FUNCTIONAL LIVER TESTS IN LIVER DISEASE

ALI JIBRAN MECCI

A thesis submitted to Queen Mary, University of London in partial fulfilment of the

requirements of the MD (res)

October 2022

Declaration of Authorship

I, Ali Jibran Mecci, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below. I attest that I have exercised reasonable care to ensure that the work is original and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material. I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis. I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university. The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without the prior written consent of the author.

Signature:

Date: 02/10/2022

Acknowledgements

First and foremost, I would like to thank all the patients that have participated in this work. Their selflessness and commitment allows for improvements in our understanding and treatment for themselves and future generations. The financial support of LAPResearch UK has allowed me to continue this work until completion.

I could not have completed this thesis without the constant guidance, endless enthusiasm and support through all the ups and downs of life and research given by Prof Graham Foster. There is no way to say thank you enough from taking me as an FY1 doctor and keeping faith in me until this point. I would like to also thank Mr Robert Hutchins for all his work and support throughout.

Having completed my medical degree at QMUL, the Royal London Hospital has always been a second home to me and it brings me great satisfaction that I was supported by so many staff both clinically and within research teams. This includes the Liver centre, the transfusion centre and the respiratory team. The collaboration with Dr Kaya Banu, Dr Danie Watson and Dr Catherine Rang was invaluable in taking a surgeon to some semblance of understanding of complex diseases.

Working with HCV Research UK allowed me to take leaps ahead in my understanding of larger datasets, statistics and collaboration and I am grateful to Prof Will Irving and Jenny Benselin for obliging me throughout.

My journey through this project has taken many twists and turns, from basic science, to gaining my animal welfare license and finally clinical work. I would like to thank all those at

Abstract

The liver is subject to several insults, whether from ingested chemicals, pathogens or genetic diseases. Many of these are transient and full function returns. However, chronic injury may lead to scarring, the volume and effect of which may be viewed along a spectrum inclusive of fibrosis, cirrhosis and eventually death. Several complications may arise from this process, including decompensation and hepatocellular carcinoma. Recognising this damage in a timely, objectively reproducible and, most importantly, safe manner is clinically critical and great strides in this regard have recently been made. Several non-invasive technologies have been produced, the most ubiquitous being transient elastography (TE), for detecting fibrosis. To measure the actual function of the remaining liver, indocyanine green excretion allows for direct and minimally-invasive testing.

This thesis focusses on the use of non-invasive testing to identify significant liver disease in a variety of different disorders. I have studied clinically relevant changes in liver fibrosis and function and related these to conventional liver function tests in an attempt to develop clinically useful markers of liver function that can be used by clinicians to risk stratify patients at risk of liver disease.

Hepatitis C treatment has recently been revolutionised with the introduction of direct-acting antivirals, not only allowing a greater level of treatment success but also widening those patients we could treat to include those with cirrhosis. However, the benefits of therapy to those with advanced fibrosis are unclear and it is not established which patients are likely to undergo functional recovery following viral clearance nor is it clear which patients are at greatest risk of liver cancer. We studied liver fibrosis and indocyanine green excretion in a cohort of patients with advanced fibrosis and here I show that patients (n=43) with mild

functional impairment (ICGR15<20.9%) are likely to recover but others are unlikely to undergo significant functional recovery. I studied the massive HCV Research UK database to identify risk factors for post-treatment development of HCC and I showed that virological treatment failure and pre-existing liver lesions predispose to future development of liver cancer.

Recent medical advances have improved the prognosis for patients with cystic fibrosis and sickle cell disease. This improved life expectancy has unveiled new long-term complications of these disorders, including liver disease. To investigate the prevalence of liver disease in adults with these genetic disorders I conducted two clinical audits using non-invasive testing and here I show that the use of a TE in a sickle cell disease clinic adds value and identifies people with liver fibrosis. However, in an adult cystic fibrosis clinic the use of TE was of limited value as some patients with evident fibrosis were not detected by this technique and other diagnostic criteria need to be applied.

In summary this work has identified prognostic factors for patients with hepatitis C and cirrhosis who have undergone effective antiviral therapy and demonstrated the value of non-invasive testing in haematological conditions whilst identifying weaknesses in the use of TE in those with cystic fibrosis.

Communications Arising from Research

Peer-reviewed publications

- Mecci AJ, Cheung M. Treatment of Hepatitis C. **eLS,** 2017, DOI:10.1002/9780470015902.a0024786

- Mecci AJ, Kemos P, Leen C et al. The association between hepatocellular carcinoma and direct-acting anti-viral treatment in patients with decompensated cirrhosis. **Alimentary Pharmacology & Therapeutics,** 2019, DOI: 10.1111/apt.15296

- De Silva S, Li Wenhao, Kemos P, Brindley H, Mecci AJ et al. Non-invasive markers of liver fibrosis in fatty liver disease are unreliable in people of South Asian descent. **Frontline Gastroenterology**, 2017, DOI: 10.1136/flgastro-2017-100865

Poster presentations at international conferences

- Mecci AJ, Kemos P, Benselin J et al. Hepatocellular Carcinoma progression and survival following Hepatitis C Virus anti-viral therapy. **EASL HCC Summit, 1-3 March 2018, Geneva, Switzerland**

- Mecci AJ, Kemos P, Foster GR. Patients with cirrhosis show an improvement in dynamic liver function following the eradication of hepatitis C virus with direct acting antivirals. **EASL International Liver Congress, 23-26 June 2021, online meeting**

- Mecci AJ, E Maxan, Kemos P et al. Diagnosis of Sickle Cell Liver Disease may be aided by Non-invasive tests. **EASL International Liver Congress, 11-15 Apr 2018, Paris, France**

Contents

List of Abbreviations

AAR	Aspartate aminotransferase and alanine aminotransferase ratio
AFP	Alpha-foetoprotein
ALBI	Albumin to bilirubin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APRI	AST to platelet ratio index
ARFI	Acoustic radiation force impulse
AST	Aspartate aminotransferase
AUC	Area under curve
AUROC	Area Under the Receiver Operating Characteristic curve
BCLC	Barcelona-Clinic Liver cancer
BSPGHAN	British Society of Paediatric Gastroenterology
CAP	Controlled Attenuation Parameter
CDC	Centers for Disease Control and Prevention
CF	Cystic Fibrosis
CFLD	Cystic fibrosis-related liver disease
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
COPD	Chronic obstructive pulmonary disease
CSPH	Clinically significant portal hypertension
CT	Computed tomography
CTP	Childs-Turcotte-Pugh
DAA	Direct-acting antivirals
DBS	Dried blood spot
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
EAP	Expanded access programme
EASL	European Association for the Study of the Liver
ECOG	Eastern cooperative oncology group
EFSUMB	European Federation of Societies for Ultrasound in Medicine and Biology
ELF	Enhanced Liver Fibrosis score
ELITA	European Liver and Intestine Association
ERCP	Endoscopic retrograde cholangiopancreatography
FAST	Focused Assessment with Sonography for Trauma
FBC	Full blood count
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GEC	Galactose elimination capacity
GGT	ɣ-glutamyl transpeptidase

