested in RCT	59	54*	8.0*	51.1*
Turco, L. et al [240]	2018	151	151	HVPG	Prospective cohort	68	60	8.0	18.4
LIVER STIFFNESS									
Asesio, N. et al [241]	2022	455	455	Liver stiffness Albumin Bilirubin Platelets INR	Retrospective cohort	72	58*	n/a	n/a
Dillon, A. et al [242]	2018	244	244	Liver stiffness Albumin Platelets INR MELD score	Prospective cohort	32	56	7.5*	35.5*
Gidener, T. et al [243]	2021	829	194	Liver stiffness	Retrospective cohort	37	64*	n/a	60.0*
Gidener, T. et al [244]	2022	1269	277	Liver stiffness MELD score CPS	Retrospective cohort	57	57*	8.0*	122.5*
Jindal, A. et al [245]	2022	626	626	Liver stiffness Albumin	Prospective cohort	72	51	n/a	26.0*
Kim, B.K. et al [246]	2012	217	217	Liver stiffness Platelets	Prospective cohort	65	50	n/a	42.1*
Merchante, N. et al [247]	2012	239	239	Liver stiffness MELD score CP score	Prospective cohort	90	44*	7.0*	20.7*
Merchante, N. et al [248]	2017	446	446	Liver stiffness	Prospective cohort	88	49*	7.0*	49.0*
Merchante, N. et al [249]	2015	275	275	Liver stiffness	Prospective cohort	89	44*	7.0*	32.0*
Semmler, G. et al [250]	2022	1173	755	Liver stiffness	Retrospective cohort	n/a	n/a	n/a	55.4*
Wang, J.H. et al [251]	2014	220	220	Liver stiffness Bilirubin AST/ALT	Prospective cohort	61	57*	n/a	36.9*
Zarski, J.P. et al [252]	2020	219	219	Liver stiffness	Retrospective case-control	64	58*	n/a	68.4
PHYSIOLOGICAL									
Berzigotti, A. et al [253]	2011	161	161	BMI	Prospective RCT	59	54	8.7	59.0*

Gomez, E.V. et al [254]	2014	402	402	Mean Arterial Pressure (MAP) Platelets AST/ALT INR	Prospective cohort	39	59*	9.0*	35.9*
Henrique, D.M.N. et al [255]	2021	55	55	6-minute-walk-test CPS	Prospective cohort	65	56	n/a	12.0
Siramolpiwat, S. et al [256]	2021	152	152	LFI Albumin Bilirubin MELD score CPS	Prospective cohort	57	63	9.2	14.9
Wang, S. et al [257]	2022	822	214	LFI	Prospective cohort	66	55	n/a	14.4*
IMAGING									
Berzigotti, A. et al [258]	2008	127	127	Spleen size Albumin	Retrospective cohort	60	59	10.0	53.0
Elkassem, A.A. et al [259]	2022	191	191	Liver surface nodularity	Retrospective cohort	65	n/a	n/a	51.6*
Fallahzadeh, M.A. et al [260]	2021	70	35	Hepquant-SHUNT test	Prospective cohort	66	57*	6.0*	50.4*
Kondo, T. et al [261]	2016	236	110	Portal haemodynamics Albumin AST/ALT MELD score	Retrospective cohort	55	67*	10.0*	33.2*
Kwon, J.H. et al [262]	2021	1027	1027	Liver-to-spleen volume ratio MELD score CPS	Retrospective cohort	66	51	<10.0	116.0*
Lee, M.H. et al [263]	2013	107	107	201Tl heart-liver radioactivity uptake ratio Platelets	Retrospective cohort	75	55	n/a	45.5*
Smith, A.D. et al [264]	2017	830	326	Liver surface nodularity	Retrospective cohort	66	53*	8.5*	50.7*
Tae, H.-J. et al [265]	2014	209	209	Thalium Shunt Index	Prospective cohort	n/a	n/a	n/a	49.6*
Tapper, E.B. et al [266]	2020	274	111	Subcutaneous fat density MELD score	Prospective cohort	56	58	11.3	60.6*
Yang, W. et al [267]	2021	292	197	T2 mapping (gadoxetic acid-enhanced MRI) Albumin MELD score	Retrospective cohort	80	63	7.9	18.7
Yu, Q.et al [268]	2022	689	689	Spleen volume	Retrospective cohort	64	54	6.7	37.6*
MISCELLANEOUS									
Boonpiraks K. et al[269]	2024	457	457	Diabetes CP score MELD	Retrospective cohort	59	64	9.9	54.0
Calvaruso, V. et al [270]	2015	118	118	Collagen proportionate area (CPA) Albumin Platelets	Prospective cohort	58	57	n/a	72.0*

Jain, D. et al [271]	2021	168	168	Thick fibrous septa Albumin Bilirubin Platelets INR Creatinine MELD score CPS	Retrospective cohort	76	49	8.8	50.0*
Lisotti, A. et al [272]	2016	154	154	ICG-R15 Platelets	Prospective cohort	66	60*	8.0*	39.0*
Yoo, H. et al[273]	2024	101	101	Child Pugh A INR APRI	Retrospective cohort	53	51	9.2	60.0*

Table 3-1: Characteristics of all studies included in review, subclassified by biomarker category

*Biomarkers that predicted decompensation are highlighted in green, versus those that were not significant predictors which are highlighted in red. Median values are indicated with an *. If information is not available, it is denoted with n/a.*

Most studies investigated multiple biomarkers. As explained in the methods, the number of biomarker studies and biomarker patients were also recorded with each study recorded multiple times depending on the number of biomarkers assessed and different cohorts studied. These collated results can be seen in Table 3-2. Based on biomarker studies, the most well-studied biomarkers were platelets (n=17), MELD (n=17) and albumin (n=16).

Type of marker	**Number of biomarker studies**	**Number of biomarker patients**
BLOOD MARKERS	*Total n = 113*	*Total n = 87,220*
Bacterial infection / translocation	2	354
Albumin	16	14,509
ALBI score (albumin, bilirubin)	2	502
ALBI-FIB4 score (albumin, bilirubin, age, AST, ALT)	3	5,008

CHESS-ALARM score (age, platelets, gender, LSM)	1	633
ABIDE model (AST/ALT, bilirubin, INR, T2DM, oesophageal varices)	1	543
EPOD score (albumin, platelets, bilirubin)	1	6,049
Fibrosis markers (ELF score [TIMP-1, PIIINP and hyaluronic acid], Serum hyaluronan, Collagen IV, Laminin)	4	1,629
Hematological markers (Von Willebrand factor, Hematological indices, Prekallikrein)	3	448
Renin, proBNP and copeptin	1	307
Serum ferritin	1	101
Serum miR-181b-5p	1	105
Platelets	17	15,003
AST/ALT	7	1,663
INR	9	7,344
Creatinine	2	5,291
ALP	1	91
Gamma GT	1	688
Bilirubin	8	12,801
Golgi protein-73	1	632
Insulin-like growth factor 1	1	148
Prognostic liver secretome signature (PLSec: [VCAM-1, IGFBP-7, gp130, matrilysin, IL-6, CCL-21, angiogenin and protein S])	1	122
CPS	11	6,683
MELD score	17	6,819
APRI score	1	101
HVPG	*Total n = 7*	*Total n = 1440*
HVPG	7	1,440
LIVER STIFFNESS	*Total n = 14*	*Total n = 4319*
Liver stiffness	14	4,319
PHYSIOLOGICAL	*Total n = 5*	*Total n = 984*
BMI	1	161
Mean Arterial Pressure	1	402

6-minute walk test	1	55
Liver frailty index	2	366
IMAGING	*Total n = 11*	*Total n = 3129*
VASCULAR:		
Thalium Shunt Index	1	209
Portal haemodynamics on Doppler ultrasonography	1	110
Hepquant-SHUNT test	1	35
Spleen volume-based non-invasive tool	2	816
NON-VASCULAR:		
Liver Surface Nodularity Measurement via CT	2	517
Liver-to-Spleen Volume Ratio via CT	1	1,027
201Tl heart-liver radioactivity uptake ratio	1	107
Subcutaneous Fat Density (via Analytic Morphomics® CT)	1	111
T2 mapping in gadoxetic acid-enhanced MRI	1	197
MISCELLANEOUS	*Total n = 5*	*Total n = 998*
Collagen proportionate area	2	219
Diabetes	1	457
Thick fibrous septa on liver biopsy specimens	1	168
Indocyanine green retention test	1	154
TOTAL	**155 biomarker studies.**	**98,090 biomarker patients**

Table 3-2: Summary of biomarker studies and biomarker patients subclassified by biomarker category

Biomarker categories

As agreed *a priori* the studies were split into 6 different biomarker categories depending on which was the primary biomarker focus of the study if multiple biomarkers were studied. The number of studies per category in decreasing

order was as follows; blood-based biomarkers (n=23), liver stiffness (n=12), imaging (n=11), HVPG (n=7), physiological markers (n=5) and miscellaneous (n=5). Whilst not all studies will be explored in this section, the biomarkers with the most evidence will be highlighted.

With regard to blood-based biomarkers, all 16 studies that assessed albumin determined that lower serum levels were significant predictors of decompensation. Indeed, two studies both reported a cut-off of <3.6g/dL.[220,261] Whilst platelets had the joint greatest number of studies (n=17), one study demonstrated that platelets do not predict decompensation, whilst four of the remaining studies demonstrated significance at the univariable level of analysis, but this was lost at the multivariable level.[211,234,238,239,241] Seven out of nine studies demonstrated that increased INR/prothrombin (PT) were significant predictors of decompensation, whilst all 8 bilirubin studies exhibited positive results with one study proposing a cut-off of >18 μmol/l.[220]. Finally, the aspartate aminotransferase (AST)/ alanine transaminase (ALT) ratio showed significant results in six out of seven studies, with contradictory results demonstrated for ALT.[233,261]

Nine different scoring systems were studied; ABIDE, ALBI, ALBI-FIB4, CHESS-ALARM, CPS, ELF, EPOD, MELD and VITRO. An explanation of what each score is composed of can be seen in Table 3-3. The most well-studied score was the MELD, with 13 out of 17 studies concluding that it is a significant predictor of decompensation with a threshold of ≥10 proposed in one study.[262] Whilst, the other scoring systems have fewer studies supporting

them, the majority of them are suggested to be superior to MELD in their respective analyses.

Scoring models	Constituents
ABIDE	INR, AST/ALT ratio, type 2 diabetes, presence of oesophageal varices, total bilirubin
ALBI	Bilirubin, albumin
ALBI-FIB4	ALBI score, AST, ALT, platelets
CHESS-ALARM	Age, gender, platelets, liver stiffness measurement (LSM)
CPS	Ascites, bilirubin, albumin, PT, and encephalopathy
ELF	Tissue inhibitor matrix metalloproteinase 1 (TIMP-1), procollagen type III aminoterminal peptide (PIIINP) and hyaluronic acid
EPOD	Platelet count, albumin, bilirubin concentration
MELD	Bilirubin, serum creatinine and INR
VITRO	Von Willebrand factor antigen, platelets

Table 3-3: Explanation of constituents of different scoring systems

With regard to liver stiffness, all studies demonstrated that increasing measurements can predict decompensation over varying time periods until 4 years. Various thresholds have been suggested ranging from ≥13kPA to ≥40kPA but most studies suggest cut-offs in the twenties.[237,245–250] With respect to other markers of fibrosis, both increased splenic stiffness (>54kPA) and increased ELF test results were demonstrated to be predictors of decompensation.[234,252]

All 11 imaging studies included demonstrated significant findings. With regards to imaging available in routine practice, a liver-spleen ratio <2.9, increased spleen size and increased liver surface nodularity all demonstrated positive findings.[258,259,262,264,268] With respect to routine vascular imaging available, portal haemodynamics on doppler ultrasonography also demonstrated significant predictive potential.[261]

With regards to HVPG measurements, all 7 studies demonstrated that increasing levels are associated with an increased risk of liver-related events. Thresholds of ≥12-16mmHg have been reported, as well as the protective effect of having HVPG <10mmHg which is associated with a 90% chance of being decompensation-free until 4 years.[235,236,239,240] Rincón et al also demonstrated a marginal improvement in a model combining HVPG and albumin versus HVPG alone (AUROC 0.727 versus 0.704).[238]

Finally, with regard to physiological parameters, obesity has been suggested to be associated with the highest risk of decompensation, followed by a moderate risk with overweight patients and the lowest risk among those with a normal body mass index (BMI).[253] The liver frailty index (LFI) has been demonstrated to independently predict decompensation as too does the six-minute walk test with a threshold of <401.8m.[255–257]

Quality assessment

Using the QUIPS framework 21 studies (34%) were assessed as having a low risk of bias, 26 (41%) moderate risk and 16 (25%) high risk as demonstrated in Table 3-4. With respect to biomarker categories, 5/23 (22%)

blood-based biomarker studies, 5/12 (42%) liver stiffness studies, 4/11 (36%) imaging studies, 1/7 HVPG (14%) and 0/5 (0%) physiological studies were deemed high risk. When observing the studies at high risk of bias, key areas of potential bias included weak prognostic factor measurement, a lack of multivariable analyses, a lack of accounting for confounding variables, and incomplete descriptions of subjects lost to follow-up, including if there were any important differences in those who completed studies compared to those who did not.

Study	Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome measurement	Study confounding	Statistical Analysis and Reporting	Overall risk of bias
Allen, A.M. et al., 2022	Low	Moderate	Moderate	Low	Low	Moderate	Moderate
Are, V.S. et al., 2020	Moderate	Low	Low	Low	Low	Low	Low
Asesio, N.et al., 2022	Low	Low	Low	Low	Low	Low	Low
Bajaj, J.S.et al., 2022	Moderate	Moderate	Low	Low	Low	Low	Moderate
Berzigotti, A. et al., 2011	Low	Low	Low	Low	Low	Low	Low
Berzigotti, A. et al., 2008	Low	Low	Low	Low	Low	Low	Low
Boonpiraks K. et al., 2024	Low	High	Low	Low	Moderate	Low	Moderate
Calvaruso, V. et al., 2015	Low	Low	Low	Low	Low	Low	Low
Calzadilla-Bertot, L. et al., 2021	Low	Moderate	Low	Low	Low	Low	Low
Chen, Q. et al., 2023	Low	Moderate	Low	Low	Low	Low	Low
Colecchia, A. et al., 2014	Low	Moderate	Low	Low	Low	Low	Low
Cordova, C. et al., 1986	High	Moderate	High	Low	High	High	High
Dillon, A. et al., 2018	Low	Moderate	Moderate	Low	High	Low	High
Elkassem, A.A. et al., 2022	Moderate	Moderate	Moderate	Moderate	Low	Low	Moderate
Fallahzadeh, M.A. et al., 2021	Low	Moderate	Low	Low	Low	Low	Low
Fujiwara, N. et al., 2022	Moderate	High	Low	Low	Low	Moderate	High

Garcia Garcia de Paredes, A. et al., 2021	Low	Low	Low	Low	Low	Moderate	Low
Gatselis, N.K. et al., 2020	Moderate	Moderate	Moderate	Moderate	Low	Low	Moderate
Gidener, T. et al., 2021	Low	Moderate	Low	Low	Low	Low	Low
Gidener, T. et al., 2022	Moderate	Moderate	Low	Low	Low	Low	Moderate
Gomez, E.V. et al., 2014	Low	Moderate	Low	Low	Low	Low	Low
Guéchot, J. et al., 2000	Moderate	Moderate	Low	Low	Low	Low	Moderate
Guha, I.N. et al., 2019	Low	Moderate	Moderate	Moderate	Low	Low	Moderate
Hartl, L. et al., 2021	Low	Moderate	Moderate	Low	Low	Low	Moderate
Henrique, D.M.N. et al., 2021	Low	Moderate	Low	Low	High	Low	Moderate
Hsu, C.Y. et al., 2021	Low	Moderate	Low	Low	High	Low	Moderate
Innes, H. et al., 2022	Low	Moderate	Low	Low	Low	Low	Low
Jain, D. et al., 2021	Low	Moderate	Low	Low	Low	Low	Low
Jindal, A. et al., 2020	Low	High	Low	Low	Low	Low	Moderate
Jindal, A. et al., 2022	Low	High	Low	Low	Low	Low	Moderate
Joly, J.G. et al., 1971	High	High	High	Moderate	High	Moderate	High
Kim, B.K. et al., 2012	Low	Moderate	Low	Low	Low	Low	Low
Kondo, T. et al., 2016	Low	High	Low	Low	Low	Low	Moderate
Kwon, J.H. et al., 2021	Low	Low	Low	Low	Low	Low	Low
Lee, M.H. et al., 2013	Low	High	Low	Low	Low	Low	Moderate
Lisotti, A. et al., 2016	Low	Moderate	Moderate	Low	High	Moderate	High
Merchante, N. et al., 2018	Low	High	Moderate	Moderate	High	Low	High
Merchante, N. et al., 2012	Low	High	Moderate	Moderate	Low	Low	High
Merchante, N. et al., 2017	Low	Moderate	Moderate	Moderate	High	Low	High
Merchante, N. et al., 2015	Low	Moderate	Moderate	Moderate	Low	Low	Moderate
Navadurong, H. et al., 2023	Low	Moderate	Low	Moderate	Moderate	Low	Moderate

Pérez-Latorre, L. et al., 2014	Low	High	Low	Low	Low	Low	Moderate
Qamar, A.A. et al., 2009	High	High	Moderate	Low	Low	Moderate	High
Rincón, D. et al., 2013	Low	Moderate	Moderate	Low	Low	Low	Moderate
Ripoll, C. et al., 2007	Low	Moderate	Low	Low	Low	Low	Low
Saeki, C. et al., 2023	Low	High	Low	Moderate	Moderate	Low	Moderate
Schneider, A.R.P. et al., 2022	Low	Moderate	Moderate	Moderate	Low	Low	Moderate
Schwarzer, R. et al., 2020	Low	High	Moderate	Moderate	Low	Low	High
Semmler, G. et al., 2022	Moderate	High	High	Moderate	High	Low	High
Siramolpiwat, S. et al., 2021	Low	Moderate	Low	Low	Low	Low	Low
Smith, A.D. et al., 2017	Moderate	Moderate	Low	Low	High	Low	High
Tae, H.-J. et al., 2014	Moderate	High	Low	Low	Low	Moderate	High
Tapper, E.B. et al., 2020	Moderate	Moderate	Low	Low	Low	High	High
Tornai, D. et al., 2021	Low	High	Moderate	Low	Low	Low	Moderate
Turco, L. et al., 2018	Low	Moderate	Low	Low	Low	Low	Low
Wang, J.H. et al., 2014	Low	Moderate	Low	Low	Low	Low	Low
Wang, S. et al., 2022	Low	Moderate	Low	Moderate	Low	Low	Moderate
Wong, Y.J. et al., 2022	Low	Moderate	Low	Moderate	Low	Low	Moderate
Yang, W. et al., 2021	Low	Moderate	Low	Low	Low	Low	Low
Yoo, H. et al., 2023	Low	Moderate	Low	Moderate	Low	Low	Moderate
Yuan, L. et al., 2019	Low	High	Low	Low	Low	Low	Moderate
Yu, Q. et al., 2022	Low	High	Moderate	Moderate	High	Low	High
Zarski, J.P. et al., 2020	Low	Moderate	High	Moderate	Low	Low	High

Table 3-4: Summary of quality assessment of studies using QUIPs framework

Meta-analysis

A meta-analysis was performed as demonstrated in Figure 3-3, with log transformation performed due to skewed data. Elevated INR was the strongest predictor of decompensation with a pooled effect size of 0.76, followed by decreased albumin with an effect size of -0.35. However, the majority of weighting in the pooled estimate was allocated to the platelet studies (92.9%). Furthermore, a high I^2 result of 96.3% was obtained suggesting significant statistical heterogeneity. A funnel plot was also generated as demonstrated in Figure 3-4 with an Egger's test ruling out significant publication bias (p=0.58). It was not possible to do a meta-analysis to predict decompensation at specific time points due to a lack of clear reporting of follow-up times in the included studies.

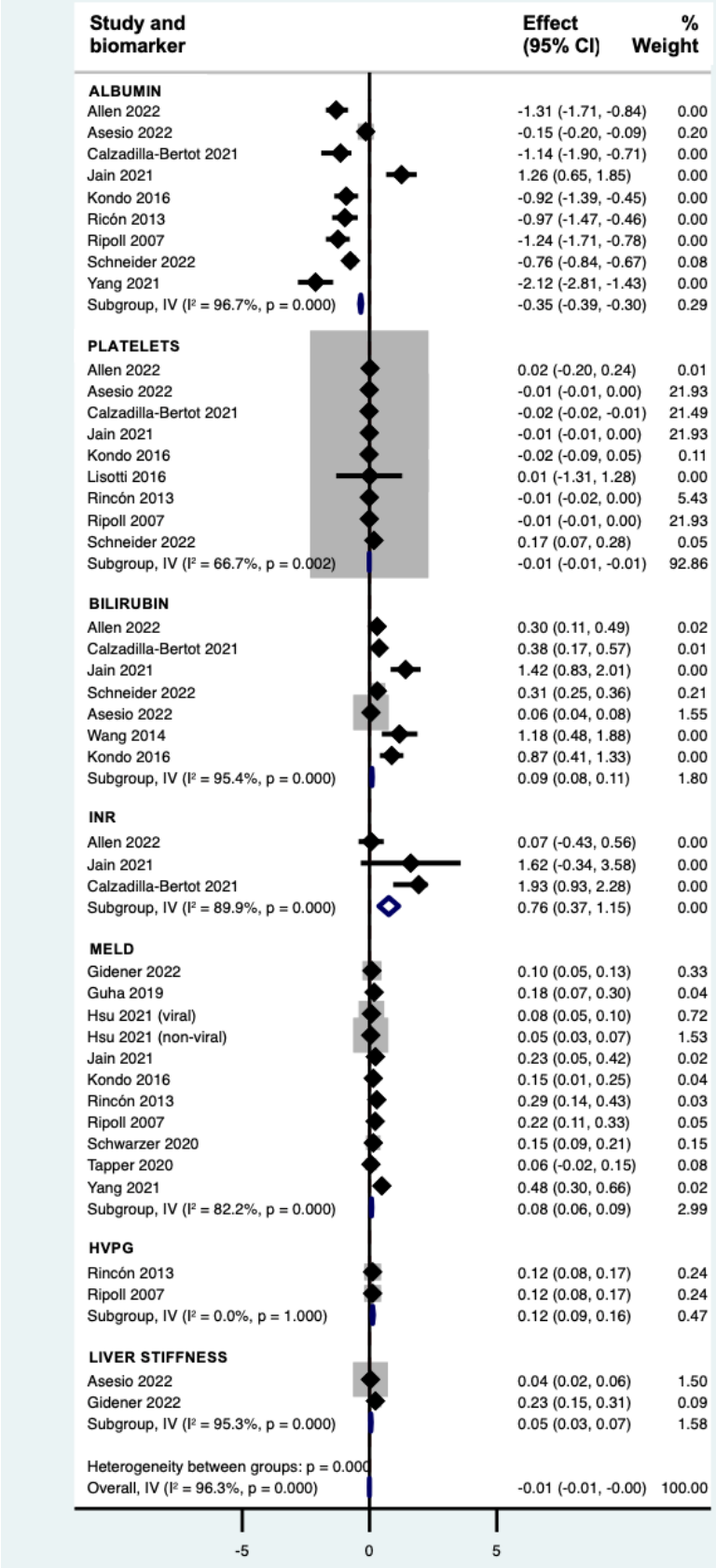


Figure 3-3: Forest plot for studies predicting decompensation categorised by biomarker (log-transformed).

The shaded boxes are proportional to the weighting of each study.

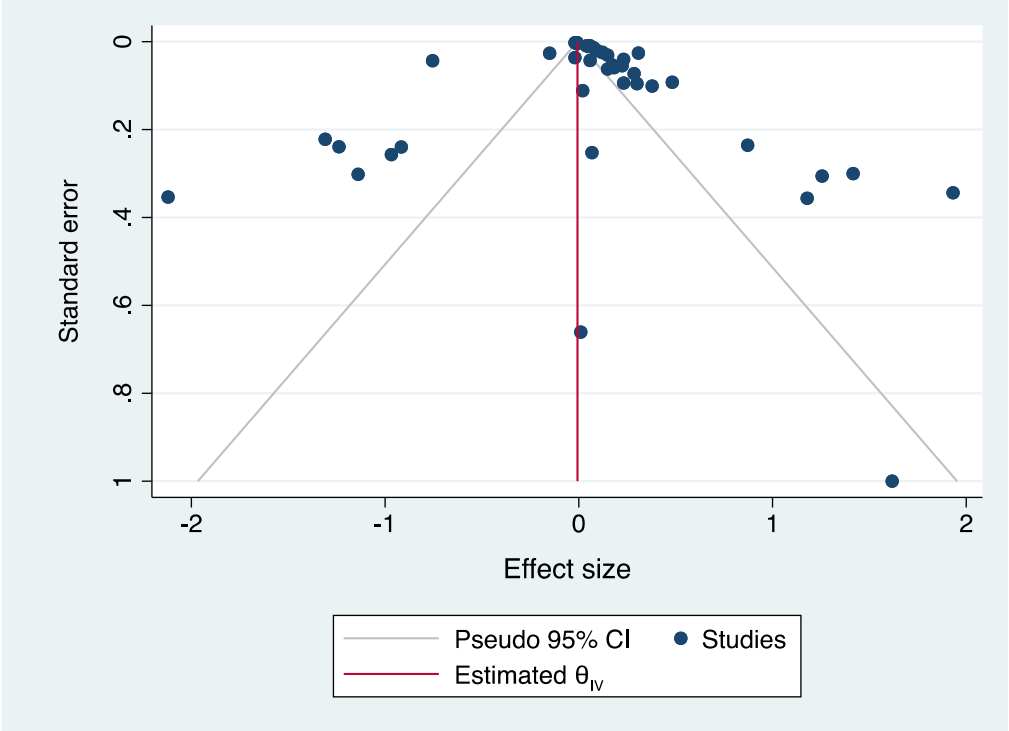


Figure 3-4: Funnel plot for studies predicting decompensation

3.4 Discussion

This systematic review and meta-analysis on biomarkers predicting future decompensation in patients with compensated cirrhosis has identified three important conclusions. Firstly, blood-based biomarkers and in particular platelets, MELD and albumin seem to be the most extensively researched. Secondly, based on the meta-analysis the strongest biomarker to predict decompensation is INR followed by albumin. Thirdly, high statistical heterogeneity in the meta-analysis and almost 25% of studies having a high risk of bias highlights the need for future studies to have robust and standardized methodology.

The fact that both an increasing INR and decreasing albumin are leading predictors of liver-related outcomes is not surprising given that they both reflect the synthetic function of the liver.[274] As liver disease severity increases there is impaired synthesis of clotting factors and albumin, hence the incorporation of both INR and albumin in the CPS in predicting cirrhosis mortality.[76] Indeed the utility of INR is further demonstrated by its incorporation into two further prognostic scoring systems for cirrhosis, the MELD and CLIF-C AD scores.[78,84] Whilst bilirubin did not exhibit as strong an effect size in the meta-analysis, all studies investigating bilirubin as a biomarker demonstrated positive results. Given that with worsening liver disease severity, there is increased synthesis and impaired clearance of bilirubin, it is logical that it has been incorporated into CPS and MELD scores as well.

Whilst the results of the meta-analysis are insightful, caution must be taken due to the high level of statistical heterogeneity (I^2 =96.3%) which is potentially due to inconsistent definitions of decompensation and varying patient populations. Whilst concerns have been raised over the validity of I^2 as a measure of statistical heterogeneity, these findings are supported by significant study bias as highlighted in the QUIPs assessment, as well as the funnel plot in Figure 3-4 suggesting potential methodological and clinical heterogeneity between studies.[275] A further comment regarding the meta-analysis is that the majority of the weighting was allocated to the platelet studies due to larger sample sizes and smaller confidence intervals. It is logical that worsening thrombocytopaenia would be a predictor of decompensation due to decreased hepatic thrombopoietin production and

increased sequestration of platelets within the spleen, with platelets being a surrogate marker of portal hypertension.[276] Indeed, this explains their incorporation into recent novel scoring systems (ALBI-FIB4, CHESS-ALARM, VITRO and EPOD). However, the pooled effect size of the platelets was small in our meta-analysis, with 1 negative study and 4 studies not showing it to be an independent predictor at the multivariable level. Finally, whilst the funnel plot suggested possible publication bias due to asymmetry, this was not confirmed by the Egger's regression test. This suggests that alternative factors such as issues with study methodology which may have exaggerated effect size, or alternatively true heterogeneity between the study populations may exist.

INR, albumin, bilirubin and platelets all demonstrate desirable qualities of a biomarker in terms of being biologically plausible, sensitive, validated, easy to measure, stable and inexpensive.[89] However, they lack specificity and are influenced by other co-morbidities, malnutrition, malabsorption, malignancy and medications.[274] Furthermore, it is unlikely that a single biomarker will suffice, but more likely a combination of biomarkers that target different pathophysiological mechanisms driving decompensation. It is this premise that has led to the evolution of different scoring systems. Whilst the MELD score has been the most well-studied and validated, there are limitations. There have been several modifications over time, including the addition of sodium as well as the latest version (MELD 3.0) incorporating gender and albumin.[79,80] However, despite these modifications, concerns still remain as patients with low scores are still at high risk of liver-related death, and it

seems to underestimate mortality in the sickest cohort of patients with acute on chronic liver failure.[82,83]

New scoring systems have emerged over recent years all demonstrating superiority over existing scores including the MELD score, albeit older versions of the score. Many of the scores are composed of liver function tests and markers of synthetic function already detailed in this discussion section in varying combinations. Other variables that have been included are the presence of type 2 diabetes and oesophageal varices as a marker of portal hypertension in the ABIDE score, and Von Willebrand Factor antigen in the VITRO score as a marker of endothelial dysfunction. Whilst these novel scores are promising, they have only been developed in recent years and require further validation to justify their use in predicting decompensation.

All HVPG studies in this review demonstrated statistically significant findings. This is not surprising given that portal hypertension is the most common haemodynamic abnormality caused by liver cirrhosis and is the main cause of decompensation. Currently, HVPG is the most accurate, reliable, and reproducible measure of portal hypertension.[4] Furthermore, compared to the blood-based biomarker category which had a significantly higher risk of bias, only one of the HVPG studies was deemed high risk. This emphasises the robustness of these studies and the reliability of their results, particularly as they are reproducible. Only 1 paper evaluated HVPG in combination with another biomarker, albumin, and this demonstrated only mild improvement.[238] However, despite its efficacy, HVPG is invasive, costly, and

can be hard to justify in clinically well patients with compensated cirrhosis given the risk of procedural complications.[229]

With a shift towards the development of non-invasive biomarkers, liver stiffness has grown in increasing popularity. Indeed, the recent Baveno guidelines have suggested a rule of 5 for liver stiffness by transient elastography (TE) [5-10-15-20-25kPA] should be used to denote progressively higher risks of decompensation regardless of the aetiology of liver disease.[4] Additionally, liver stiffness has also been incorporated in the novel CHESS-ALARM score. However, when focusing on the studies highlighted in this review, a large range of different cut-offs have been proposed. Furthermore, over 40% of studies exhibited a high risk of bias, so caution must be taken with their interpretation. Finally, questions remain over the best technique, whether that be ultrasound based such as TE or acoustic radiation force impulse (ARFI), which are cheaper but operator dependent, versus other techniques such as magnetic resonance elastography which are more time intensive and expensive, but potentially more accurate.[277]

The remaining categories of imaging, physiological and miscellaneous markers all displayed significant potential. However, their use in clinical practice is currently limited by the scarce number of studies with small sample sizes. The physiological markers highlighted in this review (body mass index, mean arterial pressure and liver frailty index) are non-invasive and easy to measure and crucially none exhibited a high risk of bias. Similarly, most of the imaging studies used ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) techniques, which

are already readily available in clinical practice. However, as per all imaging techniques, costs and time taken must be considered, as well as radiation exposure with CT imaging. Furthermore, some imaging studies, such as those involving nuclear medicine or advanced imaging techniques, are unlikely to be incorporated into clinical practice in the foreseeable future. Finally, the majority of the miscellaneous category also required liver histology, which is not likely to be indicated in most patients with compensated cirrhosis who are clinically well.

The main limitations of this review are that there was significant heterogeneity between the different studies. Firstly, the populations were heterogenous with some studies evaluating the risk of first decompensation, whilst others included patients who may have had previous decompensation; these cohorts are increasingly being recognized as two separate populations.[4] Secondly, this review was made more challenging by the evolving definition of decompensation over time. The most recent Baveno guidelines were the criteria used in this review, and they define decompensation by the development of ascites, HE, or variceal bleeding only. Crucially they have excluded jaundice which is in previous EASL guidelines, and infection which was used in previous large European multicentre cirrhosis trials (CANONIC and PREDICT) due to the theory that infection is not a true decompensating event itself, but rather a precipitant.[19–21] Therefore, some studies which used different definitions historically may not have been included in this review. Despite this, we feel this is a high-quality study which has a stringent methodology and has yielded important findings. In addition, two conceptually different types of decompensation

have recently been described; acute decompensation which occurs rapidly and tends to be associated with hospitalisation, and non-acute which occurs insidiously over months/years.[28] It is likely that future biomarker studies will need to study these populations separately.

In summary, whilst the novel biomarkers highlighted in this review have not yet clearly outperformed current scoring systems, we highlight key biomarkers to help guide future research. A single biomarker in isolation will not be the answer to this crucial unmet need. These scores will need to be composed of several components that target different pathophysiological pathways that drive decompensation including portal hypertension, systemic haemodynamics, systemic inflammation, metabolic dysfunction, and the microbiome. Indeed, whilst not the focus of this review, dynamic scores which can predict prognosis over time as well as response to therapies are not only desirable but should be actively pursued. It is also worth noting that the role of modifiable risk factors such as alcohol intake, smoking and diabetic control which have not been addressed here, would likely have significant impacts on the incidence of decompensation. Future research should explore mixed modality scores targeting non-modifiable as well as modifiable risk factors, although which exact combinations remain elusive currently. Furthermore, creating such a composite score will be a challenge to both develop and validate, and it is imperative that it is available to all to prevent inequity in healthcare, overcoming socioeconomic, rural and ethnic disparities. Crucially, given that different aetiologies exhibit substantial differences in the risk of decompensation, these tests must be tailored to individuals as opposed to using a ‘one size fits all’ model.[278]

Whilst creating individualized models with multiple components may seem unattainable, there is an increase acceptance across healthcare settings that this is what we must strive for. In order to achieve this, greater national and international collaboration is imperative, generating large data sets that can employ techniques such as machine learning, deep learning and artificial intelligence. Finally future biomarker studies should be conducted with rigorous methodology. The creation of a biomarker study checklist or guidelines would ensure that robust and comparable data is generated. Only then will we be able to successfully predict and hopefully prevent decompensation.

Chapter 4 : Evaluating the prognostic role of lipid abnormalities and fat mass in decompensated cirrhosis.

4.1 Background

Malnutrition has been estimated to be present in 20% of patients with compensated cirrhosis and between 60-90% of individuals with decompensated cirrhosis.[21,84,279] This is due to reduced dietary intake secondary to anorexigenic effects of inflammatory cytokines and malabsorption secondary to impacts on the gut through portal hypertension, resulting in increased lipolysis of fat and proteolysis of skeletal muscle.[58,59]. These effects lead to fat wasting and sarcopenia, named protein-energy malnutrition (PEM). The group of particular concern are those with decompensated cirrhosis where median survival reduces from 10 to 2 years compared to individuals who are compensated, and re-admission rates following acute decompensation are as high as 30-50%.[15,24,174]

PEM has been associated with increased mortality and as well as decompensation events in patients with liver cirrhosis.[64–66]. This effect is partly due to a loss of muscle mass which is termed sarcopaenia.[280] Sarcopaenia is a surrogate marker for severe malnutrition and a dominant component of frailty.[60] As well as muscle depletion, loss of fat mass is likely to be important, with evidence showing it may be protective against sarcopaenia as an alternative essential energy source.[62] Indeed, there is evidence to suggest that in earlier stages of cirrhosis it is predominantly fat

wasting that occurs, which may drive the muscle depletion in more advanced stages of the disease.[63]

The liver plays a crucial role in lipid regulation and homeostasis. It is involved in de novo lipogenesis, metabolism of lipids through beta oxidation, storage of lipids, transport of lipids through low density lipoprotein (LDL) to adipose tissue, as well as excretion of excess lipids in bile.[281] In cirrhosis there are pathological alterations in lipid synthesis, secretion and catabolism.[67] It has been demonstrated that both LDL and high-density lipoprotein (HDL) levels decrease with worsening liver disease severity.[282,283] HDL has been well studied and has clearly demonstrated a role in predicting future decompensation events, as well as mortality in both decompensated cirrhosis and ACLF.[284–287] It has also been shown that there is defective secretion of LDL as well as impaired function due to reduced apoprotein B-100 synthesis, which is a crucial protein component of LDL.[283,288] Despite this clear pathophysiological basis, the role of LDL as a prognostic biomarker in decompensated cirrhosis has not been thoroughly evaluated. Given LDL is inexpensive, easy to measure, stable and minimally invasive, it has many features of an ideal biomarker and therefore warrants further investigation.