HBB	β-globin gene
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HCVRUK	HCVResearch UK
HIV	human immunodeficiency virus
HR	Hazards Ratio
HVPG	Hepatic Venous Pressure Gradient
ICG	Indocyanine Green
ICG15	ICG Retention after 15 minutes
IFN	Interferon
INR	International normalised ratio
IQR	Inter-quartile range
ITU	Intensive treatment unit
IU	International Units
IV	Intravenous
IVC	Inferior vena cava
IVDU	Intravenous drug user
kPa	Kilopascal
LFT	Liver function test
LIC	Liver iron concentration
MCP	monocyte chemotaxis protein
MCV	Mean corpuscular volume
MDT	Multi-disciplinary team
MELD	Model for end stage liver disease
MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
NHHN	Non-hypervascular hypointense nodules
NHS	National health service
NHSE	NHS England
NICE	National institute for clinical excellence
NPA	Non-Pseudomonas Aeruginosa
NPV	Negative Predictive Value
NRES	National Research Ethics Service
NTM	Non-tuberculous mycobacteria
OGD	Oesophago-Gastro-Duodenoscopy
PA	Pseudomonas Aeruginosa
PDR	Plasma disappearance rate
PEG	Percutaneous endoscopic gastrostomy
PLF	Predicted liver fibrosis
PLT	Platelets
PPI	Proton pump inhibitor
PPV	Positive Predictive Value

pSWE	Point shear wave
LiRADS	Liver Reporting & Data System
RBC	Red blood cells
RECIST	Response evaluation criteria in solid tumours
RF	Radio frequency
RFA	Radiofrequency ablation
RNA	Ribonucleic acid
ROC	receiver operating characteristic curve
SCD	Sickle cell disease
SCLD	Sickle cell liver disease
SPH	Significant portal hypertension
SVR	Sustained virological response
SVR12	SVR after 12 weeks
SVR24	SVR after 24 weeks
SVR48	SVR after 48 weeks
TACE	Transcatheter arterial chemoembolization
TGF	Tissue growth factor
TIA	Transient ischaemic attack
TIPS	Transjugular Intrahepatic Portosystemic Shunt
TJ	Transjugular
UDCA	Ursodeoxycholic acid
UKELD	United Kingdom Model for End-Stage Liver Disease
ULN	Upper Limit of normal
US	Ultrasound
VCTE	Vibration Controlled Transient Elastography
VEGF	Vascular endothelial growth factor

List of Figures

List of Tables

1. <u>Introduction</u>

1.1 <u>Liver Fibrosis</u>

Here I shall detail the prevalence and pathophysiology of liver fibrosis and cirrhosis to better understand the nature of the disease as well as its clinical importance. Finally, I shall look at the new work examining its reversibility and the reasons why diagnosis is so important.

1.1.1 <u>Epidemiology</u>

Liver fibrosis describes the deposition of scar tissue within the liver, which initially occurs as a healing response. As this process persists, collagen deposition impedes normal liver function and eventually leads to the disruption of normal liver structure, formation of regenerative nodules and distortions of hepatic vasculature; this is defined as cirrhosis and may lead to portal hypertension with associated complications and eventually death.

Cirrhosis can result from various aetiologies that are not mutually exclusive and may accelerate fibrosis formation. Exact estimates for prevalence are difficult to quantify due to the indolent nature, meaning many patients either go undiagnosed or are advanced upon presentation. National Institute for Clinical Excellence (NICE) estimates that in the UK, liver fibrosis affects 600,000 people, with 10% or 60,000 suffering from cirrhosis [1, 2], though even this volume is controversial due to the manner in which it is coded [3]. Worldwide there are estimated 1.03 million deaths per year from liver disease with 170,000 deaths per year occurring in Europe [4-6].

1.1.2   Causes

Any disease affecting the liver may damage it. The commonest causes of cirrhosis in the UK are viral hepatitis, non-alcoholic fatty liver disease, or alcohol-related liver disease. Systemic diseases such as haemochromatosis, Wilson's disease, primary biliary cirrhosis, sclerosing cholangitis, Budd-Chiari syndrome or cystic fibrosis may also affect the liver. Several genetic diseases such as autoimmune hepatitis, alpha-1-antitrypsin deficiency, sickle cell anaemia and finally, medications. It is important to note that cirrhosis may take many years to develop and symptoms may not be evident until the late stages of diseases, so all patients suffering from these diseases should be screened for cirrhosis and followed up regularly [7].

1.1.3   Pathophysiology

There are various genetic factors which may aid in the rate of fibrosis progression and include Interferon gamma receptor 2 (IFNGR2) [8] and PNPLA3 gene [9, 10] among others. Once wound healing within the liver is triggered, various cytokines and growth factors such as tissue growth factor β (TGF-β), hepatic stellate cells and portal or perivascular fibroblasts differentiate into myofibroblasts which produce an increase in extra-cellular matrix from collagens I, III, IV, glycoproteins, elastic fibres and various glycans. In normal circumstances, proteolytic enzymes such as matrix metalloproteinases (MMPs) 1, 3 and 13 break down and remove these structures. As repeated damage occurs, fibrogenesis becomes favoured and tissue inhibitors of MMP (TIMPS) types one and two are produced. Over time, this leads to restructuring of liver architecture with the production of regenerative nodules, the obstruction of sinusoidal fenestrations and thus decreased access to hepatocytes, (capillarisation), and the loss of hepatocyte microvilli [11-16]. This eventually leads to vascularised fibrotic septa linking portal tracts with each other as well as portal veins and

leaving hepatocyte islands surrounded by these septa as well as fibrosis of the central veins. As this develops, fewer functional hepatocytes are available and fibrosis increases to form thick inter-nodular septum with small nodules.

The increase in intrahepatic vascular shunts associated with fibrosis is exacerbated by the insufficient release of vasodilators such as nitric oxide with increased vasoconstrictors such as adrenergic stimulations, thromboxane A2, activation of the renin-angiotensin system and anti-diuretic hormone [4, 17]. The combination of increased intra-hepatic vascular resistance, along with increased splachnic blood flow, results in portal hypertension; a major complication of cirrhosis [4, 18].

1.1.4   Clinical Manifestations of cirrhosis

As portal pressure builds, the increased pressure causes resultant splenomegaly, which may manifest in reduced platelet count; reduced serum albumin levels imply worsened hepatic synthetic function. Even at this stage, a patient may remain well, and only when the liver function starts to fail, in decompensated cirrhosis, do symptoms and signs become apparent. Patients may show jaundice indicating liver failure or clinical signs of portal hypertension such as ascites or present with complications such as variceal bleeding and hepatic encephalopathy. Liver failure may also affect other systems, such as renal or respiratory systems. Cirrhotic patients are also at increased risk of developing hepatocellular carcinoma (HCC) [19] and therefore should undergo screening every six months in accordance with international guidelines. [20, 21]. [4, 7]

Once patients are at risk of chronic liver disease or cirrhosis, they should be staged to ascertain how advanced the disease is. The most important part for treatment is the correction

of the causative aetiology and early consideration for liver transplantation. Cirrhosis was previously considered irreversible, but this has recently been challenged and increases the significance of early diagnosis. [4]

1.1.5   Cirrhosis Staging

1.1.5.1   Histological Grading

The grading of liver damage allows planning of patient treatment and ongoing surveillance if required. This can be performed either histologically or with regard to patient status. Histologically various scoring systems have been described, such as the Knodell scoring system, Scheuer and Batts- Ludwig but the most popular are Ishak and METAVIR systems [15, 16, 22-25]. The METAVIR system is described for Hepatitis C and considers an activity score ranging from no activity to severe activity and is coupled with the more frequently quoted fibrosis stage of:

• F0—No fibrosis.

• F1—Portal fibrosis without septa.

• F2—Portal fibrosis with few septa.

• F3—Numerous septa without cirrhosis.

• F4—Cirrhosis.

The problem with this system is the lack of differentiation for cirrhotic patients and thus, some express a preference for Ishak scoring of:

• 0 No fibrosis

• 1 Expansion of some portal areas with or without septa

• 2 Expansion of most portal areas with or without septa

• 3 Expansion of most portal areas with occasional portal to portal bridging

• 4 Expansion of portal areas with marked bridging (portal-portal and/or portal-central)

• 5 Marked bridging with occasional nodules (incomplete cirrhosis)

• 6 Cirrhosis, probable or definitive

1.1.5.2 Patient status Grading

Various scoring systems have been produced for the clinical staging of fibrosis and particularly cirrhosis, with many of them developed for hepatitis C. The Childs-Turcotte-Pugh grade [26] is the most widely used. First described in 1964, it uses clinical symptoms in addition to blood tests to provide one-year prognoses for cirrhotic patients. It is graded at 3 levels, A, B and C and gives 1-year survivals as A - 100%, B - 80% and C - 45% [27]. For classification purposes, A is defined as compensated cirrhosis, with B and C being decompensated. Due to the use of ascites and encephalopathy grades, Childs-Turcotte-Pugh has been seen by some as subjective and thus inherent to errors, hence several other algorithms have been produced.

One of these is the model for end-stage liver disease (MELD) score. The MELD score was initially derived for stratification of patients requiring transjugular intrahepatic portosystemic shunt procedure but has since been validated in multiple liver pathologies and is widely used to stratify and prioritise patients for liver transplantation due to its excellent prognostic predictions for three-month survival [28, 29].

1.1.6 **Cirrhosis reversibility**

Following the removal of the causative agent from the liver, regression of fibrosis and cirrhosis is seen to occur. This is prevalent in hepatitis C following achievement of sustained virological response (SVR) and hopefully with other diseases being brought under control, such as in cystic fibrosis (CF) and sickle cell disease (SCD). Following removal of the causative injury, pro-fibrotic chemokines are reduced with a reduction of myofibroblasts, whilst restorative chemokines such as MMP are produced with an increase in macrophages, dendritic cells, NK cells and gamma delta T cells [30-34]. Finally, the production of new functional vessels is important and accordingly, there is an increase in vascular endothelial growth factor (VEGF) and others such as CXCL9 [35, 36].

These features have been described histologically by Wanless et al. as perforated septa, independent microcirculation per nodule, reduction in portal tract collagen and moving towards macronodular cirrhosis [37]. These are termed as the hepatic repair complex and have been built on by Sun et al. to produce the Beijing Criteria to classify patients as either definitely progressive, indeterminate or definitely regressive, and they have proposed how this can be used for different disease states. [38, 39].

Overall, this regression of liver disease is of the utmost interest in ongoing hepatic care as the opportunity to reverse the ill effects could be revolutionary for patients.

1.2   Liver tests

1.2.1   Introduction

For the appropriate treatment of chronic liver disease, knowledge of the extent of fibrosis is essential, alongside the ability to screen and diagnose the complications from advanced disease, such as hepatocellular carcinoma. Here I shall detail various technologies used from the current gold standard of liver biopsy to various standardised tests of ultrasound, computer tomography and magnetic resonance imaging and the various ways and physics behind elastography of the liver. Finally, we shall cover functional liver tests currently in use, from indocyanine green to breath and urine tests.

1.2.2   Liver biopsy

Liver biopsy is the current gold standard for achieving a histological diagnosis of fibrosis. This test is invasive with risks of bleeding and inadvertent damage of surrounding structures; patients with ascites and thrombocytopenia due to liver disease are particularly at risk. Interpretation may be limited by the amount of tissue obtained. However, even good-sized liver fragments represent only a minority of liver tissue and may not be representative {Ratziu, 2005 #2628}. Practically, liver biopsy requires pre-procedure patient assessment, post-procedure recovery and is therefore an expensive test.

1.2.3   Non-invasive assessment of liver fibrosis

Several techniques have been used to try and achieve the goal of fibrosis assessment without the need for biopsy. These range from using basic imaging modalities which are currently used, such as ultrasound, computer tomography (CT) and magnetic resonance imaging

(MRI), to using more specific technologies such as elastography to amalgamating various blood tests to produce algorithms and scores. These are to aid clinicians in stratifying these complex patients to provide the best treatment at the best time.

1.2.3.1 **Ultrasound**

Ultrasound technology utilises sound waves of a frequency higher than can be heard by the human ear. This gives a non-invasive, painless view of the body which can be seen in real-time and is considered safe and relatively inexpensive. The machines used are also generally portable, thus can be performed either at the patient's bedside or in a well-organised clinic.

Ultrasound image is produced by sound waves being induced into the body by a transducer. Reflections or echoes of sound waves passing through the body are detected by the transducer probe. The time taken for the wave to return and the relative strength and volume of waves returned, as waves pass through substances of different acoustic impedance – such as air or tissues – is processed as images of different brightness.

Within liver disease, there are multiple qualitative indicators which may indicate cirrhosis or resultant portal hypertension. The liver may be seen to be initially enlarged during hepatitis or show coarsened echogenicity [40]. A small liver, increased nodularity, or irregular margins are hallmark signs of a cirrhotic liver. Increased venous pre-load of the liver creates signs of portal hypertension that can be seen on the US; these are large collateral veins, increased thickness of the lesser omentum, splenorenal anastomosis, splenomegaly and ascites. Portal blood flow may also be seen using doppler or B-flow modes and may thus indicate thrombosis. US scans are very good at the detection of various lesions within the liver, such

as cysts, haemangiomas or cancers; these may be further investigated by cross-sectional imaging, which we shall discuss in the following sections [41, 42].

Ultrasound scans are quick, cheap, painless, real-time, and usually the first-line test for imaging the liver. There are, however, downsides, such as the system being very user dependent both in being able to produce the images as well as the correct interpretation of images and therefore may be unreliable, especially with small defects or lesions as these may be missed. The examination may be hampered for various reasons, such as the patient's body habitus or overlying bowel gas casting a shadow behind and thus making views challenging to obtain. Ultrasounds are unable to penetrate past dense tissues such as bone and therefore, if a patient has a small shrunken liver which is mainly underneath the rib cage, it can be challenging to obtain adequate views; unfortunately, these are the cases requiring the closest monitoring. Ultrasound scans are qualitative in nature and are further hindered as some of these signs show variation for different people, thus making diagnosis difficult and possibly not matching the clinical picture [43]. Hence accurate assessment of fibrosis is not possible with ultrasound, although in advanced cirrhosis, the diagnosis can usually be established with reasonable accuracy. For these reasons, there has been a drive towards imaging modalities which allow quantification of fibrosis and reproducibility, with the primary way being elastography which will be detailed subsequently. Also, images do not give an indication of liver function and various tests have been produced for this. However, as detailed above, ultrasound continues to play a central role in HCC surveillance with excellent attributes.