Given the morbidity and mortality associated with malnutrition in liver cirrhosis, regular nutritional assessment is imperative. However, this is often resource heavy, time-consuming, and often difficult to prioritise in an overstretched healthcare system, especially in the outpatient setting. Dual-energy X-ray absorptiometry (DEXA) is the reference standard for nutritional assessment but is often unavailable, expensive and uses ionising radiation,

whereas anthropometric measures often underestimate malnutrition in this population.[289] Whilst blood-based markers such as LDL are important, the utility of remote monitoring has not been evaluated in this area and is in keeping the NHS long term plan to digitise health care and deliver sustainable healthcare into the community.[290] Bioelectrical Impedance Analysis (BIA) is a quick, convenient, and cost-effective way of measuring body composition and has demonstrated accuracy in the cirrhotic population.[289,291] BIA provides fat mass (FM) and fat-free tissue mass (FFM) and works on the principle that electrical conductivity is reduced in fat tissue due to increased resistance (impedance), in contrast to FFM which has much more rapid conduction.[292] A limitation of studies to date in cirrhosis is that they have only tended to assess BIA at a single time point.

In summary, the literature to date has suggested a potential prognostic and monitoring role for markers of fat and lipid metabolism in patients with decompensated cirrhosis which warrants further evaluation. In this chapter, through two sub-studies, I will aim to evaluate lipid derangement as well overall fat mass changes in patients following hospitalisation with AD. The specific aims of each sub-study are highlighted below.

LDL: sub-study 1

- To determine the ability of LDL to predict liver-related outcomes following AD, including infection, ACLF development, readmissions and 90-day mortality.

Fat mass: sub-study 2

- To investigate whether regular monitoring of fat remotely in the community is feasible following AD.
- To examine whether fat mass changes correlate with markers of malnutrition as well as liver disease severity.

4.2 Methods

LDL: sub-study 1

Study design and participants

Patients included in this study were participants in the PREDICT study.[20] This was a prospective, observational, multicentre European study with patients recruited between March 2017 to July 2018. Ethical approval was obtained by the institutional review board at each centre. The inclusion and exclusion criteria are detailed below.

Inclusion criteria

1. Participant age ≥ 18 years old.
2. Non-elective admission due to AD, defined by the development of ascites, HE, GI bleeding, and infection, or any combination (infection alone did not constitute an AD).
3. Cirrhosis was defined by a combination of standard clinical criteria, ultrasonographic and endoscopy findings, as well as histology.

Exclusion criteria

1. Patients with acute or subacute liver failure without underlying cirrhosis

2. Patients with cirrhosis who developed decompensation in the postoperative period following partial hepatectomy
3. Pregnancy
4. Evidence of current malignancy except for non-melanocytic skin cancer and hepatocellular carcinoma within Milan criteria
5. Presence or history of severe extra-hepatic diseases, e.g., chronic renal failure requiring haemodialysis, severe heart disease (NYHA > II), severe chronic pulmonary disease (GOLD > III), severe neurological and psychiatric disorders
6. HIV-positive patients
7. Previous liver or other organ transplantation
8. Admission/referral of more than 72 hours before inclusion
9. Patients who declined to participate, or who could not provide prior written informed consent and without a legal surrogate decision maker, and it appeared unlikely that the patient would regain consciousness or sufficient ability to provide delayed informed consent
10. Physician's denial (e.g. the investigator considered that the patient would not follow the protocol scheduled).

Data collected

Data collected included: patient demographics; aetiology of liver disease; type of decompensation event; medication history and blood test results at baseline and 7 days after enrolment. During admission, new events including new/ progressive decompensation and infection were also recorded 1 week after inclusion.

Patients were prospectively followed up for 3 months. Clinical data regarding readmissions as well as ACLF development were recorded. ACLF was defined as per the EF CLIF ACLF cirtiera.[293] Finally, data on liver transplantation and death was also collected at 3 months.

Statistical analysis

Data analysis was performed using STATA (StataCorp. 2019. Stata Statistical Software: Release 17. College Station, TX: StataCorp). Summary statistics were performed on patient demographics, aetiology of liver disease, medication history, disease severity scores and blood test results. A non-parametric assumption was used for all statistical tests. Any missing data were excluded from the analysis. A Pearson's chi-squared test was used to test for statistically significant differences in nominal or ordinal data. A Mann-Whitney U test was used to test for statistical significance in variables of continuous data. The correlation between variables was assessed by Spearman rank correlation. Univariable and multivariable regression analysis was also performed for outcome data.

Fat mass: sub-study 2

An analysis was conducted on patients being remotely monitored at the Royal Free Hospital between August-December 2020, with CirrhoCare, in partnership with CyberLiver Limited. 20 patients were monitored remotely for 12 weeks post hospital discharge for acute cirrhosis decompensation. This was the first ever study of digital, multi-modal monitoring, at home for management of advanced cirrhosis.[156] It involved monitoring a range of vital signs (sensor-technology), fluid balance (bioimpedance-scale) and higher

mental function (smartphone-app) in the patient's home, all key metrics perturbed in advanced cirrhosis. The data was uploaded in real-time securely to CyberLiver's secure cloud and platform. The actionable, decision-assisted, analytical algorithms, then suggested interventions on a clinician dashboard (assessing clinical outcomes), for community-based therapy.

BIA data was obtained using a Withing's bioimpedance scale. Participants were prompted via the CirrhoCare App to measure their weight and body composition at the same time every day, by standing in the centre of the scales in bare feet and minimal clothing. The scales provided measurements of total weight, fat mass, muscle mass, bone mass and hydration status. The average of all fat mass measurements taken during the 1st week of the study for each participant was calculated to provide an average week 1 fat mass. The same was done for week 8 to calculate a week 8 fat mass. Food intake data was collected through self-reporting. Patients entered the number of cooked meals and number of total meals they had daily. Alcohol intake during the study period, if applicable, was also recorded.

The Clinical Frailty Score (CFS) was used to assess frailty with a score of 5 or more being used to define frailty, which is consistent with the literature.[294,295] Sarcopaenia was assessed by the Skeletal Mass Index (SMI) from CT imaging either during admission or <3 months prior to admission.[296] SMI has been validated and gives a robust measure of whole-body muscle mass by measuring the cross-sectional area of muscle at the level of the third lumbar vertebrate, normalized to the patient's height. Sarcopaenia was

defined as per the American Association for the Study of Liver Diseases (AASLD) guidelines (SMI: men<50cm^2/m^2, women <39cm^2/m^2).[280]

Each participant had routine face-to face clinical assessments and blood tests performed at baseline and end of participation. Clinical examination was also undertaken including assessments of frailty and cirrhosis decompensation. Liver severity scores: CPS, MELD and CLIF-C AD were then calculated at baseline and follow-up.[76,78,84] Information regarding any clinically significant events or hospital re-admissions during the trial period were also obtained.

Data analysis was performed using IBM SPSS (Version 28) software. Summary statistics were performed on patient demographics, aetiology of liver disease, medication history, disease severity scores and blood test results. A non-parametric assumption was used for all statistical tests. A Pearson's chi-squared was used to test for statistically significant differences in nominal or ordinal data and the Mann-Whitney U test was used for continuous data. Spearman's test was used to assess for correlations.

4.3 Results

LDL: sub-study 1

Summary

232 patients were included in this study. A summary of their baseline and day 7 characteristics can be seen in Table 4-1. At baseline 157 patients (68%) had ascites, 75 (32%) had HE and 30 (13%) had GI bleeding. 85 (37%)

patients were diagnosed with alcohol related hepatitis either by either NIAAA criteria or histology, and only 8 patients had HVPG measurements performed.

By day 7, 20 (9%) of patients developed a new infection and 28 (12%) developed new/ progressive decompensation. During the 3-month follow-up period 32 (14%) patients developed ACLF, 62 (27%) had readmissions and 13 (6%) died.

Characteristics	Baseline	Day 7
Age*	58 (51-66)	
Male	154 (66%)	
White	226 (97%)	
Aetiology -Alcohol -MASH -MetALD -HCV -Cryptogenic -Other	 150 (65%) 15 (6%) 13 (6%) 13 (6%) 10 (4%) 31 (13%)	
Beta-blockers	107 (46%)	
Rifaximin	35 (15%)	
Diuretics	165 (71%)	
Statins	17 (7%)	
WBC (x10^9/L)*	6.1 (4.3-9.3)	5.7 (4.1-8.8)
CRP (mg/L)*	15 (6-37)	11 (5-24)
Platelets (x10^3/L)*	100 (64-163)	113 (76-183)
INR*	1.4 (1.2-1.6)	1.3 (1.2-1.5)
Albumin (g/dL)*	2.8 (2.5-3.3)	3.0 (2.6-3.3)
Bilirubin (mg/dL)*	2.2 (1.3-5.3)	1.8 (1.0-3.7)
AST (U/L)*	53 (33-91)	52 (35-78)
ALT (U/L)*	26 (18-42)	29 (20-43)
ALP (U/L)*	127 (89-185)	127 (97-183)
GGT (U/L)*	112 (48-289)	110 (55-271)
Creatinine (mg/dL)*	0.8 (0.7-1.1)	0.8 (0.7-1.0)
Sodium (mEq/L)*	136 (132-139)	136 (133-139)
HDL (mg/dL)*	22.7(12.7-32.0)	21.8 (12.7-34.0)
LDL (mg/dL)*	72.0 (50.2-96.5)	74.2 (48.3-95.0)
CLIF-C AD score*	50 (46-56)	48 (44-54)

Table 4-1: Characteristics of study cohort at baseline and day 7

**Median values provided with interquartile range*

Baseline and day 7

There was no difference in baseline LDL or HDL in those who were on statins versus those who were not (58.4 vs 72.0 mg/dL, p=0.463 and 25.6 vs 22.1, p=0.448, respectively). No significant correlations were noted between LDL and blood test parameters. In contrast, significant, although modest correlations were noted between baseline HDL and WBC (r= -0.205, p=0.014) as well as CRP (r= -0.307, p=0.002). The same findings were also noted for day 7 HDL and day 7 WBC (r= -0.322, p<0.001) and day 7 CRP (r = -0.357, p<0.001).

When assessing new events by day 7, baseline LDL showed a trend towards lower results in those who subsequently developed infection versus those that did not (57.0 vs 73.4 mg/dL, p=0.098). This trend was also maintained in univariable logistic regression (OR 0.98, 95% CI 0.96-1.00, p=0.097). In contrast, neither HDL nor LDL/HDL ratios demonstrated any predictive capacity.

Readmissions, ACLF development and 90-day mortality

When analysing readmissions in the 3-month follow-up period, LDL demonstrated its strongest signal with day 7 LDL levels being significantly lower in those who were readmitted versus those who were not (55.0 vs 78.6 mg/dL, p=0.003). Indeed, day 7 LDL remained an independent predictor of readmissions in both univariable and multivariable analysis as demonstrated in Table 4-2.

When addressing 90-day transplant free mortality there was a trend towards lower baseline LDL levels in those who died compared to those who survived (42.5 vs 75.8 mg/dL, p=0.058) and this was maintained in univariable logistic regression (OR 0.97, 95% CI 0.94-1.00, p=0.097). When looking at delta LDL changes between day 7 and baseline, there was a trend towards a reduction in those who died, compared to increased levels in those who survived (-12.3 vs 3.9 mg/dL, p=0.074). A similar trend was noted in delta LDL with regards to ACLF development with patients who developed ACLF having a median reduction of 7.7mg/dL compared to a gain of 3.8mg/dL in those that remained free from ACLF (p=0.053). No significant results were noted for either HDL alone or LDL/ HDL ratios when looking at outcome measures.

	Univariable analysis		***Multivariable analysis***	
	OR (95% CI)	*p value*	*OR (95% CI)*	*p value*
Age	***1.03 (1.01-1.07)***	***0.007***	*1.03 (0.99-1.07)*	*0.137*
Sex	*0.61 (0.32-1.16)*	*0.130*		
Beta blockers	*1.24 (0.69-2.21)*	*0.474*		
Statins	*2.04 (0.74-5.61)*	*0.169*		
Day 7 CRP	*1.00 (0.99-1.01)*	*0.981*		
Day 7 platelets	*1.00 (0.99-1.00)*	*0.092*		
Day 7 albumin	***0.53 (0.30-0.92)***	***0.024***	***0.50 (0.25-0.98)***	***0.044***
Day 7 CLIF-C AD	***1.08 (1.04- 1.13)***	***<0.001***	***1.09 (1.03-1.15)***	***0.003***
Day 7 HDL	*1.00 (0.98-1.02)*	*0.863*		

Day 7 LDL	***0.99 (0.98-1.00)***	***0.019***	***0.99 (0.97-1.00)***	***0.010***

Table 4-2: Univariable and multivariable logistic regression of variables predicting hospital readmissions

Fat mass: sub-study 2

15 patients were included for analysis with complete BIA data available for 8 weeks. Table 4-3 shows a summary of their baseline characteristics. All patients had ascites to varying degrees at recruitment with only 1 individual diagnosed with refractory ascites. 6/15 (40%) patients had low-grade HE at baseline (grade 1), with all individuals assessed as having the capacity to participate in the monitoring program by the investigating team.

Characteristic	Value
Age	63 (52-68) years
Male	11/15 (73%)
Alcohol aetiology of cirrhosis	12/15 (80%)
Frailty present	8/15 (53%)
CFS	5 (3-5)*
BMI	24.9 (22.7-26.6) kg/m^2*
CPS	8 (8-9)*
MELD score	12 (10-19)*
CLIF-C AD score	49 (46-58)*
CRP	8 (2– 19) mg/L*
WBC	5.3 (3.6-10.5) x10^9*
Urea	4.8 (2.7-6.4) mmol/L*
Creatinine	80 (60-99) umol/L*
Albumin	33 (30-35) g/L*
Week 1 Fat mass average	13.94 (10.6-17.1) kg*

Table 4-3: Baseline characteristics of participants

*Median values provided with interquartile ranges

Bioimpedance data

BIA data provided total body weight values which were the sum of fat mass, bone mass, and muscle mass with hydration status determined separately. As muscle mass trends directly mirrored hydration, and muscle is the only hydrous element of the three body compartments, it was concluded that the muscle mass component would be included within total body water. The values for muscle mass were therefore deemed unreliable as liver cirrhosis patients often have significant fluid accumulation, and therefore this was not analysed. Bone mass trends were also found to be influenced by trends in hydration and so analysis of trends in this component was not undertaken. Although bone is an anhydrous body compartment, it is a very small mass in comparison to hydration, and as such, readings may have been affected by large fluid shifts which do occur in this population. By contrast, fat mass trends did not mirror hydration trends and were, therefore, used for analysis.

Frailty

8/15 (53.3%) of patients were defined as frail. The median CFS score was 5 (IQR 5-5.5) in the frail group vs 3 (IQR 3-4) in the non-frail group. The body mass index (BMI) was similar across both groups (24.8 vs 25.1) with no significant differences in admission WBC, CRP Albumin, Urea and creatinine noted. The admission CLIF-C AD showed a trend towards being higher in the frail cohort compared to the non-frail group (53 vs 46, p=0.072). In addition,

3/15 patients had hospital re-admissions during the study period due to decompensation of cirrhosis and all these individuals were frail.

The average week 1 and week 8 fat masses were higher in the non-frail cohort in comparison to the frail cohort (17.1 vs 13.0kg, 17.9 vs 14.6kg respectively), although this was not statistically significant. There was no significant difference in change in fat mass over the study period for the two groups.

Sarcopaenia

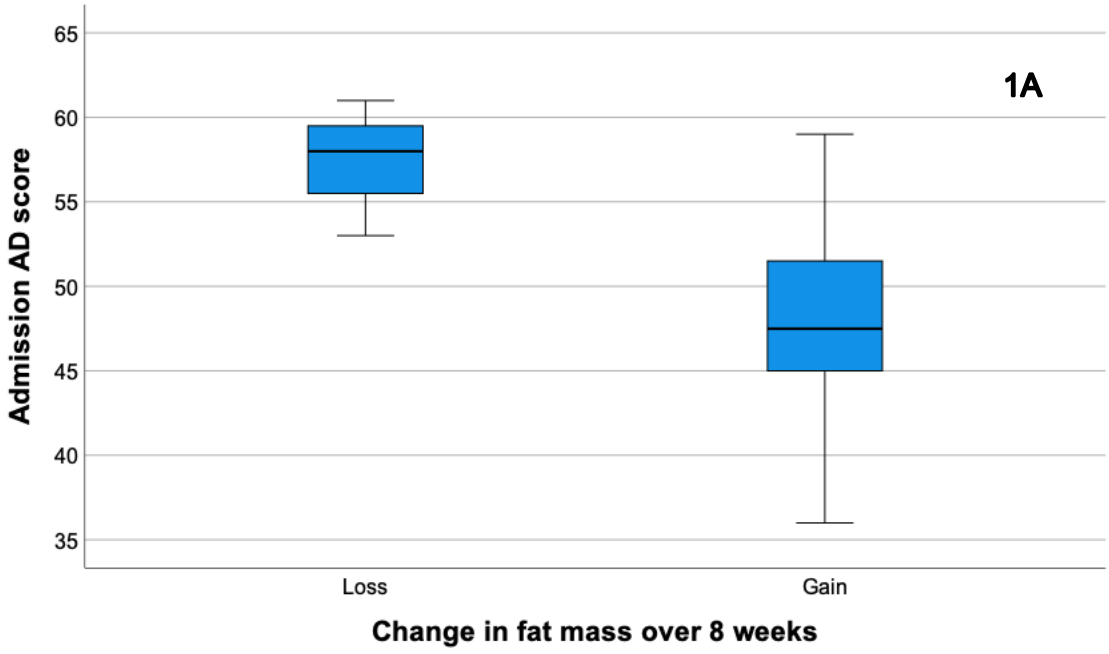
9/15 patients had CT imaging available for assessment of sarcopenia within the specified time frame. 6/9 (66.7%) patients had sarcopaenia based on the AASLD guidelines imaging criteria [280]. Baseline BMI was significantly lower in the sarcopaenic group vs non-sarcopaenic group (23 vs 28 kg/m^2, p=0.048). In addition, there was a trend towards a lower creatinine in the sarcopaenic group (74 vs 99 μmol/L, p=0.09). Whilst median CLIF-C AD score was higher in the sarcopaenic cohort, this was not statistically significant (53 vs 47). There were no other significant differences in baseline blood results and hospital re-admission rates between the two groups.