1.2.3.2 Computer tomography and Magnetic Resonance Imaging

Computer tomography (CT) scanning is a cross-sectional imaging modality using X-rays used to view organs within the body. Magnetic Resonance Imaging (MRI) utilises the manipulation of the magnetic moment found within hydrogen atoms to produce a cross sectional image of the body.

CT scans can now be performed very quickly and images of the abdomen are taken within a single breath hold. CTs are generally available in most hospitals though the scans are more expensive and more difficult to organise than ultrasound scans. The major disadvantages are radiation exposure, artefacts and cost.

For the diagnosis of cirrhosis and distinction between fibrosis, CT has shown a diagnostic accuracy of 71.9%, sensitivity of 77.1% and specificity of 67.6%, with the use of collateral vasculature, nodular liver surface and caudate vs right lobe ratios being the best individual predictors [44, 45]. As US has a sensitivity of 66%, one would not use CT as a diagnostic tool specifically for cirrhosis, though it may be diagnosed if a scan is performed for another aetiology. CT may also show other signs of cirrhosis, such as splenomegaly.

For the diagnosis of primary liver cancers, especially HCCs, both CTs and MRIs may be used.

For patients with suspicion of HCC or patients with advanced cirrhosis in whom US has proven difficult, MRI is a useful non-ionising imaging form, which is deemed gold standard for HCC diagnosis and localisation [42, 46]. Diagnosis of HCC in cirrhotic patients is by non-invasive guidelines and based upon the vascularity of HCC (detailed above). This is

made prominent following administration of an intravenous contrast such as gadobenate

dimeglumine (MultiHance®) or gadoxetic acid (Primovist®) with follow-up to demonstrate

washout from the lesion.

1.2.3.3 **Shear wave elastography**

1.2.3.3.1 **Introduction**

Classical scans do not reliably identify liver fibrosis [43, 47, 48] and a better test is required.

Elastography is defined by the European Federation of Societies for ultrasound in medicine

and biology (EFSUMB) as "a type of remote palpation that allows measurement and display

of biomechanical properties associated with the elastic restoring forces in the tissues that act

against shear deformation" [49]. This aims to unify various iterations of elastography,

whether via a quasi-static or dynamic method of production, as well as the output method,

whether this is graphical and therefore qualitative or numerical and therefore quantitative.

1.2.3.3.2 **Strain elastography**

Strain elastography utilises movements within the body either due to physical palpation on

the body or internal physiological movements to produce a non-quantitative image [50].

1.2.3.3.3 <u>Elastography Physics</u>

Elastography was first postulated by Sarvazyan et al. [51]. The over-arching premise for elastography is, tissue which is stiffer moves less when a force is applied; known as Hooke's Law. The movements induced are slight and its measurement is by sensitive techniques, notably ultrasound or MRI, with either graphical or numerical output.

Two types of waves propagate through tissue; high frequency compressional waves are longitudinal and transmit via the compression and extension of adjacent tissues in the direction of movement. These ultrasound waves are shown as a change in position frame-to-frame in a graphical manner [49, 52-54] (Figure 1.1a). Alternatively, low frequency shear waves are sinusoidal and transmit via perpendicular movement of tissues in the direction of movement [49, 52-54] (Figure 1.1b).
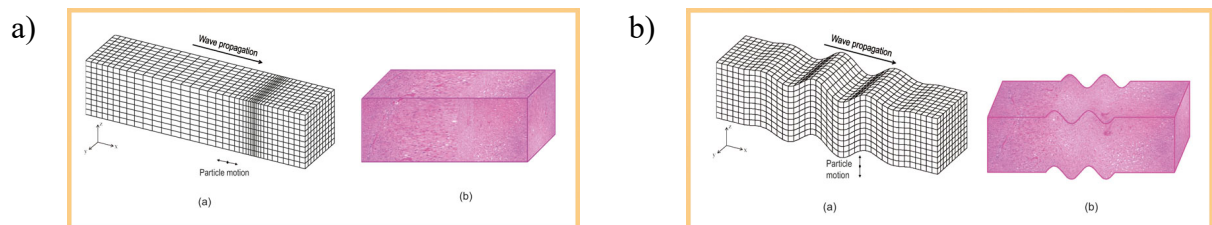
Figure 1.1: Types of waves instituted through tissue a) a compressional wave propagates along the tissue, whereas b) displays a shear wave, which propagates at 90 degrees transverse to the tissue [53]

Shear waves cause movement and slight deformation within liver tissue and this speed can be measured. When Hooke's Law and Young's modulus are applied a reading for liver stiffness can be given [52, 53, 55-57]. Further details can be found in Appendix 1.

1.2.3.3.4 <u>Transient elastography (Fibroscan®)</u>

Vibration Controlled Transient Elastography (VCTE) is a technology sold through Echosens® as Fibroscan®. This self-contained system utilises transient shear waves and ultrasound imaging to provide a numerical value based upon the speed of shear wave propagation through tissue (see Figure 1.2) [58, 59]. Further details can be found in Appendix 1.

Following the introduction of TE in 2003 [55], a failure rate of 2-10% was reported; usually related to obesity [60-62]. To overcome this, an XL probe was designed, producing lower frequency and greater amplitude waves with deeper focal length [63]. Although the manufacturer's guidelines suggest there is no difference between these probes, several guidelines recommend using a cut-off of 1.5kPa lower for the XL probes [52, 53, 64].
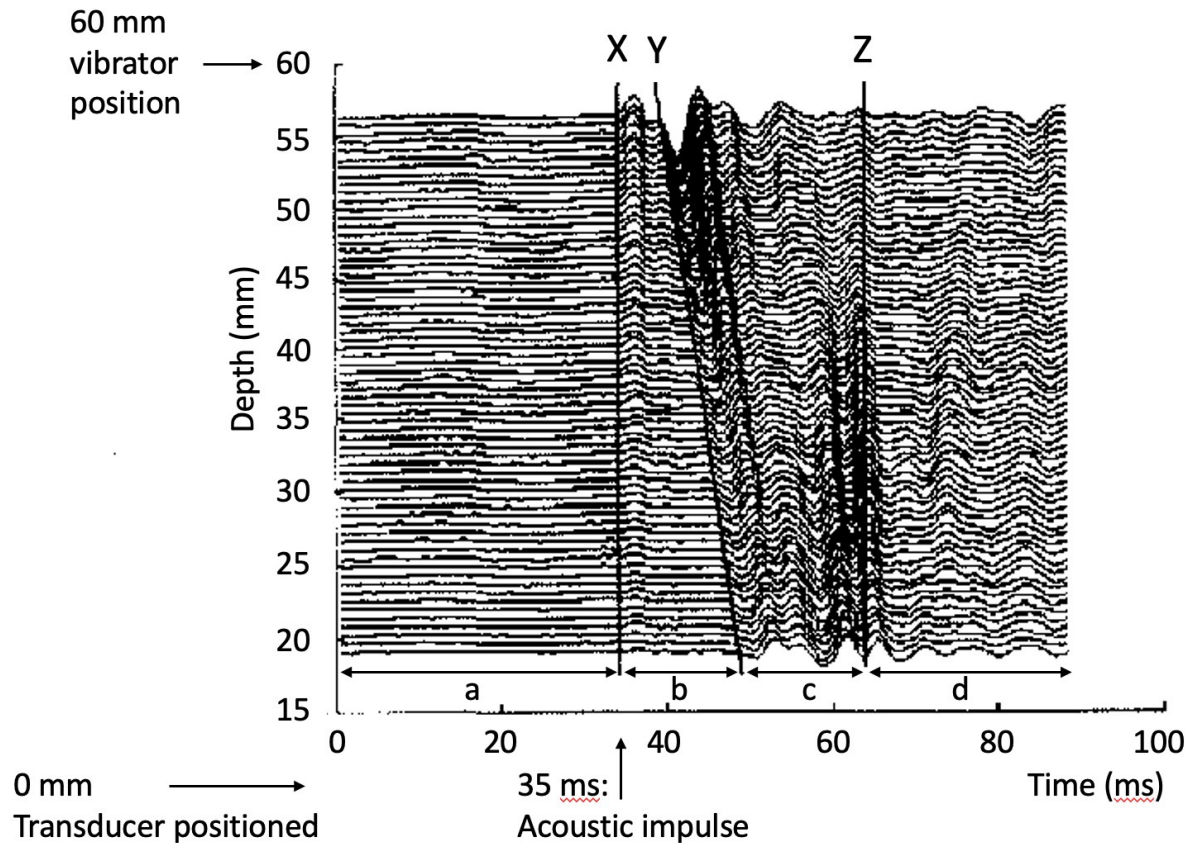
Figure 1.2: Shear wave propagation through tissue shown as displacement at varying depths against time. Zone a - the transducer is placed on the patient with tissues at rest. At 35ms, vibration is introduced to the tissue. Zone b is the region showing compressional wave displacement. Line Y indicates where the shear wave first transitions through the medium, as can be seen with the displacement throughout all layers. Zone c shows displacement by the shear waves and is the transient area used for calculating transient elastography. Line z delineates the region where measurements can no longer be taken due to reflections from boundaries, as seen within zone d, termed backscatter. mm = millimetre, ms: millisecond [65, 66].

Putting all of these together, Fibroscan® measurements should be taken between intercostal spaces, with the A-mode (amplitude mode) used to avoid a large skin to liver capsule length and ensure there are no obstructions i.e. roughly a straight line. The M-mode (motion mode) plots sequential A-mode images and can be used similarly. When the shear wave is discharged manually an elastogram is produced, depicted as a black portion within this image. As the x-axis is time, the faster the shear wave moves through the tissue, the more vertical this section is. A linear regression is automatically plotted and if the value is below r=0.85, this reading is deemed unreliable [55]. 10 valid measurements should be taken with no more than an extra three invalid measurements within this run read as IQR/M. Luciderme et al. showed an IQR/M of over 0.21, leading to an over-estimation of fibrosis [67].

Validation of Fibroscan® in patients began in 2004 by Ziol et al., who performed a multicentre, blinded trial where 251 HCV-positive patients underwent liver biopsy and Fibroscan® within six months. These were compared by independent pathologists using the METAVIR scoring system and in cases of discrepancy reviewed for steatosis and activity. This showed AUROC values of 0.76 for F2 and 0.93 for F4 with positive predictive value (PPV) and negative predictive value (NPV) of 56% and 88% for F2 and 97% and 78% for F4 [68]. This study included those cross-infected with hepatitis B and HIV. A further study by Platon et al. excluded these patients, though did include less stringent biopsy volumes of six tracts as opposed to the ten required by Ziol. This allowed for a much larger cohort of 1,202 patients with corresponding higher AUROC values of F>= 1 - 0.879 and F4 - 0.97 though they also used slightly lower cut-off values of 9.1kPa for F3 and 13.2kPa for F4. The PPV and NPV for F2 were 90.1% and 70.2% and for F4 86.5% and 97% respectively [69]. Colletta et al. reported on forty patients with early liver disease following HCV diagnosis. These patients had two liver biopsies at a median period of 6.5 years apart, with the second coupled with a Fibroscan®. They found a good correlation between low-grade fibrosis and Fibroscan® scores, which also decreased the need for repeat biopsies in these patients [70]. A meta-analysis by Friedrich-Rust et al. looked at fifty studies comparing TE to liver biopsy and reported a corrected AUROC for F2 of 0.91 and F4 was 0.99 [71]. The most standardised values used for fibrosis and cirrhotic values are taken from a paper by Castera et al., which compares the use of Fibroscan® with serum tests such as Fibrotests and APRI scoring. They found Fibroscan® to be most accurate, with values for F3- 9.5kPa and F4 of 12.5kPa. They showed a sensitivity of 87% and a specificity of 91% for patients with F4 staged liver disease. Again, these patients were all HCV-exclusive patients with their scans correlated with biopsies [72]. Afdhal et al. tried to further address this problem, performing a 2-phase study and found a value of 12.8 showed F4 patients with 76% sensitivity and 85% specificity

[73] and these approximate values were confirmed when looking at a further 200 Italian patients [62]. Two meta-analyses looked at 9 and 40 studies, respectively, and found sensitivities of 87% and 83%, with specificities of 91% and 89% for cirrhotic patients [74, 75]. It is noted that many of the patients used in these studies are HCV positive, so some caution should be used when applying TE to other diseases. However, this was assessed by the American Gastroenterological Association Institute, which recommended the cut-off of 12.5 kPa (+/-1) though this was based on a selection of studies which used this cut-off and therefore supply an inherent bias. Nevertheless, their reasoning for this was the ongoing effect of incorrect diagnosis being kept below 5% for high-risk individuals being falsely reassured. For decompensated cirrhosis, a value of 19.5 (+/-2) was felt to be the point for patients to receive an OGD for oesophageal varices diagnosis [76]. Baveno VI guidelines dictate the use of <20kPa with a platelet count of <150,000 as having a very low risk for oesophageal varices and can, therefore, avoid having gastroscopy [77]. These cut-offs are true for HCV cirrhosis; however, lower cut-offs for Hepatitis B Virus (HBV) have been suggested for 10kPa [78, 79].

Fibroscan® has been shown to be an easily trainable modality. Initially, operators undergo a 4-hour training session with an Echosens® representative. Following this, training on healthy individuals and then pathological scans are performed. The most significant increase in competence is following ten scans, though this does steadily increase with acceptable performance after 50-60 scans, with most definitions of an experienced operator having performed over 500 scans [80-85].