The average week 1 and week 8 fat masses were lower in the sarcopaenic group compared to the non-sarcopaenic group (12.5 vs 17.1kg, 13.8 vs 17.9kg respectively). The sarcopaenic cohort gained 1.44kg over the study period compared to 0.8kg in the non-sarcopaenic group which corresponded with a higher number of cooked meals (2.5 vs 1) and total meals (3 vs 2) per day. However, none of these changes were significant.

Fat mass changes

A comparative analysis was performed between those individuals that gained fat mass during the 8-week study period vs. those that lost fat mass (12 vs 3 participants). There was no significant difference between the number of meals and cooked meals per day between the two groups (2.5 vs 2 and 1.5 vs 2). In addition, no difference in frailty was noted across the two groups with the median CFS score being 5 in both groups. No significant correlations were noted between week 1, week 8 and change in fat mass in relation to admission blood test results, sex or liver disease severity scores.

However, of note, admission CLIF-C AD score and WBC were significantly higher in individuals who lost fat compared to those who gained fat (58 vs 48, p=0.048 and 11.2 vs 5.0 $x10^9$, p=0.031), which can be seen in Figure 4-1.



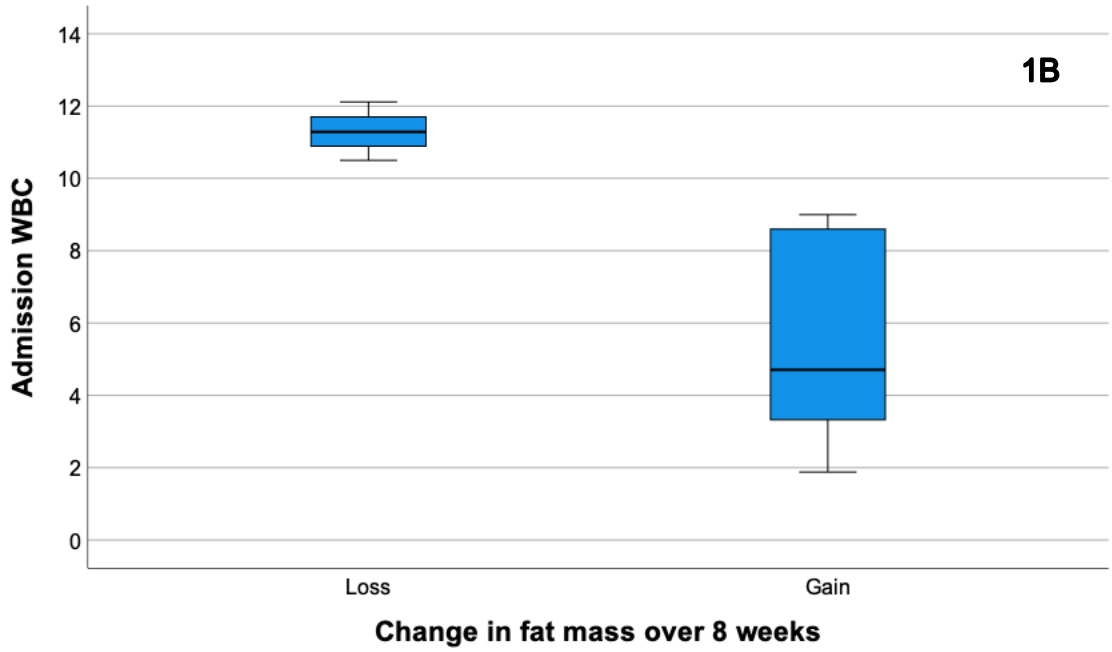


Figure 4-1: Graphs showing differences in admission CLIF-C AD scores (1A) and admission WBC (1B) in individuals who lost weight over 8 weeks versus those who gained weight.

4.4 Discussion

These two studies have highlighted the crucial role of lipid metabolism and fat homeostasis in patients following AD. Sub-study 1 has demonstrated that lipoproteins, and in particular low LDL, is a significant predictor of hospital readmissions. It may also provide a potential signal for other liver-related events such as infection, ACLF development and mortality, although this did not reach significance in this study. In contrast, sub-study 2 took a more macro approach looking at overall fat mass and demonstrated for the first time that fat mass could be monitored remotely and serially in patients with decompensated cirrhosis. Furthermore, a relationship between fat mass, systemic inflammation and liver disease severity was also demonstrated.

LDL: Sub-study 1

With regards to sub-study 1, the main positive finding was the ability of LDL to independently predict readmissions in both univariable and multivariable analysis. The PREDICT study revealed a cohort of patients following AD who were at high risk of readmissions and death, termed unstable decompensated cirrhosis (UDC).[20] The authors demonstrated that this group had exhibited significant features of portal hypertension as demonstrated by significant circulatory dysfunction, gastrointestinal bleeding and TIPS requirement. Whilst this study did not assess mechanistic pathways, as liver disease severity increases with corresponding worsening of hepatic synthetic function and portal hypertension, the decreased LDL likely occurs as a consequence of impaired apoprotein synthesis and LDL secretion.[283] Whilst statins have been demonstrated to lower portal hypertension in a number of studies as well as their known LDL lowering effects, it is worth noting that no difference in LDL was noted between those taking statins and those not in this study, although the numbers were small.[297–299]

Whilst the findings were not significant, the potential link to infection prediction as well as ACLF development and mortality are worth discussing. Whilst historically, portal hypertension was thought to be the predominant force behind decompensation and liver-related mortality, there is an increasing understanding of the synergistic role of systemic inflammation, as being the main driving force behind ACLF development.[20,51] LDL has been demonstrated to play a role in the removal of bacterial toxins, as well as lipopolysaccharides (LPS) from gram-negative bacteria and lipoteichoic acid from gram positive bacteria.[300] Indeed, in animal models, where artificially

high endogenous LDL levels are created by deleting the LDL receptor, protection against the effects of LPS and gram-negative infection were shown.[301] These findings are also consistent with human studies, with a large cohort study of almost 30,000 patients demonstrating that low LDL was associated with higher rates of sepsis.[302] Therefore, although we cannot conclusively determine this, we can hypothesise that lower LDL levels as a consequence of impaired hepatic function could reduce the protective effects on immune dysfunction and systemic inflammation, therefore increasing the risk of liver-related events. In this study sample size was likely an issue, with an insufficient number of events and an adequately powered study is warranted.

Fat mass: Sub-study 2

This study demonstrated that BIA data can be obtained accurately and remotely in the decompensated cirrhosis population over a prolonged time period. BIA has previously been studied in the cirrhotic population and has demonstrated that it could play an important role in nutritional assessment as well as correlating with mortality.[303–305] However, these studies have predominantly assessed FFM (bone, muscle, and total body water) and only at a single time point. This pilot study is the first to demonstrate the potential benefit of monitoring fat mass in this population and shows that it can be monitored accurately in the patient's home on a daily basis. Patients with decompensated cirrhosis frequently suffer from complications such as ascites and peripheral oedema, with significant fluid shifts following abdominal paracentesis. There is concern in the field about fluid retention affecting the accuracy of BIA results, although some studies have

demonstrated it can be used in patients with ascites.[303–306] Indeed, in this study muscle and bone mass tended to mirror hydration status and, therefore, were not analysed as they were felt to be inaccurate. By comparison, fat mass was not affected by hydration status, and therefore would seem to be the ideal component of BIA analysis to use in the decompensated cirrhosis population.

Frailty is a broad syndrome which involves decreased physiological reserve, resulting in increased vulnerability to health stressors and predisposes to adverse health outcomes.[307] In cirrhosis, there has been a tendency to focus on physical frailty as opposed to the traditional care-of the elderly definition, which is more of a global construct.[280] 53% of patients is this study were defined as frail which is higher that the 17-43% reported in the literature.[308,309] This is potentially because patients were recruited to this study following an admission with an acute decompensation of cirrhosis and, therefore, were likely to be deconditioned with more advanced disease. Indeed, all hospital re-admissions were in the frail cohort and there was a trend towards higher recruitment CLIF-AD scores in comparison to the non-frail individuals. This aligns with the literature which shows that frailty is associated with increased morbidity and cirrhosis complications.[310,311]

The proportion of sarcopaenic patients in the study is consistent with the literature which suggests an incidence of 30-70%.[61] Our results show that the sarcopaenic group had a significantly lower creatinine than the non-sarcopaenic group. This is not surprising given that skeletal muscle mass is the main determining factor of creatinine generation and therefore a low

muscle mass would lead to reduced creatinine levels.[312] Lower week 1 and week 8 average fat masses were also noted in the sarcopaenic group, although non-significant, which is potentially due to the small sample size. This is logical given the link between protein and lipid metabolism. Cirrhosis is a condition with impaired responses to fasting and accelerated starvation responses. Sarcopaenia results in reduced metabolic reserves and in order to preserve muscle, adipose tissue is metabolised preferentially.[313] Indeed, there is evidence that preventing fat wasting may be protective against sarcopaenia.[62] The lower combined muscle and fat mass in the sarcopaenic cohort would explain why this cohort had a significantly lower BMI than the non-sarcopenic group. We did not demonstrate any significant difference in weight change and meal intake over the study period in the sarcopaenic vs non-sarcopaenic cohort. Although we are not able to prove this, we theorise that regular monitoring did have a positive impact on patient behaviour including diet, but this would need to be explored in further prospective studies.

This study demonstrated that both admission WBC and CLIF-C AD score were significantly higher in those individuals that lost fat mass over the study period compared to those who gained it. This is not necessarily surprising as it is known that decompensated cirrhosis is associated with systemic inflammation and this progresses with liver disease severity.[20,21]. It is also known that that this hyperinflammatory state is an energetically expensive process.[68] This higher level of catabolism with increasing liver disease severity is associated with increased mobilisation and oxidation of fat substrates and higher levels of PEM.[69,70] It therefore follows that those with

more severe liver disease are more likely to lose fat mass over time. Whilst this theory is established in the literature, previous studies have tended to assess fat mass at a single time point. This seems to be the first study to demonstrate this relationship in the decompensated cirrhosis population with regular fat mass monitoring in the community, and suggests this could be used as a biomarker, to assess disease stability or/and response to management.

Limitations

The main limitations of both studies were the sample sizes and that they may have lacked power to detect some clinically significant findings. With regards to the lipid study, further assessment of other components of the lipoprotein pathway or even lipidomics may have provided more insights into the pathophysiology underlying the findings. Similarly, with regards to the fat mass study, it would have also been advantageous to have another formal nutritional assessment at baseline, such as anthropometry or DEXA scan for comparison, albeit these were not within the CirrhoCare study protocol, from which the data for analysis was derived.

Conclusions

In conclusion, these two studies highlight the importance of fat and lipid metabolism following AD and how they can provide importance prognostic information. Indeed, there is even the potential for these biomarkers to be used collaboratively. As demonstrated in sub-study 1, LDL measurements during hospitalisation with AD can help predict liver-related events following discharge. LDL demonstrates biological plausibility with decreased levels

linked to hepatic dysfunction and disease severity, as well as exhibiting a protective role in immune dysfunction and systemic inflammation. Furthermore, the low cost, reproducibility and widespread availability makes this an ideal screening biomarker. This could theoretically flag high risk patients who would then warrant remote monitoring of fat mass and nutritional reserve in the community. This study highlighted the importance of fat mass given its association with severity of acute decompensation as well as systemic inflammation. BIA is ideal to monitor fat, as it is safe, rapid, and requires little to no training and can be repeated. Further prospective studies are required to validate both of these biomarkers to help prevent malnutrition and complications in the cirrhosis population who are vulnerable and at high risk of morbidity and mortality.

Chapter 5 : CL-ART: a novel smartphone application that can help predict future hospitalisation secondary to cirrhosis acute decompensation.

5.1 Background

Liver disease is a globally leading cause of morbidity and mortality. The subset of particular concern are individuals with AD of cirrhosis, which is associated with a high risk of deterioration and hospitalisation.[4,21,84] The short-term readmission rates following hospitalisation with AD are between 30-50%, with a significant burden on patients and carers as well as deleterious effects on quality of life. [23,24,314] HE is the most serious and prevalent complication of liver cirrhosis, occurring in up to 30-40% of patients.[315,316] Onset of OHE, characterised by disorientation, lethargy or asterixis (grade 2) is associated with a poor prognosis, with 1-year mortality rates of over 60% which is higher than any other decompensating event.[317,318] Furthermore, following an episode of OHE, there is a significant reduction in health-related quality of life, increased risk of hospitalisation, as well as a 40% chance of HE recurrence within 1 year.[319–321]

Being able to determine one's risk of future decompensation, and particularly OHE, would enable closer monitoring, lifestyle modification, earlier treatment and perhaps the possibility of preventing associated complications such as falls and motor vehicle accidents.[322] Many cognitive tests have been developed to detect HE, including subtle cognitive deficits, termed covert HE (CHE), which is the combination of minimal hepatic encephalopathy (MHE) and grade 1 HE, as it is a strong risk factor for OHE development.[323] These

include pencil-and-paper tests, such as the Psychometric Hepatic Encephalopathy Score (PHES), electroencephalography (EEG) and critical flicker frequency (CFF). However, these tests often require resources which are not widely available, or in the case of neurocognitive tests are time-consuming, show significant variability in their diagnostic capabilities, and crucially have not been validated in predicting future liver-related events.[324,325]

Innovative solutions are required, and in this regard, digital healthcare could help reduce the inequality in management as well as improve associated morbidity and mortality. Indeed, digitising care and incorporating technology is in keeping with governmental policy in the UK with the NHS Transformation Directorate, and the Department of Health and Human Services in the United States (US).[290] The most well-known example of this is the EncephalApp, which a smartphone-based test which has been validated for the diagnosis of MHE.[151,152] The limitations of this test are that it conventionally took 10 minutes to complete, although a shorter version has now been validated, and it is not suitable for colour-blind individuals.[165]

The CL-ART is a novel smartphone App that was developed for the CirrhoCare program.[156] With its rapid testing (<30 seconds) and high useability feedback from the pilot study, this test could be a valuable new tool in cirrhosis management. This study aims to compare the performance of CL-ART with other established cognitive tests and investigate its ability to predict future HE as well as other cirrhosis decompensation events.

5.2 Methods

We conducted a multi-centre prospective study evaluating cognitive function in patients with liver cirrhosis at the Royal Free Hospital in London and Aarhus University Hospital in Denmark. Patients were recruited from the outpatient clinic, day-case unit and hepatology wards between October 2021 and November 2022. Data was collected using medical notes, laboratory, radiology and histology reports, clinic letters and discharge summaries. In addition, a cohort of healthy controls were also recruited.

Inclusion criteria for patients

1. Men and women of age ≥ 18 years old.
2. Cirrhosis defined by standard clinical criteria, ultrasonographic findings and/or histology. Cirrhosis of any aetiology was included. However, patients with cirrhosis due to autoimmune hepatitis had to be on a stable corticosteroid dose for ≥3-month period before study inclusion.
3. Subjects able to give informed consent.

Exclusion criteria for patients

1. Subjects with ACLF according to the criteria published by *Moreau et al*, Gastro 2013.[21]
2. Subjects with active bacterial or fungal infection who had received less than 24 hours of appropriate antibiotic/ antifungal treatment.
3. Subjects with active or recent gastrointestinal bleeding (unless controlled for >48 hours).

4. Current overt hepatic encephalopathy, defined as grade II-IV hepatic encephalopathy according to the West-Haven classification.[326]
5. Conditions that could impact cognitive function:
    - Active hepatitis C which has not been treated
    - Established neurological disorders
    - Subject undergoing active alcohol withdrawal treatment
    - Subject intoxicated or under the influence of illicit drugs as per clinician assessment
    - Treatment with antipsychotics or other psychotropic drugs with sedative effects
6. Subjects with active hepatocellular carcinoma or a history of hepatocellular carcinoma that was in remission for less than six months for uninodular HCC, or for less than 12 months for multinodular HCC within Milan criteria.
7. Subjects with a history of significant extrahepatic disease with impaired short-term prognosis, including:
    i) Congestive heart failure New York Heart Association Grade III/IV
    ii) COPD GOLD >2
    iii) Chronic kidney disease with serum creatinine >2mg/dL or under renal replacement therapy.
8. Subjects with current extrahepatic malignancies including solid tumours and hematologic disorders.
9. Subjects with mental incapacity, language barrier, or any other reason considered by the investigator precluding adequate understanding, cooperation, or compliance in the study.

10. Refusal or inability to give informed consent.

Baseline assessments

Data collected at baseline included patient demographics, aetiology of liver disease, history of previous decompensation events, comorbidities, and medication history. Routine blood tests were taken including renal profile, liver function tests, coagulation profile, full blood count, albumin, and venous ammonia. The following liver disease severity scores were also calculated; MELD Na, CPS and CLIF-C AD score.[76,78,84]

Each participant was asked to perform three cognitive tests in single sitting as detailed below and demonstrated in Figure 5-1.

1.EncephalApp Stroop Test (approximately 10 minutes to complete)

This test was chosen as it is the most well-studied App-based test in the cirrhosis literature and has been extensively validated in the diagnosis of early HE. [151,152] Participants performed the test on a smartphone provided by the research team. The test presents users with a series of runs where they have to identify the colour of the printed text.