Another strength of Fibroscan® is the inter-observer reproducibility, shown to be in the region of 98% in a small study of 26 patients [73]. This level was agreed upon by Fraquelli et

al., who looked at 200 patients receiving liver biopsies and repeat scans [62] and this value was further amplified by a Romanian study [86]. Boursier et al. found that it was slightly lower at 93%. This study used four experienced judges, split into 2 separate groups. One important outcome was following the categorisation of liver stiffness scores to the METAVIR equivalence, a much greater discrepancy appeared of around 15% though they did find this was higher at the F2 level with the lowest discrepancy at the higher F4 level, which goes with many properties for Fibroscan® [87].

The cost-effectiveness of TE has been examined in several studies and was brought together in a recent systematic review looking at eight overall studies, five of which came from the UK. They concluded that TE was cost-effective, especially on a healthcare system scale, particularly in more advanced disease, when compared to liver biopsy. This was true mainly for HCV and most likely for hepatitis B though further work is needed as well as for other diseases [88].

TE has many positives, satisfying many of the virtues we look for in giving an easily trainable system which provides a quantitative readout which is reproducible as well as being painless, quick and cheap for the patient, whilst ensuring an instantaneous result which may be used to affect treatment immediately for patients. However, there are still drawbacks to this technology. Primarily, the Fibroscan® machine is incapable of performing a 2D imaging ultrasound scan with B mode readout of the patient's abdomen, which is required for all cirrhotic patients on a 6-monthly basis. This may require patients having to attend multiple appointments. Additionally, measurements taken are of a shallower depth than may be attempted by other methods. Also, as the mechanical shear waves cannot pass through a liquid, this thus renders TE unusable if the patient has ascites. Due to several other causes of

ascites, such as portal thrombosis, malignant ascites and renal fluid retention, the ability to rule out liver cirrhosis is removed. Active and particularly acute hepatitis has an effect on liver stiffness, likely due to congestion of the liver parenchyma with oedematous fluid; meaning scans performed on patients with transaminases over five times the normal limit should be interpreted with caution and repeated when these have reduced [89-93]. Similarly, this is also true for right-sided heart failure [94] and extra-hepatic cholestasis [95]. Also, the ingestion of food within the preceding two hours causes a falsely raised TE result, so this is actively discouraged [96, 97]. The results gained are user-dependent as technique is important; the probe should be placed perpendicular to the skin, or if incorrectly positioned, it may affect results with the only way to recheck the results is test repetition. Following introduction of the XL probe, more obese patients can receive a valid TE measurement though there continues to be a proportion where this is still unsuitable. Finally, patients with small intercostal spaces may have difficulty or pain due to the mechanical nature of the shear wave production, and in the worst cases, the ribs may affect the readings by deflecting shear waves, giving an incorrect result. A small probe has been manufactured to assist with this, although this is mainly used in the paediatric setting.

1.2.3.3.5 <u>Shear wave elastography (pSWE)</u>

All other types of ultrasound shear wave elastography are based on the principles of acoustic radiation force impulse (ARFI) being produced in various ways and detected by ultrasound [98, 99]. Waves are focused at a specific point then converted to mechanical energy, producing a small amount of movement within the tissues. Shear waves move away from the focus point and when this is taken for a single ARFI impulse, this is termed a point shear wave elastography (pSWE) with readings given in real-time over an ultrasound image. The

advantage over TE is the region of interest may be manipulated and so areas such as vessels and ascites may be avoided. Also, images may be stacked to produce a 3D reading. The downside for pSWE is the production of heat, meaning only three readings may be taken.

The readings for pSWE measurement are given in metres per second (m/s) and range from 0.5-4.4m/s. As stated by Friedrich-Rust et al., after a meta-analysis of eight studies where the individual patient's data was requested, F2 was deemed to be <1.34m/s and for F4 to be 1.8m/s with sensitivities of 79% and 92% and specificity of 85% and 86% respectively [98]. These values were refined to 1.35m/s and 1.87m/s in a larger systemic review two years later [100]. A large multicentre international study looked at 914 patients with HCV and correlated with liver biopsies to find valid measurements in 99.6% off cases with cut-off values of 1.37 (+/-0.48)m/s for F2 and 2.23 (+/- 0.71)m/s for F4 with a correlation of 0.65 (p<0.01) [101].

1.2.3.3.6 <u>Supersonic Shear Imaging (SSI)</u>

Supersonic shear imaging (SSI) utilises ARFI waves at differing depths to form a Mach cone which is then read on US imaging and elastography calculated (Figure 1.3).
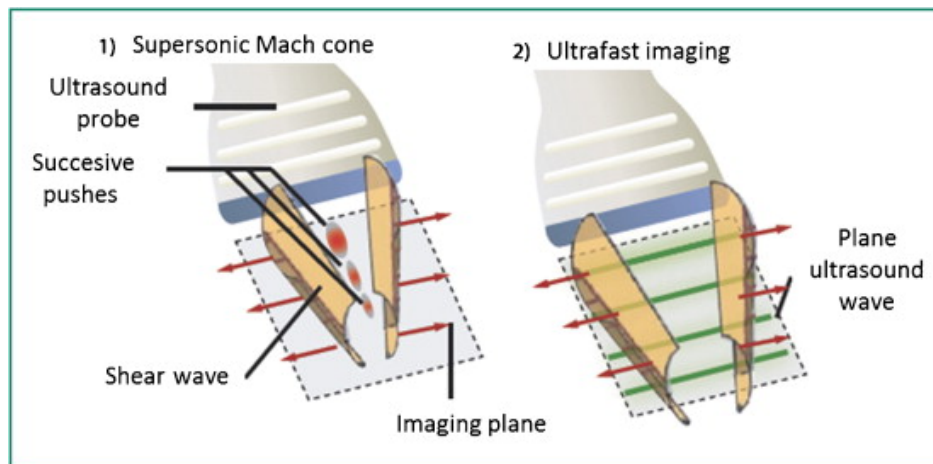
Figure 1.3: Production of Supersonic shear waves (SSI). Ultrasounds are successively focused at different depths to create pushes by radiation pressure. The constructive interferences of shear waves form a supersonic Mach cone (in which the speed of the source is greater than the speed of the generated wave) and a quasi-plane shear wave is created; 2: the ultrasound machine then switches into an ultrafast imaging mode to follow the shear wave that is propagating through the medium. [50]

Recently, Herrmann et al. performed a pooled meta-analysis of 1,134 patients from 13 sites using histology as the gold standard and showed cut values for F2 of 7.1 and F4 of 13.0 with sensitivities of 94.8% and 79.4% with specificities of 39.9% and 83.6% respectively with comparable results to TE [102].

1.2.3.3.7 <u>Magnetic resonance elastography (MRE)</u>

Magnetic resonance elastography (MRE) is based upon MRI technology but amalgamates the basics of elastography which we have explored already. MRE requires three steps; the production of shear waves by a driver system, the acquisition of wave motion data and processing of inversion algorithms to produce images for analysis. Positives for MRE are that the whole liver may be imaged, reducing sampling bias, may be used despite ascites and images are stored and so may be re-evaluated for disease progression. The limitations of MRI persist, including expense, acceptability to patients and metalwork. Additionally, scores are

produced following manual delineation and are thus prone to miscalculation and have limited use in iron-overload states e.g. SCD. [103, 104]

MRE has been evaluated within a pooled meta-analysis by Singh et al., who acquired data from an overall 531 patients from 12 studies. They found cut-offs for F2 as <3.66kPa and F4 as>4.71kPa with sensitivities of 73% and 91% and specificities of 79% and 81%, respectively. The AUROC was also calculated as 0.84 for F1 and 0.92 for F4 [105]. These overall values closely reflected early studies into MRE [106].