2.CyberLiver Animal Recognition Test (CL-ART) (less than 30 seconds to complete)

Another App based test performed on a smartphone as detailed above. The test involves recognising and naming animals appropriately.[156]

3. Psychometric Hepatic Encephalopathy Score (PHES) (approximately 20 minutes to complete)

The final test was selected as it is the current 'gold standard' pencil-and-paper test battery for the evaluation of patients with liver cirrhosis for hepatic encephalopathy. The test is made up of 5 individual components.[326]

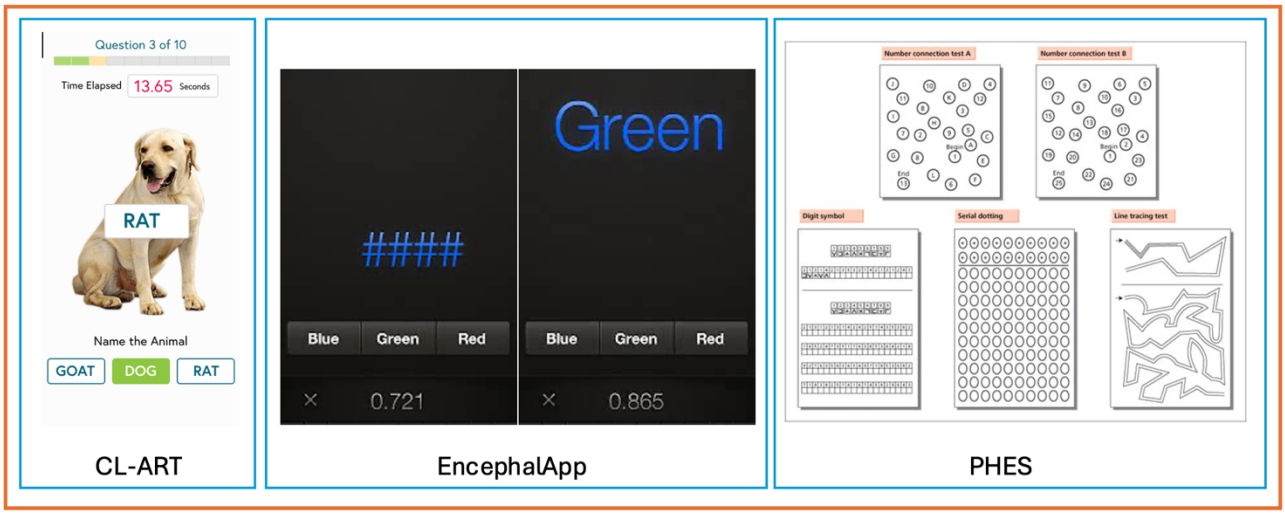


Figure 5-1: Images of the three cognitive tests performed

On completion of testing, participants were requested to complete a useability questionnaire for each of the three tests on the provided smartphone. The data (compliant with General Data Protection Regulation [GDPR] legislation) was stored on a secure CyberLiver Cloud and ISO13485:2016-certified, CE marked, platform as per the CirrhoCare study.[156]

Follow up

No further visits or assessments were scheduled after the baseline visit. However, patients were followed up for 6 months to acquire clinical follow-up data. The data collected included mortality, outpatient covert episodes of HE, acute decompensation events requiring hospital admission, newly prescribed rifaximin, TIPS insertion and orthotopic liver transplantation (OLT).

Ethical and regulatory approval

All individuals provided written informed consent and the study was approved by the London – Brighton & Sussex Research Ethics Committee (IRAS ID 285666; REC number 20/HRA/3843; NCT05045924) in accordance with the Declaration of Helsinki.

Statistical analysis

A sample size of 105 patients was determined based on a 20% incidence of OHE, a 5% 2-sided alpha, 90% power and 10% dropout rate for follow-up. Data analysis was performed using STATA (StataCorp. 2019. Stata Statistical Software: Release 17. College Station, TX: StataCorp). Summary statistics were performed on patient demographics, aetiology of liver disease, comorbidities, disease severity scores and blood test results. A non-parametric assumption was used for all statistical tests. Any missing data were excluded from the analysis. A Pearson's chi-squared test was used to test for statistically significant differences in nominal or ordinal data. A Mann-Whitney U test was used to test for statistical significance in variables of continuous data, and the Kruskal-Wallis test was used when three or more groups were analysed. The correlation between variables was assessed by Spearman rank correlation. AUROC curves were calculated to determine the

diagnostic ability of tests to predict future decompensation events and the Youden index was used to determine optimal thresholds. Regression analysis was performed for outcome data.

5.3 Results

Summary statistics

A total of 105 patients and 48 healthy controls were recruited to the study. With regards to baseline characteristics, there was a higher percentage of males in the patient cohort compared to the controls (68% vs 58%, p=0.265), with similar median ages (58 vs 56, p=0.853). The control cohort had a greater median number of years of education (17 vs 13, p<0.001). Whilst, both CLART and PHES did not significantly correlate with the level of education, EncephalApp test score results were influenced by education (r= -0.282, p=0.004).

The median time taken to complete all 3 cognitive tests was significantly shorter in the controls compared to cirrhosis patients; CL-ART (15.38 vs 23.59s, p<0.001), EncephalApp (148.19 vs 210.78s, p<0.001) and total PHES score (0 vs -3, p<0.001).

Baseline characteristics for the patients can be seen in Table 5-1. 84 patients (80%) were decompensated at recruitment with 40 (38%) having a previous episode of HE. There was no significant difference in any cognitive score between decompensated and compensated individuals. 12 patients (18%) had an EEG within 1 month of recruitment, of which two suggested a degree

of HE. There was no statistical difference in the time taken to complete all three cognitive tests between the compensated and decompensated groups.

Within the 6-month period, 36 patients (34%) had admissions with AD with a range of 1-5 admissions. 12 individuals (11%) had admissions primarily due to HE and 14 patients (13%) had admissions with AD, where the primary reason for admission was non-HE related, but they also developed HE. 20 (19%) individuals experienced outpatient episodes of non-overt HE in the community. 6 patients underwent liver transplantation during follow-up and 19 patients died, of which 15 (14%) were liver-related, and of which two were an unknown cause.

Characteristic	Total (n=105)	No future admission with HE (n=93)	Future admission with HE (n=12)	p value
Age	58 (50-63)	64 (56-67)	57 (49-62)	0.062
Male	71 (68%)	62 (67%)	9 (75%)	0.562
Ethnicity - White - Asian - Eastern European - Other	 74 (70%) 11 (10%) 8 (8%) 12 (12%)	 64 (69%) 11 (12%) 7 (7%) 11 (12%)	 10 (84%) 0 (0%) 1 (8%) 1 (8%)	0.556
Education (years)	13 (11-16)	13 (11-16)	13 (10-15)	0.235
Aetiology - Alcohol - MASLD - Autoimmune - Other	 69 (66%) 10 (9%) 7 (7%) 19 (18%)	 61 (66%) 9 (10%) 7 (8%) 16 (17%)	 8 (67%) 1 (8%) 0 (0%) 3 (25%)	**0.050**
Previous HE	40 (38%)	32 (34%)	8 (67%)	**0.030**
Decompensated at onset	84 (80%)	75 (81%)	9 (75%)	0.645
Diabetes	17 (16%)	14 (15%)	3 (25%)	0.379
Cardiac history	21 (20%)	17 (18%)	4 (33%)	0.220
Respiratory history	6 (6%)	4 (4%)	2 (17%)	0.082
CKD	8 (8%)	6 (6%)	2 (17%)	0.209
Antibiotic prophylaxis	9 (9%)	9 (10%)	0 (0%)	0.260
Lactulose prescription	56 (53%)	46 (49%)	10 (83%)	**0.027**
Rifaximin prescription	31 (30%)	25 (27%)	6 (50%)	0.098

Beta blocker prescription	27 (26%)	24 (26%)	3 (25%)	0.952
Sodium (mmol/L)	136 (133-139)	136 (133-139)	138 (134-140)	0.322
Bilirubin (μmol/L)	30 (16-61)	30 (16-62)	32 (23-44)	0.669
Albumin (g/L)	33 (29-37)	33 (29-37)	31 (29-36)	0.687
INR	1.3 (1.2-1.5)	1.3 (1.2-1.5)	1.3 (1.2-1.6)	0.486
Platelets ($x10^9$/L)	115 (74-178)	120 (75-181)	88 (67-122)	0.203
CRP (mg/L)	8 (4-20)	9 (4-21)	5 (4-13)	0.783
WBC ($x10^9$/L)	6.1 (4.4-7.9)	6.2 (4.4-7.8)	5.1 (3.5-8.7)	0.481
Creatinine (μmol/L)	70 (58-98)	69 (58-96)	75 (61-104)	0.381
ALT (unit/L)	34 (24-48)	35 (24-57)	32 (23-37)	0.331
AST (unit/L)	57 (42-83)	59 (42-86)	50 (41-59)	0.291
Ammonia (μmol/L)	41 (30-64)	39 (29-60)	75 (58-99)	**<0.001**
MELD Na	16 (11-19)	15 (11-19)	16 (15-20)	0.333
CPS	8 (7-10)	8 (7-10)	9 (7-10)	0.964
CLIF-C AD	48 (44-53)	48 (43-52)	50 (47-53)	0.240
CL-ART (seconds)	23.59 (19.35-28.29)	22.70 (19.00-27.04)	31.49 (27.13-43.02)	**<0.001**
EncephalApp (seconds)	210.78 (180.59-277.04)	205.24 (179.33-257.86)	287.36 (264.89-315.51)	**<0.001**
PHES total	3 (-7 to -1)	-3 (-6 to -1)	-8 (-10 to -6)	**0.002**

Table 5-1: Baseline characteristics of the total cohort, as well as baseline characteristics of individuals when split into those admitted due to HE during follow-up compared to those that were not hospitalised.

The p values reflect statistical analysis of those with HE-related admissions versus those who were admission-free. Median values are shown with IQRs.

Cognitive testing in diagnosis of MHE

A good correlation was demonstrated between the CL-ART and EncephalApp (r=0.816, p<0.001) and CL-ART and PHES (r= -0.652, p<0.001) as demonstrated in Figure 5-2. One patient could not complete the EncephalApp due to being colour-blind. An analysis was performed to determine the ability of the cognitive tests to diagnose early HE, termed MHE. The PHES test was used as a gold standard with a PHES core of fewer than -4 points being used as the threshold for MHE.[327] Using this cut-off, 40 patients (38%) had a diagnosis of MHE. The median CL-ART score was significantly higher in those with MHE compared to those without HE (29.35 vs 20.96s, p=<0.001), as was true for EncephalApp (277.95 vs 189.91s, p<0.001) and ammonia levels (54 vs 37μmol/L, p=0.07). The CL-ART demonstrated a high ability to diagnose MHE with an AUROC of 0.85 (95% CI 0.78-0.93), compared to EncephalApp (0.86, 95% 0.78-0.93) and ammonia (0.64, 95% CI 0.53-0.76).

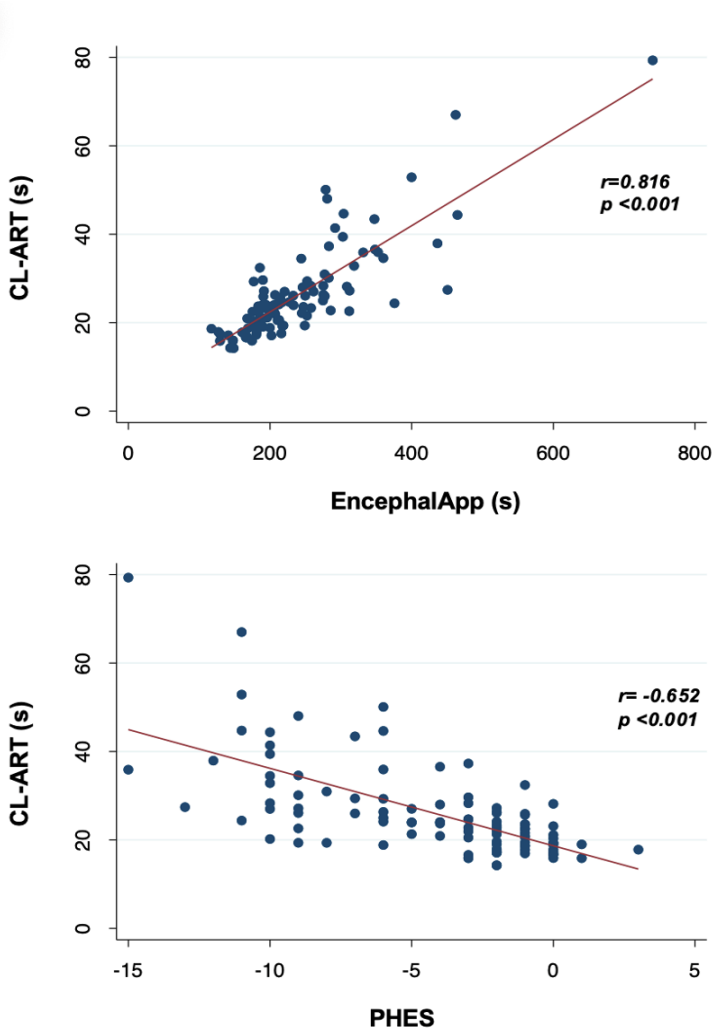


Figure 5-2: Correlation analysis between CL-ART and EncephalApp (top) and CL-ART and PHES (bottom)

Cognitive testing and future decompensation events

Analysis was performed on the performance of cognitive testing in those individuals who developed outpatient CHE episodes within the 6-month follow-up period in the community. Baseline CL-ART results were significantly higher in those who developed future outpatient HE episodes compared to those who did not (30.59 vs 22.99s, p=0.008). The same was observed for EncephalApp (275.32 vs 203.66, p=0.007) and ammonia (64 vs 39 μmol/L, p=0.02), with no statistical difference in PHES noted.

When looking at all cause decompensation, CL-ART was significantly higher in individuals hospitalised due to any AD during follow-up compared to those who were not admitted (27.1 vs 21.3, p<0.001). This was also true for EncephalApp (276.36 vs 192.88, p<0.001), PHES (-6 vs -2, p=0.002) and ammonia (58 vs 37 μmol/L, p=0.007). CL-ART and EncephalApp demonstrated a good ability to predict future AD-related hospitalisations, both with an AUROC of 0.75. The performance of PHES and ammonia were slightly inferior with an AUROC of 0.68 and 0.67 respectively.

The baseline characteristics of patients who were admitted due to HE during follow-up versus those that were not can be seen in Table 5-1. It can be seen that in those who had subsequent admissions, a higher proportion of patients had previous episodes of HE and were on lactulose. The baseline cognitive tests and ammonia were also noted to be significantly higher in this cohort. In terms of predicting future admissions primarily due to HE, the CL-ART demonstrated the strongest AUROC (0.84, 95% CI 0.76-0.92), followed by EncephalApp (0.82 95% CI 0.73-0.91), ammonia (0.81, 95% CI 0.71-0.92) and then the PHES (0.77, 95% CI 0.65 – 0.89). Using the Youden index the optimal cut-off to predict future HE-admissions was 26 seconds with a sensitivity of 92% and specificity of 70%. The predictive value of using a threshold CL-ART score of 26s is demonstrated in Figure 5-3, with a significantly higher incidence of HE-related admissions seen with a baseline score of ≥ 26s. Indeed, the CL-ART remained an independent predictor of future admissions due to HE in both univariable and multivariable analysis (Table 5-2). When creating alternative multivariable models substituting CL-

ART for either EncephalApp or PHES, neither of the other cognitive tests maintained significance.

23 patients (22%) were admitted with AD during follow-up, either primarily due to HE, or due to another cause but developed HE. Baseline CL-ART, EncephalApp, PHES and ammonia scores were also significantly higher in this cohort compared to the non-hospitalised group with strong predictive AUROCs (0.77, 0.78, 0.71 and 0.77).

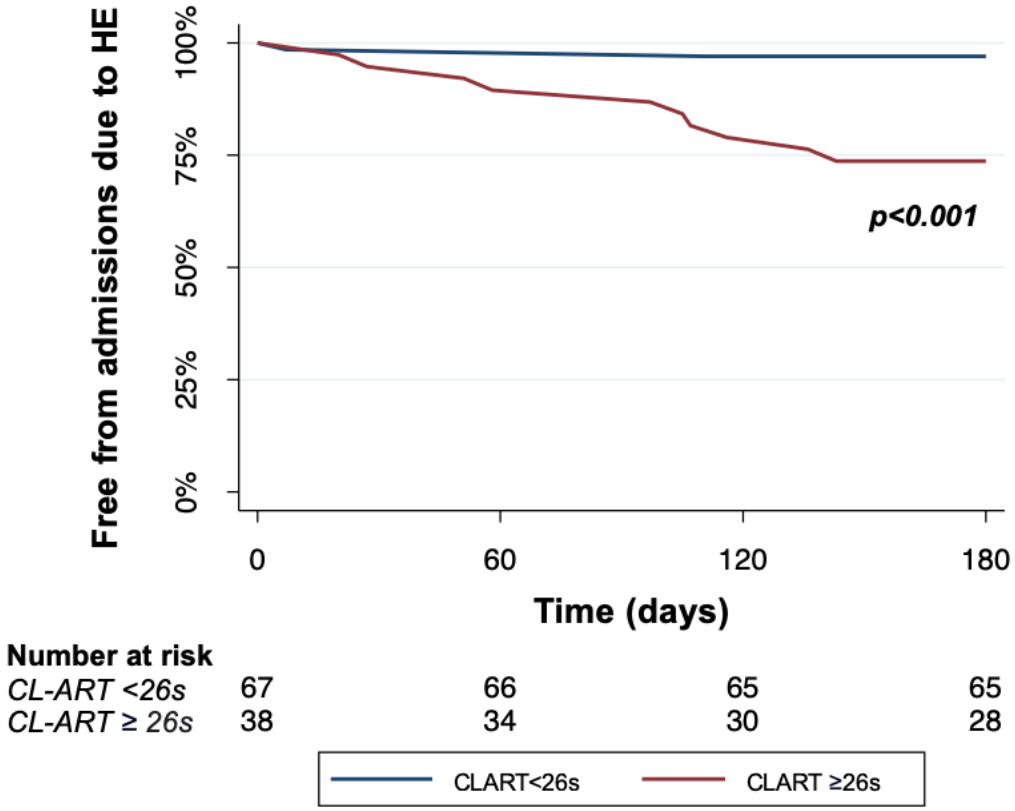


Figure 5-3: Kaplan Meier curve demonstrating the incidence of HE-related admissions during follow-up when split into two cohorts, using a CL-ART threshold of 26s

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Sex	1.50 (0.38-5.94)	0.564		
Age	1.05 (0.98-1.12)	0.136		
Diabetes	1.88 (0.45-7.82)	0.385		
Rifaximin	2.72 (0.80-9.22)	0.108		
Beta blockers	0.95 (0.24-3.84)	0.952		
Previous HE	**3.81 (1.07-13.63)**	**0.040**	1.47 (0.33-6.66)	0.613
Sodium	1.07 (0.93-1.25)	0.305		
Bilirubin	1.00 (0.99-1.01)	0.818		
Albumin	0.99 (0.91-1.10)	0.996		
WBC	0.94 (0.76-1.16)	0.541		
CRP	1.00 (0.96-1.03)	0.902		
Creatinine	1.00 (0.99-1.01)	0.365		
Ammonia	**1.03 (1.01-1.05)**	**0.001**	**1.03 (1.01-1.06)**	**0.006**
EncephalApp	**1.01 (1.00-1.01)**	**0.045**		
PHES	**0.81 (0.70-0.95)**	**0.007**		
CL-ART	**1.07 (1.02-1.12)**	**0.009**	**1.07 (1.01-1.13)**	**0.030**

Table 5-2: Univariable and multivariable analysis of factors predicting future admissions during follow-up due to HE

Mortality

CL-ART demonstrated an ability to predict liver-related mortality over the 6-month period in univariable analysis (OR 1.05 [95% CI 1.00-1.09], p=0.041), as did EncephalApp (OR 1.01 [95% CI 1.00-1.01, p=0.014) and PHES (OR 0.86 [95% CI 0.75-0.97], p=0.017). However, none, remained significant in a multivariable analysis.

Feedback from cognitive tests

The results from the patient useability questionnaires completed at the end of cognitive testing can be seen in Figure 5-4.

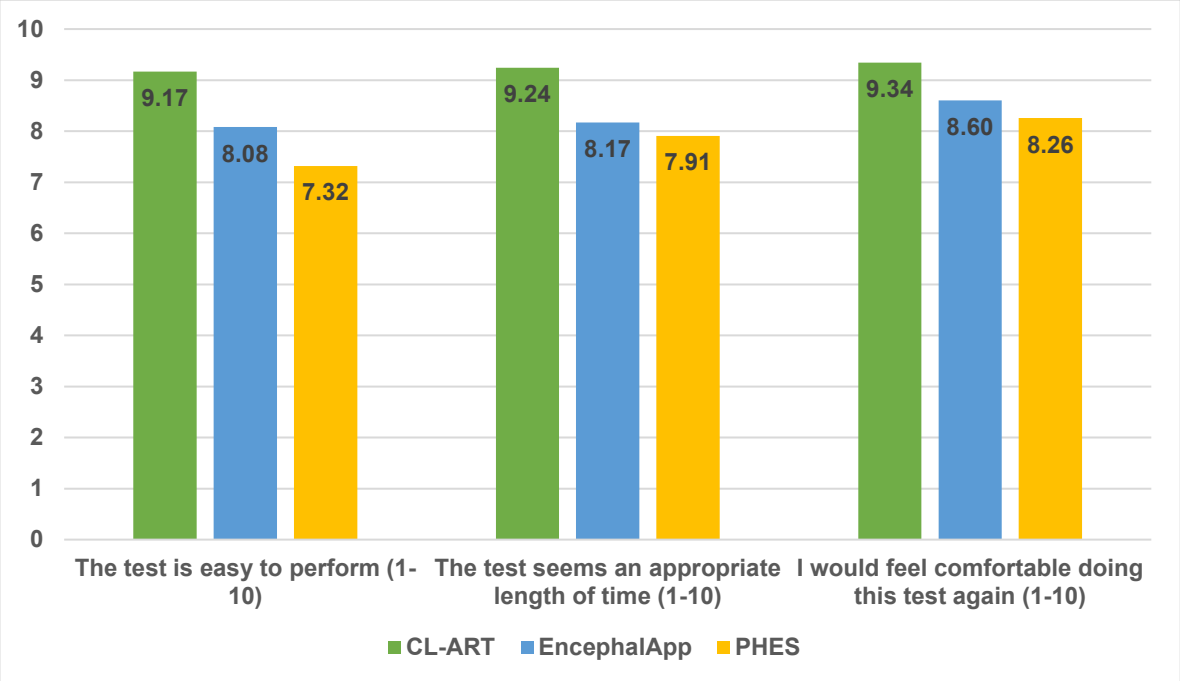


Figure 5-4: Mean participant useability feedback results for CL-ART, EncephalApp and PHES

Scale of agreement from 1-10, where 1 represents the most negative response and 10 represents the most positive response.

5.4 Discussion

The incidence of cirrhosis acute decompensation requiring admission, and in particular HE, is associated with significant morbidity and mortality. The current lack of validated biomarkers with high sensitivity and specificity to predict future HE episodes needs to be urgently addressed. Studies have suggested various biomarkers including bilirubin, albumin, CRP, ammonia, and IL-6, but none have yet translated into clinical practice for this purpose.[26,328–330]

This study aimed to determine whether the CL-ART correlated with established cognitive tests and whether it could help predict future hospitalisation with AD, with a specific focus on HE. The key findings of the study are that CL-ART demonstrated non-inferiority in diagnosing MHE when compared to EncephalApp and PHES (the gold standard), but with shorter testing times and higher patient useability scores across all questions asked. Crucially the CL-ART demonstrated an ability to independently predict future hospitalisation due to HE with a threshold of 26 seconds providing a high sensitivity and specificity. Furthermore, it demonstrated a potential signal in predicting subsequent hospitalisation due to all-cause decompensation.

The cohort of patients in this study seems to be representative of the wider cirrhotic population with median age, a predominant alcohol aetiology, incidence of previous HE, and hospitalisations with AD being consistent with international data.[20,25,200,315] The majority of the cohort was decompensated at recruitment, but it is worth noting that there was no significant difference in cognitive test results between the decompensated and compensated cohorts.

This does suggest that the tests are generalisable and could be applied across the spectrum of chronic liver disease, but this must be taken with caution as the study was not powered for sub-group analysis.

An important finding of this study was the comparable performance of the CL-ART to the PHES and EncephalApp in diagnosing MHE using PHES as the gold standard. Indeed, in the CirrhoCare pilot study where the CL-ART was first used, it demonstrated utility in assisting in the diagnosis of HE in the community, which is also supported by results in this study. Furthermore, as per review by two independent expert hepatologists, this may have prevented disease progression and even hospitalisation in a small number of cases.[156] Currently, the EncephalApp and CL-ART are the only cognitive digital biomarkers available in the diagnosis of HE. Whilst the EncephalApp is well validated, it is time-consuming, although as stated previously a shortened version has now been developed which takes 1 minute to complete.[165] Unfortunately, this was only published after study recruitment had commenced and therefore a direct comparison with CL-ART was not possible in this study. The other limitation of the EncephalApp is in individuals who are colourblind. Red-green colour blindless has been reported to be as prevalent as 8% in the male population, although significantly less in the females.[331] Indeed, one patient in our cohort could not complete the test for this reason.

Whilst an array of HE tests have been developed, recent EASL guidelines have advocated the use of the Animal Naming Test (ANT), which involves listing as many animals as possible in 60 seconds, as a screening test for

CHE.[332] This was not used as a cognitive test comparator in this study as both the CL-ART and ANT involve either the recognition or naming of animals, and performing one test would likely impact the participant's performance on the subsequent test and therefore lead to unreliable results.

The principal finding from this study was the ability of CL-ART to predict subsequent hospitalisation due to HE in both a univariable and multivariable analysis. Whilst both PHES and EncephalApp demonstrated a predictive capacity in univariable analysis, they did not remain significant in multivariable models. In contrast, the CL-ART threshold of 26 seconds in this study demonstrated a good specificity, but crucially an excellent sensitivity of 92%. Given the potential to use CL-ART remotely in cirrhosis management, having a high sensitivity is critical to ensure episodes of HE are not missed given the associated morbidity and mortality. Whilst there is some data to suggest both the EncephalApp and ANT can predict future OHE episodes, both tests seem to be impacted by level of education, unlike the CL-ART.[153,333] The EncephalApp demonstrated a significant correlation, albeit weak, with years of education in our results, and the ANT has shown to be influenced by education less than 8 years.[333] Ehrenbauer et al recently conducted a study where cirrhosis patients completed 6 cognitive tests at baseline, including PHES, EncephalApp and ANT. Interestingly, only PHES and ANT remained independent predictors of OHE development within 1 year in multivariable models, but ANT lost significance when adjusted for age and education.[325] However, contraindicating this is a recent large multicentre study where PHES demonstrated no ability to predict future OHE in those with MHE. [334]Given the time-consuming nature of PHES, as well as its

complexity and lack of ability for remote use, this study highlights the urgent need for cognitive biomarkers that can be used remotely to deliver sustainable hepatology care.

A signal was also demonstrated by the CL-ART in predicting all-cause decompensation with significantly higher baseline test scores in patients who were subsequently hospitalised. Whilst this study is not assessing pathophysiological mechanisms, it is known that systemic inflammation and portal hypertension, which are key drivers of decompensation, can have negative impacts on brain function through disruption of the blood-brain barrier and subsequent neuroinflammation.[51] This would provide a potential explanation for slow cognitive test scores in those at high risk of decompensation. Finally, whilst all cognitive tests demonstrated a predictive capacity for liver-related mortality in univariable analysis, none remained significant in multivariable analysis. However, the number of events was low and therefore the study likely lacked sufficient power to detect such differences.

The main limitations of this study are that it involved a heterogenous population including both compensated and decompensated individuals, although as stated previously there was no significant difference in cognitive test results between the two populations. Due to the limited sample, subgroup analysis on compensated cirrhosis, pre-ACLF, unstable decompensated and acute decompensated cohorts was not possible, even though these are likely distinct populations with different outcomes. Having said this, one could argue that using a heterogenous population more

accurately reflects a real-world cohort of cirrhosis patients. Another important limitation is that the cognitive tests were only performed at a single time point. Testing at multiple time points would have allowed delta changes to be assessed and correlated with outcomes. However, this will be addressed in the CirrhoCare randomised controlled trial, funded by the National Institute for Health and Care Research, which is currently recruiting across sites in the United Kingdom as well as a separate multicentre Indian sub-study which is ongoing.

In conclusion, this study demonstrates that CL-ART can diagnose HE and predict hospitalisation due to all acute cirrhosis decompensation with highest sensitivity and specificity for HE-related admissions. Its rapid testing, smartphone application and high useability mean it can be used remotely, and therefore play a crucial role in predicting decompensation, enabling early community intervention.

Chapter 6 : DAS: A novel dimethylarginine scoring system to predict liver-related events following acute decompensation of cirrhosis

6.1 Background

Portal hypertension is the most common haemodynamic abnormality caused by liver cirrhosis and is the main cause of complications including ascites, variceal bleeding and encephalopathy.[4] Portal hypertension is caused by increases in intrahepatic resistance and portal blood flow, with intrahepatic resistance being due to hepatic fibrosis as well as endothelial dysfunction resulting in increased intrahepatic vascular tone.[335] Endothelial dysfunction has been demonstrated to be characterised by insufficient NO synthesis.[336] Asymmetric dimethylarginine (ADMA) is a competitive endogenous inhibitor of endothelial NO synthase (eNOS) and has been demonstrated to be associated with eNOS dysfunction and reduced NO in decompensated cirrhosis and acute liver failure.[337,338] Other important components of this pathway are L-arginine, from which NO is synthesised and symmetric dimethylarginine (SDMA), which is a stereoisomer of ADMA and interferes with NO synthesis by competing with L-arginine for transport across cell membranes.[339]

Mookerjee et al demonstrated that patients with decompensated cirrhosis and alcohol-related hepatitis had significantly elevated plasma ADMA and SDMA levels with subsequent eNOS dysfunction which correlated with

increased portal hypertension and mortality. Indeed, a combined score of ADMA and SDMA was superior to traditional liver scoring systems such as the CPS and MELD in predicting outcomes. [340] Additionally, a recent randomised controlled trial demonstrated that administration of 5-Methyltetrahydrofolate (5-MTHF), which enables the degradation of ADMA, showed a significant reduction in HVPG with a corresponding decrease in ADMA.[341]

Unique to SDMA, is a correlation with renal dysfunction in cirrhotic patients, perhaps due to impaired hepatic metabolism of ADMA, leading to renal vasoconstriction, increased renovascular resistance and therefore increased SDMA retention.[342] This is of particular importance given the lack of validated biomarkers to predict renal impairment, yet its incidence is reported as high as 30-50% in patients hospitalised with cirrhosis.[343]

Whilst a signal has been demonstrated for the role of both ADMA and SDMA individually in decompensated cirrhosis, these have yet to be validated in large populations. In this study, we aim to prospectively explore the ability of a novel scoring system, termed DAS, which combines these two biomarkers, in predicting liver-related events following acute decompensation (AD) of cirrhosis.

6.2 Methods

Study design and participants

Patients included in this study were participants in the PREDICT study.[20] This was a prospective, observational, multicentre European study with patients recruited between March 2017 to July 2018. Ethical approval was obtained by the institutional review board at each centre. The inclusion and exclusion criteria are detailed below.

Inclusion criteria

1. Participant age ≥ 18 years old.
2. Non-elective admission due to AD, defined by the development of ascites, HE, GI bleeding, and infection, or any combination (infection alone did not constitute an AD).
3. Cirrhosis was defined by a combination of standard clinical criteria, ultrasonographic and endoscopy findings, as well as histology.

Exclusion criteria

4. Patients with acute or subacute liver failure without underlying cirrhosis
5. Patients with cirrhosis who developed decompensation in the postoperative period following partial hepatectomy
6. Pregnancy
7. Evidence of current malignancy except for non-melanocytic skin cancer and hepatocellular carcinoma within Milan criteria
8. Presence or history of severe extra-hepatic diseases, e.g., chronic renal failure requiring haemodialysis, severe heart disease (NYHA >

II), severe chronic pulmonary disease (GOLD > III), severe neurological and psychiatric disorders

9. HIV-positive patients
10. Previous liver or other organ transplantation
11. Admission/referral of more than 72 hours before inclusion
12. Patients who declined to participate, or who could not provide prior written informed consent and without a legal surrogate decision maker, and it appeared unlikely that the patient would regain consciousness or sufficient ability to provide delayed informed consent
13. Physician's denial (e.g. the investigator considered that the patient would not follow the protocol scheduled).

Data obtained at baseline and during follow-up

Data collected included: patient demographics; aetiology of liver disease; type of decompensation event; medication history; blood test results at baseline and 7 days and median length of stay after enrolment. During admission, new events including new/ progressive decompensation, infection and acute kidney injury (AKI) were also recorded 1 week after inclusion.

Patients were prospectively followed up for 3 months. Clinical data regarding readmissions as well as ACLF development were recorded. ACLF was defined as per the EF CLIF ACLF criteria.[293] Finally, data on liver transplantation and death was also collected at 3 months.

ADMA and SDMA measurement

Sample acquisition and storage

Plasma samples from recruitment and day 7 (+/- 2 days) were analysed. Following collection, samples were separated by centrifugation and stored at -80°C for subsequent analysis.

Sample Preparation

20µl of sample was added to 80µl Water in a 1.5ml microcentrifuge tube. 10µl Formic Acid was added, followed by 1ml Acetonitrile. The sample was vortexed for 30 seconds then centrifuged at 15200rpm for 10 minutes at 4°C. The supernatant was transferred to a clean tube and evaporated to dryness under N2 at 55°C. The residue was reconstituted in 1ml 1% Aqueous Formic Acid containing 25ng/ml Internal Standard, and transferred to either a high-performance liquid chromatography vial or a 96 well plate for analysis.

Liquid chromatography-mass spectrometry analysis (LCMS)

The samples were analysed on a Shimadzu LCMS 8040 Mass Spectrometer attached to a Nexera LC system using the parameters describes in Table 6-1.

Parameter	**Value**
Column	Discovery HS F5 150 x 2.1mm, 3.0µm

Mobile Phase A		0.1% Formic Acid in Water					
Mobile Phase B		0.1% Formic Acid in Acetonitrile					
Flow Rate		0.25 ml/min					
Column Temperature		40°C					
Autosampler Temperature		4°C					
Injection Volume		10µl					
Run time		10 minutes					
Gradient		Time (mins)			%B		
		0 – 2.0			0		
		2.0 – 5.0			0 – 0.5		
		5.0 – 5.5			0.5 – 95		
		5.5 – 6.5			95		
		6.5 – 7.0			95 - 0		
Nebulising Gas Flow		3L/min					
DL Temperature		250°C					
Heat Block Temperature		400°C					
Drying Gas Flow		15L/min					
Transitions		Transition	Dwell time	Q1	CE	Q3	Acq. time
	SDMA	203.1 – 70.1	100	-17	-27	-13	0 - 10
	ADMA	203.1 – 70.1	100	-17	-27	-13	
	ADMA	203.1 – 46.1	100	-17	-17	-19	
	IS	136.1 – 70.1	100	-13	-16	-12	
Dilution Factor		50					

Table 6-1: The parameters for LCMS analysis using the Shimadzu LCMS 8040 Mass Spectrometer attached to a Nexera LC system

ADMA concentration was determined using the 203.1 – 46.1 transition. The SDMA concentration was calculated by subtracting the measured ADMA

concentration from the combined SDMA + ADMA concentration determined by the 203.1 – 70.1 transition. The DAS score was formulated from the addition of ADMA and SDMA results.

Statistical analysis

Data analysis was performed using STATA (StataCorp. 2019. Stata Statistical Software: Release 17. College Station, TX: StataCorp). Summary statistics were performed on patient demographics, aetiology of liver disease, medication history, disease severity scores and blood test results. A non-parametric assumption was used for all statistical tests. Any missing data were excluded from the analysis. A Pearson's chi-squared test was used to test for statistically significant differences in nominal or ordinal data. A Mann-Whitney U test was used to test for statistical significance in variables of continuous data. The correlation between variables was assessed by Spearman rank correlation. AUROC curves were calculated to determine the diagnostic ability of tests to predict future liver-related events and the Youden index was used to determine optimal thresholds. Univariable and multivariable regression analysis was also performed for outcome data.

6.3 Results

Summary statistics

409 patients were recruited to the study with summary characteristics show in Table 6-2. With regards to decompensating events at baseline, 280 patients (68%) had ascites, 125 (31%) had HE and 62 (15%) had variceal bleeding. With respect to liver-related events, 117 patients (29%) had evidence of bacterial infection and 21(5%) of patients had an AKI at baseline. 152 patients (37%) had alcohol-related hepatitis at recruitment, either determined histologically or based on the NIAAA clinical criteria. In terms of interventions, only 16 patients (4%) had HVPG measurements performed either at baseline or week 1.

During follow-up, when assessing patients 1-week post-recruitment, 45 patients (11%) developed new or progressive decompensation, 28 patients (7%) developed new bacterial infection and 12 developed new AKI (3%). The median length of stay was 7 days (IQR 4-13). 49 participants (12%) developed ACLF during follow-up (27 grade one, 16 grade two, 4 grade three, and 2 unknowns due to missing data) with median time to ACLF development being 12 days (IQR 7-48). 111 patients (27%) were re-admitted during the subsequent 3 months with a range of 1-5 admissions. With regards to survival data, 28-day and 90-day survival rates were 99% and 95% respectively.