1.2.3.3.8 <u>Fibrosis serum tests</u>

Numerous algorithms based upon routine blood tests have been produced to diagnose fibrosis and cirrhosis. Several proprietary tests have been marketed, such as Fibrotest, Fibrometer and Enhanced Liver Fibrosis score (ELF). Algorithm-based tests are cheaper and easier to calculate than proprietary tests and many have been validated for HCV. However, due to their nature, they contain many markers which are not liver-specific and thus susceptible to derangements from other diseases or states such as rheumatoid arthritis, Gilbert's syndrome or those causing derangements to platelets or AST [107-109]. They are, however, less likely to fail than ultrasound-based tests. There is a similarity because they are better at diagnosing cirrhosis than earlier stages of fibrosis. The AST to platelet ratio (APRI) was developed in 2003 [110] and a large meta-analysis performed in 2011, involving 8,739 patients from 40 studies found a cut-off or 0.7 to be indicative of significant fibrosis (sensitivity - 77%, specificity - 72%) and above 1.0 showing cirrhosis (sensitivity - 76%, specificity - 72%) with higher values also having higher positive predictive values [111]. Chou et al. performed a meta-analysis, finding most having AUROCs of between 0.52-0.86 with ELF, Fibrometer

and FIBROspect II to be most accurate for fibrosis. All tests fared better in diagnosing

cirrhosis with AUROCs of 0.65-0.91 with platelet count, Hyaluronic acid, ELF, Hepascore

and Fibrometer [112]. Below are a selection of different tests and algorithms used in HCV as

adapted from EASL clinical guidelines [113, 114] (Table 1.1).

Table 1.1: Various serum liver tests, including AUROC, sensitivities and specificities. Adapted from EASL-ALEH guidelines [113]

Test name	Constituents of test	AUROC	Sensitivity (%)	Specificity (%)
Fibrotest® (Biopredictive, Paris, France)	α-2-macroglobulin, γGT, apolipoprotein A1, haptoglobin, total bilirubin, age and gender	0.87	75	85
Forns Index	7.811 - 3.131 x ln(platelet count) + 0.781 x ln(γGT) + 3.467 x ln(age) - 0.014 x (cholesterol)	0.81	30-94	51-95
AST to Platelet Ratio (APRI)	AST (/ULN)/platelet (10^9/L) x 100	0.8	41-91	47-95
FibroSpectII® (Promotheus Laboratory Inc, San Diego, USA)	α-2-macroglobulin, hyaluronate and TIMP-1	0.83	77	73
MP3	0.5903 x log(PIIINP [ng/ml]) - 0.1749 x log(MMP-1 [ng/ml])	0.82	35-65	85-96
Enhanced Liver Fibrosis score® (ELF) (Siemens Healthcare, Erlangen, Germany)	age, hyaluronate, MMP-3 and TIMP-1	0.78	87	51
Fibrosis Probability Index (FPI)	10.929 + (1.827 x Ln[AST]) + (0.081 x age) + (0.768 x past alcohol use*) + (0.385 x HOMA-IR) - (0.447 x cholesterol)	0.77	42-85	48-98
Hepascore® (PathWest, University of Western Australia, Australia)	bilirubin, γGT, hyaluronate, α-2-macroglobulin, age and gender	0.82	63	89
Fibrometer® (Echosens, Paris, France)	platelet count, prothrombin index, AST, α-2-macroglobulin, hyaluronate, urea and age	0.89	80	84
Lok index	LogOddsLok = (1.26 * AST / ALT) + (5.27 * INR) - (0.0089 * Platelets) - 5.56 LokIndex = e(LogOddsLok) / (1 + e(LogOddsLok))	0.81	40-98	53-99
Goteborg University Cirrhosis Index (GUCI)	AST x prothrombin - INR x 100/platelet	0.85	80	70
Virahep-C model	-5.17 + 0.20 x race + 0.07 x age (yrs.) + 1.19 ln(AST [IU/L]) - 1.76 ln(platelet count [103 /ml]) + 1.38 ln(alkaline phosphatase [IU/L])	0.83	51-90	54-90
Fibroindex	1.738 - 0.064 x (platelets [104 /mm3]) + 0.005 x (AST [IU/L])	0.83	30-40	97

	+ 0.463 x (gamma globulin [g/dl])			
Fib-4	age ([yrs.] x AST [U/L]) / ((PLT [10(9)/L]) x (ALT [U/L])(1/2))	0.7	23	96.6
HALT-C model	-3.66 - 0.00995 x platelets (103 /ml) + 0.008 x serum TIMP-1 + 1.42 x log(hyaluronate)	0.81	47-88	45-92
King's Score	age x aspartate aminotransferase x international normalised ratio / platelets	0.79	86	80

γGT: Gamma-glutamyl transferase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, INR: International normalised ratio APRI: AST to Platelet Ratio, ln: Natural log, ULN: Upper limit of normal, TIMP: TIMP metallopeptidase inhibitor, PIIINP: Type III Procollagen Peptide, MM: Matrix metalloproteinase, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, yr: Year, AUROC: area under the receiver operating characteristic, %: Percentage

1.2.3.3.9 <u>Comparison of shear wave elastography techniques</u>

As we have seen, the challenge of quantifying liver fibrosis has been attacked from various angles, each with its positive and negative features. The comparison of these has occurred through multiple studies to try and delineate the best. TE is the oldest of all of these so has been validated the most and across the most diseases whilst also being used as the gold standard in certain studies. Other ultrasound imaging techniques have the same problems as TE with regard to food intake [115]. Several studies have been outlined in Table 1.2.

Table 1.2: Table of studies comparing shear wave elastography techniques

Study Name	Modalities tested	Type of study	Findings	Comment
Bota et al. [116]	TE vs pSWE vs *Biopsy	Meta-analysis	**TE** – FR – 6.6%, Se – 89%, Sp – 87% **pSWE** – FR – 2.1%, Se – 87%, Sp – 87%	Non-pooled analysis
Sporea et al. [101]	TE vs pSWE vs *Biopsy	Meta-analysis	**TE** – Se – 93.7%, Corr – 0.728, AUROC – 0.87 **pSWE** – Se – 98.8%, Corr – 0.689, AUROC – 0.93	
Friedrich-Rust et al. [117]	TE vs pSWE vs *Biopsy	Single centre	**TE** – Corr – 0.73, AUROC – 0.91 **pSWE** – Corr – 0.71, AUROC – 0.95	
Rizzo et al. [118]	TE vs pSWE vs *Biopsy	Single centre	**TE** – FR – 6.5%, F2 AUROC – 0.78, F4 AUROC – 0.8 **pSWE** – FR – 0%, F2 AUROC – 0.86, F4 AUROC – 0.89	
Cassinotto et al. [119]	TE vs pSWE vs *Biopsy	Single centre	**TE** – FR – M probe - 11.2%, XL probe – 2.3%, AUROC – 0.91 **pSWE** – FR – 0%, AUROC – 0.88	All aetiologies
Multiple studies [120-122].	TE vs pSWE		Corr = 0.72-0.92, p <0.001	