Characteristics	Baseline	Day 7	ACLF
Age*	58 (51-65)		
Male	69% (281)		
White	96% (393)		
Aetiology -Alcohol	62% (253)		

-MASH -MetALD -HCV -Cryptogenic -Other	7% (28) 4% (18) 5% (21) 4% (18) 17% (71)		
Beta-blockers	47% (194)		
Rifaximin	17% (71)		
Diuretics	67% (275)		
Statins	8% (33)		
WBC ($x10^9$/L)*	5.9 (4.2 -8.8)	5.6 (3.9-7.9)	8.8 (6.1-12.9)
CRP (mg/L)*	15.2 (6.0-36.6)	11.1 (5.0-24.8)	31.4 (17.1-50.0)
Platelets ($x10^3$/L)*	100 (64-147)	110 (70-159)	109 (74-149)
INR*	1.4 (1.2 -1.7)	1.4 (1.2-1.6)	1.6 (1.4-2.2)
Albumin (g/dL)*	2.8 (2.5-3.3)	3.0 (2.6-3.4)	2.7 (2.3-3.3)
Bilirubin (mg/dL)*	2.4 (1.4-5.8)	1.9 (1.1-4.3)	3.7 (2.0-12.8)
AST (U/L)*	55 (33-88)	51 (35-78)	61 (37-114)
ALT (U/L)*	29 (19-44)	30 (21-43)	34 (17-45)
ALP (U/L)*	125 (89-179)	126 (93-181)	116 (88-173)
GGT (U/L)*	90 (47-250)	101 (49-228)	87 (47-169)
Creatinine (mg/dL)*	0.8 (0.7-1.1)	0.8 (0.7-1.0)	2.1 (1.2-2.8)
Sodium (mEq/L)*	136 (132-139)	136 (133-139)	135 (129-140)
ADMA (µmmol/L)	1.32 (1.03-1.76)	1.30 (0.98-1.79)	1.68 (1.11-2.26)
SDMA (µmmol/L)	2.83 (1.91-4.23)	2.65 (1.81-3.70)	4.48 (2.63-6.82)
DAS score (µmmol/L)	4.21 (2.96-6.08)	4.02 (2.86-5.45)	6.31 (3.95-9.73)
CLIF-C AD score*	51 (46-57)	49 (44-55)	N/A

Table 6-2: Summary characteristics of cohort at baseline, day 7 and ACLF onset

**Median values provided with interquartile range*

Readmissions

When assessing readmissions, the baseline DAS score was significantly higher in those were who readmitted versus those were not (4.63 versus 4.03µmmol/L, p=0.004). The same observation was noted for ADMA alone (1.49 versus 1.27 µmmol/L, p=0.002) and SDMA (3.26 versus 2.64 µmmol/L, p=0.006). When analysing samples from day 7, this signal remained for DAS (4.45 versus 3.84 µmmol/L, p=0.009). Indeed, in both univariable and multivariable analysis baseline DAS remained an independent predictor of hospital readmission as show in Table 6-3.

Variable	**Univariable**		**Multivariable**	
	OR (CI)	**p value**	**OR (CI)**	**p value**
Age	**1.03 (1.01-1.05)**	**0.006**	1.02 (1.00-1.04)	0.095
Sex	0.80 (0.50-1.30)	0.370		
Aetiology	0.95 (0.87-1.05)	0.334		
Beta blocker	1.18 (0.76-1.83)	0.456		
Rifaximin	**2.32 (1.36-3.96)**	**0.002**	**2.11 (1.22-3.66)**	**0.007**
Statins	1.18 (0.54-2.57)	0.670		
Baseline infection	0.90 (0.55-1.46)	0.666		
Baseline AKI	1.08 (0.41-2.85)	0.880		

Baseline DAS	**1.14 (1.05-1.24)**	**0.002**	**1.12 (1.03-1.22)**	**0.007**
Baseline CLIF-C AD score	**1.03 (1.01-1.06)**	**0.017**	1.02 (1.00-1.05)	0.112

Table 6-3: Univariable and multivariable analysis of factors predicting hospital readmissions

The AUROC for baseline DAS to predict readmissions was 0.60 (95% 0.53-0.66). Using the Youden index the optimal cut-off for predicting for readmissions was 3.74 μmmol/L, producing a sensitivity of 70% and specificity of 45%. Using this cut-off a statistically significant difference was demonstrated in those who had subsequent readmissions versus those who remained admission free, as demonstrated in Figure 6-1.

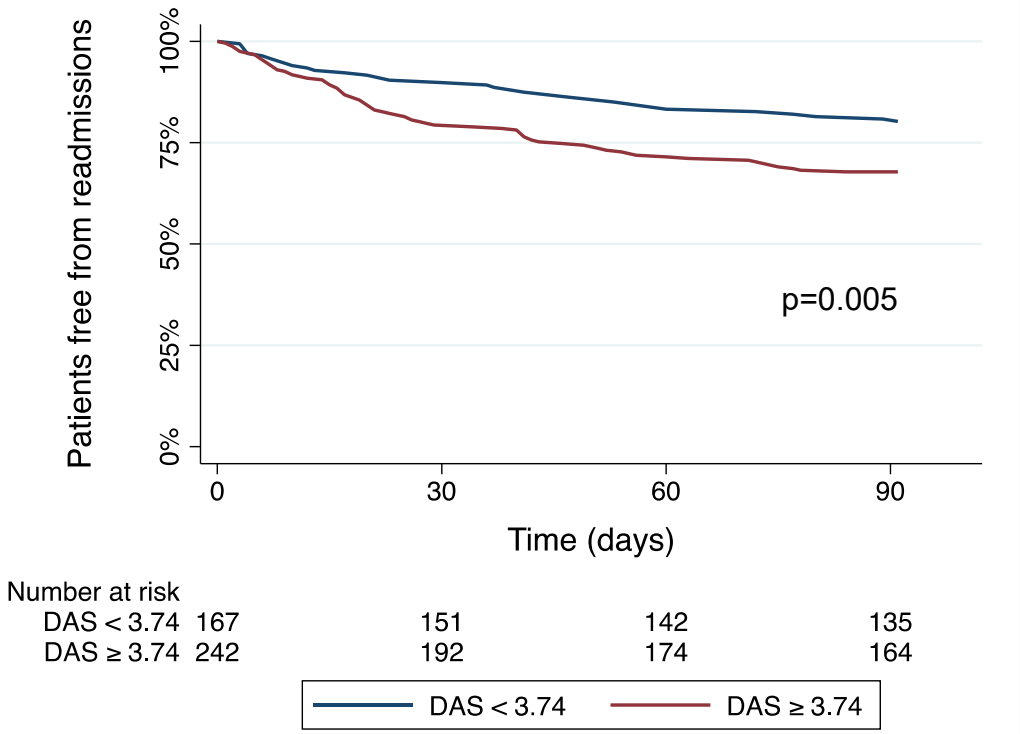


Figure 6-1: Kaplan Meier curve demonstrating the incidence of patients who remained free from readmissions during follow-up when using a DAS threshold of 3.74μmmol/L

ACLF development

With regards to participants who subsequently developed ACLF, baseline DAS scores were significantly higher in those who developed ACLF versus those who did not (5.51 versus 4.08 μmmol/L, p=0.006). The same finding was noted for baseline SDMA results (3.40 versus 2.71 μmmol/L, p=0.004) with a trend noted for ADMA (1.41 versus 1.30 μmmol/L, p=0.059). This remained true for the DAS score at day 7 with significantly higher results in those that developed ACLF (5.35 versus 3.93μmmol/L, p=0.008). Indeed, as per readmissions, the baseline DAS score remained an independent predictor of ACLF development in both univariable and multivariable analysis

as can be seen in Table 6-4.

Variable	Univariable		Multivariable	
	OR (CI)	p value	OR (CI)	p value
Age	1.02 (1.00-1.05)	0.103		
Sex	0.77 (0.40-1.51)	0.444		
Aetiology	0.98 (0.87-1.12)	0.785		
Beta blocker	1.07 (0.59-1.94)	0.817		
Rifaximin	1.26 (0.60-2.65)	0.549		
Statins	0.45 (0.10-1.95)	0.287		
Baseline infection	1.38 (0.73-2.60)	0.316		
Baseline AKI	1.79 (0.58-5.57)	0.312		
Baseline DAS	**1.12 (1.02-1.23)**	**0.022**	**1.13 (1.01-1.25)**	**0.027**
Baseline CLIF-C AD score	**1.13 (1.08-1.18)**	**<0.001**	**1.13 (1.08-1.18)**	**<0.001**

Table 6-4: Univariable and multivariable analysis of factors predicting ACLF development

The AUROC for baseline DAS to predict ACLF development within 3 months was 0.62 (95% CI 0.54-0.71). Using the Youden index the optimal cut-off for predicting for readmissions was 4.21 μmmol/L, producing a sensitivity of 70% and specificity of 52%. Using this cut-off a statistically significant difference was demonstrated in those who developed ACLF versus those who did not, as demonstrated in Figure 6-2.

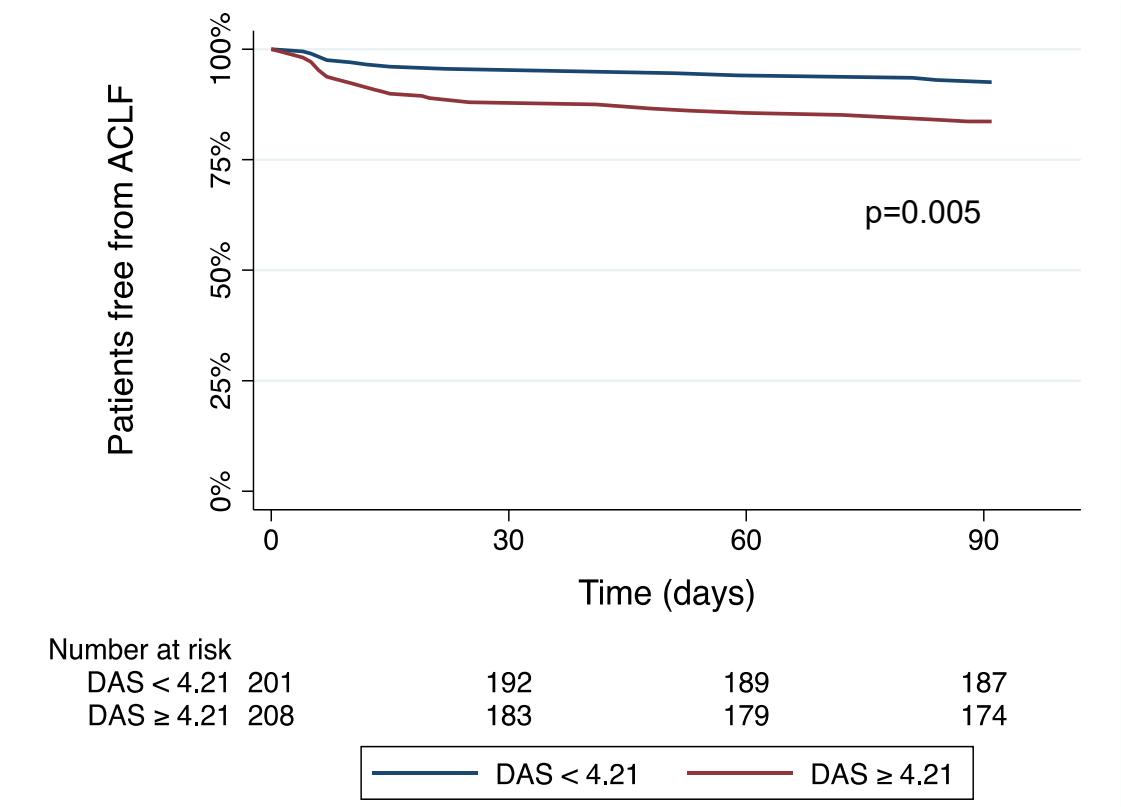


Figure 6-2: Kaplan Meier curve demonstrating the incidence of patients who remained free from ACLF during follow-up when using a DAS threshold of 4.21μmmol/L

90-day transplant free survival

When analysing survival data, significantly lower baseline DAS results were observed in those who survived versus those who died during follow-up (4.15 versus 5.48 μmmol/L, p=0.033). Once again, the same findings were noted for ADMA alone (1.30 versus 1.47 μmmol/L, p=0.016) and SDMA (2.77 versus 3.57 μmmol/L, p=0.049). Whilst a trend was noted for baseline DAS in univariable analysis to predict survival (OR 0.90, 95% CI 0.78-1.02, p=0.086), this was not sustained in multivariable analysis. Although a significant correlation was noted between baseline DAS and platelets (*r*=-0.10, p=0.04) as well as CLIF-C AD scores (*r*=0.11, p=-0.02).

Renal failure

Baseline SDMA and DAS showed a significant correlation with creatine (*r*=0.38, p<0.001 and *r*=0.32, p<0.001 respectively). Whilst no signal was demonstrated in predicting new AKI development by day 7, significant results were demonstrated in predicting renal failure development as per EF CLIF ACLF criteria. Baseline SDMA and DAS scores were significantly higher in those who developed subsequent renal failure compared to those who did not (4.23 versus 2.75 μmmol/L, p=0.009 and 5.87 versus 4.09 μmmol/L, p=0.012, respectively). This observation was maintained at day 7 with SDMA and DAS scores being significantly higher in those who developed renal failure (4.15 versus 2.59 μmmol/L, p=0.002 and 5.79 versus 3.93 μmmol/L, p=0.004). This signal was strongest at baseline, with SDMA remaining a predictor of renal failure development in both univariable and multivariable analysis (Table 6-5).

Variable	Univariable		Multivariable	
	OR (CI)	p value	OR (CI)	p value
Age	**1.04 (1.01-1.08)**	**0.024**	1.03 (0.99-1.07)	0.161
Sex	1.24 (0.55-2.76)	0.602		
Aetiology	0.92 (0.77-1.11)	0.394		
Beta blocker	1.52 (0.70-3.39)	0.289		
Lactulose	0.96 (0.44-2.06)	0.912		
Diuretics	1.24 (0.53-2.88)	0.625		
Baseline infection	1.00 (0.43-2.33)	0.997		
Baseline SDMA	**1.18 (1.03-1.36)**	**0.020**	**1.18 (1.01-1.39)**	**0.034**
Baseline CLIF-C AD score	**1.11 (1.06-1.17)**	**<0.001**	**1.11 (1.06-1.17)**	**<0.001**

Table 6-5: Univariable and multivariable analysis of factors predicting renal failure development

6.4 Discussion

This study evaluates the role of a novel dimethylarginine scoring system termed DAS. It demonstrates original findings that following hospitalisation with AD, DAS can independently predict readmissions and ACLF

development, with a signal demonstrated for mortality. Furthermore, SDMA exhibited a strong predictive capacity for subsequent renal failure.

The original PREDICT study demonstrated a cohort of patients classified as UDC, which categorised patients who were re-admitted or died following AD. These individuals had features of significant portal hypertension as demonstrated by increased circulatory dysfunction, gastrointestinal haemorrhage and TIPS insertion.[20] ADMA levels have previously been shown to be higher in patients with decompensated cirrhosis compared to compensated individuals, as well as correlate with disease severity in terms of the CPS.[337] Indeed, ADMA has been showed to correlate with the degree of portal hypertension, and has been associated with endothelial dysfunction in many conditions. [340,344] Furthermore, a recent study demonstrated that 5-MTHF which degrades ADMA, led to a reduction in portal hypertension.[341] Taken together, portal hypertension is therefore a key driver of readmissions with ADMA demonstrating a crucial role in the underlying pathogenesis.

Whilst the link between portal hypertension and ADMA has been demonstrated, its role in predicting subsequent liver-related events has not been thoroughly assessed. Only one previous study has demonstrated a link to cognitive dysfunction and HE development post TIPS, but this is the first study to demonstrate an ability to predict future admissions following AD.[345] The likely underlying mechanisms are highlighted by the significant correlations demonstrated between DAS and markers of portal hypertension (platelets) as well as liver disease severity (CLIF-C AD score). Finally, a DAS threshold of 3.74μmmol/L was demonstrated to be significant in

distinguishing patients who had readmissions versus those who did not during follow-up. Whilst the sensitivity and particularly specificity were limited, a threshold provides clinical utility which requires further validation.

Whilst much of the focus of dimethylarginine research has assessed ADMA, SMDA has independently demonstrated an ability to predict survival in decompensated cirrhosis, as well as be a marker for mortality and cardiovascular disease across healthcare conditions.[340,346] It is therefore unsurprising that this study has shown that the DAS score, which combines both dimethylarginines has demonstrated an ability to predict readmissions in both univariable and multivariable analysis. With regards to mortality, baseline DAS scores were significantly lower in those who survived compared to those who died, and indeed Mookerjee at al demonstrated a strong mortality signal for DAS previously.[340] Whilst a trend for DAS scores was noted in univariable analysis, this was not sustained. We hypothesise that this was due to the relatively small number of deaths in this study, as well as perhaps a sicker cohort in the Mookerjee et al study as reflected by 25% mortality, compared to 5% in this study.

Whilst portal hypertension was historically thought to be the sole driver of decompensation, there is an increasing understanding of the key role of systemic inflammation. There is translocation of bacteria and bacterial-by products, termed PAMPs, across the gut mucosa to the systemic circulation which drives inflammation. Indeed, portal hypertension exacerbates this inflammatory burden through increasing gut permeability.[51,52] A second cohort of patients highlighted in the PREDICT study, was the pre-ACLF

group, who developed ACLF within 3 months following AD and exhibited a high burden of systemic inflammation. Indeed, severity of systemic inflammation has shown to correlate with ACLF grade as well as independently predict ACLF development.[21,347]

Both ADMA and SDMA have been shown to be pro-inflammatory, stimulating a range of markers including nuclear factor kappa B (NF- *κB),* TNF-α, multiple interleukins as well as adhesion molecules, exacerbating oxidative stress.[345,348,349] Indeed, a novel liver dialysis device was used in ACLF and demonstrated a reduction in markers of systemic inflammation as well as both dimethylarginines, corresponding with improved disease severity scores and faster ACLF resolution.[350] Therefore, once again, given the pathophysiological basis, it is unsurprising that DAS has demonstrated an ability to independently predict ACLF development in this study, although this has not been shown previously. Additionally, a threshold of 4.21μmmol/L was shown to be significant in predicting those who developed subsequent ACLF compared to those who did not. As per re-admissions, this threshold exhibits similar benefits as well as limitations. Whilst markers of systemic inflammation were not formally assessed in this study, the underlying mechanisms as described in this section have been well studied previously.

Finally, we have demonstrated that SDMA can predict renal failure in both univariable and multivariable analysis. This signal was not demonstrated in predicting new onset AKI between baseline and week 1 post recruitment, but I hypothesise this was due to only 12 patients developing AKI, with a larger cohort being required to evaluate this. SDMA is excreted predominantly by

the kidneys, demonstrating an excellent correlation with various markers of renal function with a greater ability to detect early/ mild renal dysfunction.[351,352] SDMA has demonstrated to be significantly higher in individuals with hepatorenal syndrome compared to cirrhotic patients without renal failure, and exhibits a strong correlation with creatinine.[342] In clinical practice, creatinine is used as the standard marker of renal function and indeed in this study we demonstrated a significant correlation between SDMA and creatinine. However, there is increasing recognition of the limitations of creatinine in this cohort. It overestimates renal function due to reduced creatinine production as a consequence of impaired liver function, as well as sarcopaenia which is extremely prevalent in cirrhotic populations.[85] In contrast to creatinine, evidence suggests that SDMA is not impacted by a range of factors including muscle mass, age and gender, and therefore it may be an ideal diagnostic and predictive renal biomarker.[353]

One of the main limitations of this study is that it was not powered to determine liver-related outcomes such as mortality. Nonetheless it was a well conducted multi-centre prospective study with a large number of patients included. Secondly, in this analysis there is a lack of baseline dimethylarginine levels in healthy controls as well as patients with compensated cirrhosis for comparison. Furthermore, the DAS score has only been assessed in one cohort. Both of these issues will be addressed in the next phase of the project with a second validation cohort, as well as healthy controls and compensated cirrhosis patients planned for analysis.

In conclusion, this robust study has demonstrated novel findings that the DAS score can predict hospital readmissions and ACLF development following decompensation, as well as a predictive capacity of SDMA in renal failure. These positive findings, and the key role of the dimethylarginines in the pathogenesis of decompensation and liver-related events, warrants further evaluation in prospective studies.

Chapter 7 : Summary and Future Directions

7.1 Summary of context for thesis

Decompensated cirrhosis is a complex entity with the definition evolving over time as our understanding has increased. As stated, the onset of decompensation, defined by the development of overt ascites, overt encephalopathy and variceal bleeding drastically alters the trajectory of a patient.[4] The development of further decompensation events in the form of recurrent variceal bleeding, recurrent ascites, recurrent HE, SBP, HRS-AKI and/or jaundice, either sequentially or in combination leads to significantly worse outcomes.[4,33] Furthermore, distinguishing between NAD and AD seems to be crucial with AD requiring hospitalisation and being associated with significantly worse morbidity and mortality.[20,24,29]

The understanding of the pathophysiology driving decompensation has also expanded in recent times. Historically, portal hypertension was thought to be the predominant force due to increased intrahepatic resistance and portal inflow.[38] Over time it has been discovered that the intrahepatic resistance component is dynamic due to endothelial dysfunction, with the NO pathway being particularly implicated, as explored in this thesis.[40] More recently, the systemic inflammation hypothesis has evolved, with circulating PAMPs and DAMPs driving pathogenesis. It is now clear that portal hypertension and systemic inflammation act synergistically, with portal hypertension increasing gut permeability and translocation, precipitating decompensation with its associated morbidity and mortality. [51] Finally, the role of metabolic dysfunction with PEM has gained prominence with clear evidence that it

worsens outcomes, with a focus in this thesis on fat and lipid metabolism.[65,66] Malabsorption secondary to portal hypertension, as well as systemic inflammation, which is energetically expensive and reduces oral intake through the anorexigenic effects of cytokines, all drive malnutrition depleting muscle and fat stores, again linking these different mechanisms.[58,59] This thesis has aimed to focus on biomarkers which target each of these different pathways as well as the interplay between them.

Whilst multiple liver disease severity scoring systems have been developed and improved over time, they all have significant limitations and often underperform, other than in the original contexts in which they were developed. Furthermore, whilst most have been developed to predict mortality, there are no validated models to predict liver-related events.[221] This has set the premise for this thesis, to try and identify novel biomarkers that could be utilised in risk prediction.

When first trying to develop a new biomarker, one must first determine what the ideal qualities of a biomarker should be. Whilst not an exhaustive list, the desirable qualities include biological plausibility, sensitivity and specificity, validation, minimal invasiveness, stability, ease of measurement, low cost and being generalisable across populations.[88] As highlighted in a recently published systematic review and meta-analysis that I co-authored, a large number of new biomarkers have emerged over recent years.[140] However, none of these have been incorporated into clinical practice, likely due to a lack of clarity over which biomarkers are superior, which would work best in specific scenarios and whether they truly outperform current scoring systems.

Therefore, there remains an unmet need for novel biomarkers which target pathophysiological mechanisms of decompensation and can either be used independently or more likely in combination to develop composite scores.

As well the qualities of a biomarker already highlighted, they must be beneficial to both patient and clinician. Disparity in care already exists, with worse outcomes demonstrated in those in deprived and rural areas, which has only been compounded by the increasing prevalence of liver disease, as well as the lasting effects of the COVID-19 pandemic.[10,142] In this regard digital healthcare could be the key, enabling a wealth of patient-related biomarker data to be obtained remotely, with the opportunity for community based therapy and management to be instigated in real time. This transformative approach could truly revolutionise patient centred care and personalise interventions, which is why digitising healthcare is part of governmental policy in many countries across the globe.

7.2 Summary of findings

As already highlighted, there are a lack of prognostic and predictive biomarkers with regards to decompensated cirrhosis. The COVID-19 pandemic only exacerbated these issues by directing resources towards the virus, and away from other healthcare conditions include chronic liver disease. Whilst it was speculated that the pandemic may negatively impact the care and outcomes of individuals of individuals with decompensated cirrhosis, this had not been sufficiently assessed.[194,195] In order to try and address this question, I performed a retrospective analysis of all admissions

with AD to a tertiary hepatology and transplantation centre over 2 ½ years with the cohort separated into the pre-COVID and COVID eras. Concerningly, the key findings during the COVID-19 pandemic were of increased liver severity scores in those admitted to ITU, a trend towards increased re-admission rates and increased early mortality. The first chapter therefore set the premise for this thesis by highlighting the detrimental effects of COVID-19 and therefore justifying the need for improved biomarkers that can be deployed in clinical practice. The effects of the pandemic are likely to be long lasting and have implications on the way we care for cirrhosis patients. There needs to be a shift towards more sustainable pathways that are more acceptable to patients as well as reducing our collective carbon footprint. It is this logic that has led to a substantial portion of this thesis being focussed on digital biomarkers which can be utilised remotely, reducing the need for unnecessary hospital journeys as well as opening up diagnostic and therapeutic opportunities to those in more remote and deprived areas.

Before one can try to develop and validate new biomarkers, it is imperative to perform a thorough analysis of the literature to assess which markers have already been evaluated. Whilst I have co-authored a review assessing biomarkers that can predict outcomes in patients with decompensated cirrhosis, it is important to take a step back along the pathway to assess patients with compensated cirrhosis.[140] This is because as stated previously, the transition from compensated to decompensated cirrhosis significantly alters the trajectory of a patient and puts them at risk of further decompensation.[4,15] Following on from this, chapter 2 of my thesis attempted to address this by performing a systematic review and meta-analysis of

biomarkers that predict decompensation in patients with compensated cirrhosis. In terms of categories, blood-based biomarkers had the most evidence, with platelets, MELD and albumin having the largest number of studies. Based on the meta-analysis, INR and then albumin are the strongest predictors of decompensation but given the significant statistical heterogeneity, this must be interpreted with caution. This chapter also highlights a range of other individual markers or novel scoring systems which could all have potential but currently lack validation. Crucially, they have all been developed using components that target the underlying pathogenesis of decompensation, which is a principle that I have used in selecting biomarkers for evaluation in the subsequent chapters of my thesis.

In the third chapter, the focus was placed on the metabolic dysfunction which is widely prevalent in decompensated cirrhosis with a clear link between PEM and worse outcomes.[65,66] Whilst, the importance of malnutrition has been known for a while, robust and practical nutritional biomarkers are lacking. Given the prevalence of sarcopaenia it is unsurprising that indices assessing skeletal mass as well as handgrip strength have evolved. However, little attention has been given to fat, with fat loss being implicated earlier in disease progression and being protective against sarcopaenia.[62,63] The liver plays a crucial role in fat and lipid regulation with pathological alterations occurring in cirrhosis and reduced lipoprotein levels demonstrated.[67,283] Whilst the role of HDL in predicting liver-related events has been well established, LDL has not been sufficiently investigated, particularly given that it exhibits many features of an ideal biomarker.[285,354,355] Chapter 3 aimed to explore the prognostic and monitoring role of markers of fat and lipid

metabolism in two separate sub-studies assessing patients with AD requiring hospitalisation.

In the first sub-study, the role of LDL as a prognostic biomarker in identifying patients at high risk of liver-related events was explored in the PREDICT cohort, which was large prospective, observational, multicentre, European study. LDL demonstrated an ability to independently predict future re-admissions as well as providing a potential signal for other events such as new infection, ACLF development and short-term mortality. Whilst clear mechanistic pathways were not elicited, it is known that LDL can have protective effects over immune dysfunction and systemic inflammation.[300,302] In the second sub-study the role of BIA in assessing body composition was evaluated given its ease of use and ability to acquire measurements remotely. Whilst BIA has been used the cirrhotic population previously, this was the first study to demonstrate that it could be used remotely to take serial measurements of fat mass accurately and not be affected by fluid shifts which are prominent in this population. Furthermore, a signal was demonstrated in terms of patients with higher levels of baseline systemic inflammation and liver disease severity scores having a tendency to lose fat moss over the follow-up period which is intuitive given the increased energy expenditure required.[68,70] Both of these studies have highlighted the importance of fat and lipid metabolism in this cohort, with a potential for the markers to be used sequentially, with low LDL indicating high risk patients and BIA monitoring being deployed to such individuals in the community.

Whilst any form of decompensation significantly alters a patient's prognosis, HE is the most prevalent and carries the highest mortality.[315,317] Whilst various tests have been developed to diagnose cognitive dysfunction, they have not been validated in predicting new liver-related events. Given the ability to prevent HE progression through early detection and treatment of precipitating events and titration of laxatives, predictive biomarkers for HE remain both elusive and coveted. The CL-ART test was a novel cognitive test developed for the CirrhoCare program and was demonstrated to be rapid (<30 seconds) and have very good useability feedback.[156] The aim of this chapter was to compare the performance of CL-ART to established cognitive tests as well as determine whether it could predict future OHE as well as other decompensation events. The comparator cognitive tests chosen were the EncephalApp, as it is the most well studied App based test for HE, and the PHES as it is the gold standard for the diagnosis of early HE.[151,152,326] CL-ART demonstrated an excellent performance in diagnosing MHE when compared to the other cognitive tests, but with shorter testing times and superior participant feedback. Crucially though, it exhibited an ability to independently predict future hospitalisation due to HE with a signal to predict hospitalisation due to all-cause decompensation as well. Its rapid testing, smartphone application and high useability mean it can be used remotely, potentially playing a vital role in predicting decompensation and enabling early community-based therapy.

The final chapter in in this thesis has aimed to target components that are involved in driving portal hypertension and systemic inflammation, which as already highlighted, are the key mechanisms behind decompensation. ADMA

and SMDA are both known to reduce NO availability in the liver, consequently increasing portal pressures, as well being pro-inflammatory, being implicated in stimulating a range of mediators.[340,345,348] Whilst there is limited data studying these markers individually, the novel objectives of this chapter were to validate a novel score combining these two metabolites, termed DAS, to predict-liver related outcomes in the PREDICT cohort. This study demonstrated that DAS could independently predict readmissions and ACLF development with a mortality signal demonstrated as well. SDMA also demonstrated an ability to independently predict renal failure, which given that it does not exhibit some of the inherent weaknesses of creatinine in terms of being influenced by muscle mass, age and gender, make it a very promising biomarker.[353] Given the biological plausibility of both ADMA and SDMA, and these findings reinforcing their validity in decompensated cirrhosis, further evaluation is warranted.

In summary, in this thesis I have highlighted the need for improved biomarkers, given the deleterious impact of the COVID-19 pandemic on decompensated cirrhosis outcomes. Furthermore, given the lasting legacy of the pandemic which has changed the way we care for our patients, we must develop markers that are in keeping with and improve existing sustainable care pathways. I have drawn attention to the current biomarkers that have the strongest evidence for predicting decompensation based on the current literature, highlighting both their strengths and weaknesses. Finally, I have then studied a range of blood based and digital biomarkers that target the pathophysiological mechanisms underlying disease progression as well as each exhibiting a range of qualities which would make them desirable. Whilst

it is beyond the remit of this thesis to determine the optimal combination of biomarkers, it has made incremental progression in the field, highlighting new targets and tests of interest.

7.3 Future directions

Conducting research in the field of decompensated cirrhosis is challenging, given that this cohort of patients are very sick. It can be difficult to achieve sufficient patient retention in long term studies due their high morbidity and frailty, making it hard for patients and carers to commit to studies, let alone routine clinical appointments. This is confounded by the fact that the majority of patients have alcohol related disease with a large proportion having ongoing misuse, coupled with significant socioeconomic disparities in this population leading to challenges with engagement.[1,206] Furthermore, the evolving definition of decompensation over recent years has led to additional complexities. Most of this thesis has focussed on patients with AD, requiring hospitalisation which is recognised as a distinct cohort. However, new sub-groups are emerging in terms of AD versus NAD, which is more insidious in nature and does not tend to require hospitalisation.[29] There is an increasing acceptance that the number and type of decompensating events is important, with the greater the number leading to worse prognosis.[33] Finally, post discharge after AD, further characterisation depending on whether readmissions, death or ACLF development occur can result in further subcategories.[20] Whist these distinctions are important, as decompensated cirrhosis is a heterogenous entity, in order to sufficiently study these different groups, significant national and international collaboration will be required. In order to recruit the numbers required to detect significant differences, large

consortiums which incorporate all relevant stakeholders including clinicians, multidisciplinary team members, patient groups as well as policy makers will be needed. Importantly, these cannot just be composed of large specialist centres, but also smaller district general hospitals in remote and deprived areas to ensure the full patient spectrum is represented. This I believe is key to driving forward decompensated cirrhosis research.

Whilst the systematic review and meta-analysis I conducted highlighted key biomarkers that could potentially be incorporated into future prognostic models, it also highlighted important limitations with studies conducted to date. It highlighted significant heterogeneity in terms of patient population, study design as well as outcomes. Furthermore, it highlighted a significant risk of bias in many published studies. In order for findings from future studies to be considered reliable and replicable, it is crucial they are conducted with robust methodology. Perhaps the key to enable this to be standardised across the field is to introduce a checklist for investigators who wish to conduct biomarker studies. Indeed, I have already been part of discussions with various young and senior investigators across Europe regarding the need for such a checklist and there is widespread agreement that this required. Whether this is part of a structured Delphi protocol, or more informal process remains to be determined but is definitely part of a future research agenda.

With regards to the range of novel biomarkers that have been studied in this thesis, steps have already been planned or undertaken to progress these to the next stage. With regard to the role of lipid dysfunction, the intention is to

validate the positive LDL findings in a second cohort of patients. This cohort may either be from the ACLARA study, which was the first prospective observational study of patients hospitalised non-electively for AD in Latin America, or the DASIMAR study, a large UK based multi-centre study which also recruited patients following admission with AD.[356,357] Furthermore, EF CLIF have undertaken lipidomic studies in European cohorts of patients. Another valuable step would be to explore this analysis in order to further try and elucidate the complex mechanisms underlying lipid and lipoprotein derangement in decompensated cirrhosis.

Both fat mass as measured by BIA, and the CL-ART test as an assessment of cognitive function and predictor of future liver-related events showed positive results in this thesis. These are both crucial components of the CirrhoCare remote monitoring system. On of the limitations of the CL-ART study which has been highlighted is that the test was only performed at single time point at baseline. To address this, and determine the utility of delta changes in scores, a multi-centre sub-study in India is currently ongoing with testing being performed at multiple time points. With regards to both BIA and CL-ART, these are both undergoing further validation in the CirrhoCare RCT which is currently recruiting. In order to address some of the weaknesses in the original fat mass study, more objective and validated measures of nutritional status and frailty have been incorporated into CirrhoCare RCT, including sequential measurements of hand grip strength, liver frailty index and the Royal Free Hospital-Nutritional prioritising tool.[358,359] The aim is to recruit 214 patients in total across the UK with 107 patients undergoing daily monitoring for 12 weeks. This will generate a wealth of data, which will

enable robust algorithm development to help predict future liver-related events and enable instigation of community-based therapy. Importantly, these digital biomarkers will not be used in isolation but in combination to try and identify the risk of individual decompensating events such as ascites or HE, just as we do in clinical practice, using a range of assessments to make a diagnosis. Even more exciting is that this program will enable the potential for an individual 'digital fingerprint' to be developed. The algorithm will develop a normal baseline for a participant based on their own results and will tailor risk profiles based on their delta changes in data points from one day to the next. This truly is forward facing research, embracing technology and personalised care in one integrated approach.

Finally, whilst I have just emphasised the huge potential of digital research, this does not negate the importance of more traditional blood-based biomarkers. The last chapter is case-in-point of the importance of targeting components of pathways that are directly involved in the pathogenesis of decompensation. This study has re-emphasised the importance of the dimethylarginines and shone a light on DAS as a prognostic score. However, this is only the beginning of this journey and funding has been secured to validate these findings in the DASIMAR cohort of patients as well as a separate cohort of healthy volunteers and patients with compensated cirrhosis. Furthermore, there is an understanding that whilst the technique used to do the DAS analysis, LC-MS is well validated and reliable, it is not widely available. In order to move this story along the translational pathway and make this clinically viable, a high-throughput assay is required. To this end, the next phase of this project is to repeat a sub-set of samples using

commercially available ELISA kits for ADMA and SDMA. Whilst ADMA ELISA kits have been used in human studies, SDMA have not, being restricted predominantly to animal studies.[341] Furthermore, a direct comparison of LC-MS and ELISA has not been conducted, so this will be truly novel. Together these next phases should help confirm the prognostic role of the DAS score.

In conclusion, whilst the aim of this thesis was not to develop the optimal prognostic scoring system itself, it has highlighted novel biomarkers that should be considered, as well as important steps that should be considered in decompensated cirrhosis research. As I have already stated, a single biomarker will not be in the key, but rather a composite score composed of several components targeting different pathophysiological pathways. These should also aim to be individualised accepting that a 'one size fits all' model is no longer acceptable either to patients or clinicians. Whilst this may seem too ambitious or unattainable, we must strive to achieve this and large data sets using data modelling and artificial intelligence are likely to be instrumental. Crucially it important that these risk prediction models are available to all to prevent inequity in healthcare, overcoming socioeconomic, rural and ethnic disparities. Whilst the optimal combination of biomarkers remains elusive, I feel this thesis has made a positive contribution to the field in search of these answers.

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