Vermehren et al. [123]	TE vs pSWE	Single centre	**TE** – FR – 18% **pSWE** – FR – 0%	-Patients with ascites -Neither indicate the presence of varices
Poynard et al. [124].	TE vs SSI vs Fibrotest	Single centre	**TE** – FR – 9.5% **SSI** – FR – 14% **Fibrotest** – FR - 2.1%	
Elkrief et al. [125]	TE vs SSI vs *Biopsy	Single centre	**TE** – FR – 66%, AUROC – 0.78 **SSI** – FR – 3%, AUROC – 0.87	End stage liver disease
Sporea et al. [126]	TE vs SSI vs pSWE vs biopsy	Single centre	**TE** – Se – 72.2% **pSWE** – Se – 92.1% **SSI** – Se – 71.3%	
Degos [127]	TE vs Fibrotest, Fibrometer, APRI and Hepascore,	Multi-centre	**TE** – cirrhosis AUROC – 0.9, F2 AUROC – 0.76 **Serum tests** – cirrhosis AUROC – 0.77-0.86, F2 AUROC – 0.72-0.79	More serum tests performed than TE
Boursier et al. [128]	TE vs Fibrometer	Single centre	**TE** – Se – 63.3% **Fibrometer** – Se – 69.7% **Combination** – 86%	
Bende et al. [129]	2D-SWE vs *TE	Single centre	**2D-SWE – F2** – AUROC – 0.95, Se –	Similar Failure rate

			92.7%, Sp – 85.5% **2D-SWE – F4** – AUROC – 0.96, Se – 91.7%, Sp – 92.5% Corr – 0.83	
Guo et al. [130]	pSWE vs MRE	Meta-analysis	**pSWE** – F2 AUROC – 0.85, F4 AUROC – 0.94, se – 75%, Sp – 80% **MRE** – F2 AUROC – 0.97, F4 AUROC – 0.97, se – 87%, Sp – 94%	
Yoon et al. [131]	MRE vs SSI vs *Biopsy	Single centre	**SSI** – AUROC – 0.85 **MRE** – AUROC – 0.85 Corr. – 0.724	MRE failures due to iron deposition
Several studies [132, 133].	TE vs MRE		**TE** – AUROC – 0.9 **MRE** – AUROC – 0.9	Very high cut-offs for cirrhosis via TE (23.7kPa)

*: Gold standard, TE: Transient Elastography, pSWE: point Shear Wave Elastography, SSI: Supersonic shear imaging, 2D-SWE: 2D Shear Wave Elastography, APRI: AST to platelet ratio, MRE: Magnetic resonance elastography, FR: Failure rate, Se: Sensitivity, Sp: Specificity, Corr: Correlation, AUROC: Area Under the Receiver Operating Characteristic curve

In conclusion, there are multiple studies comparing all non-invasive tests for fibrosis and multiple options for clinicians and healthcare systems as they all give comparatively good results. Ultimately the choices are made by patient type and service provisions. TE is the easiest to learn and can be used by nurses or healthcare assistants and has the most evidence base, however, its main issue is for patients with ascites. In this scenario, either pSWE or SSI may come to the rescue, though if this is the second line, the familiarity of the radiologists

with the equipment may give inaccurate results unless this technology is used as the first line. The inherent difficulty being the need for adequate training and the already burgeoning pressure on radiology services may make this unfeasible. MRE is certainly the most reliable and accurate, but access to equipment is the main difficulty, although this may be overcome in patients requiring regular cross-sectional imagining. Finally, serum tests are more accessible though less accurate than elastography and they are probably best used in conjunction with ultrasound systems and if a discrepancy arises, patients may either have an MRE or a liver biopsy.

1.2.3.4   Functional liver tests

Imaging techniques give answers to the amount of fibrosis present in the liver but do not provide data on the liver's function. The interpretation has relied on liver function test panels. While these give an indication, none are exclusively related to the liver so can be falsely interpreted. Several other tests have been devised to overcome these, which specifically measure aspects of liver function. The clearance principle states that hepatic clearance can be calculated as; Liver blood flow X Liver extraction capacity; this is the basis of most functional liver tests [134].

1.2.3.4.1   Indocyanine green excretion test

Indocyanine Green (ICG) excretion test uses non-invasive pulse spectrophotometry to monitor the clearance of a compound, which is exclusively metabolised by the liver and, therefore, measures liver function. ICG is injected intravenously and then excreted exclusively by the liver in an ATP-dependent manner into bile. Rate of excretion is determined peripherally by pulse spectrophotometry and liver function is inferred. Only a

small fraction is detectable peripherally after 10 minutes. It does not undergo enterohepatic circulation. [134-141].

The excretion of ICG is based upon a bi-modal excretion with an initial peak showing the first pass of the dye following intravenous administration, so can be used to indicate hepatic blood flow and to infer the cardiac output. The subsequent decrease indicates the removal of the majority of ICG into the hepatocellular compartment. This is followed by another peak indicating the re-circulation phase and finally, the hepatic elimination phase [134, 142].

The use of ICG in patients with cirrhosis has been well described[143]. Typically, sinusoids are extremely permeable with the transport of large particles such as albumin being free to move into the space of Disse and thus interact with the membrane of the hepatocyte. In cirrhosis, this region has an increased deposition of collagen which reduces this permeability and is termed capillarisation [12, 13]. Huet et al. demonstrated the transition of albumin to hepatocytes to be markedly reduced due to this pathological construction. They also showed the correlation that ICG transport was also decreased, likely because it is bound to proteins in the vascular compartment [144]. This was further built upon by Kawasaki et al., who showed the decrease in the extraction of ICG from the blood by hepatocytes to be reduced by up to 85% irrespective of the direction of blood flow, though it must be noted that vascular shunting cannot be ignored [145, 146]. Once ICG has entered the cell, the issue of cytoplasmic transport is encountered, potentially inferring cellular dysfunction in the auspices of cirrhosis. Shinohara et al. found a certain amount of decrease in the transport of ICG through the cell, which may further reduce the excretion of ICG into the bile [147]. ICG excretion correlates well with the degree of fibrosis as analysed by TE and the current standard, model of end-stage liver disease (MELD) scoring system [55, 142, 148-152].

Pulse spectrophotometry relies upon a finger probe measuring near-infrared wavelengths between 805nm and 905nm, the maximum and minimum absorption wavelengths for ICG and also the lowest absorptions for haemoglobin and bilirubin (Figure 1.4) [142, 153]. The machine produces two calculations. First is a measure of the plasma disappearance rate (PDR) of ICG, based on working out the constant and backward extrapolation, expressed as a percentage per minute. The other is the retention ratio after 15 minutes ICGR15 (Figure 1.5). Both are well correlated with normal values of PDR being >18%/min and ICGR15 as <10% [154, 155]. Caution should be taken in patients with hyperbilirubinaemia with levels over 50μmol/L due to competitive uptake between ICG and bilirubin but the NTCP transporter.
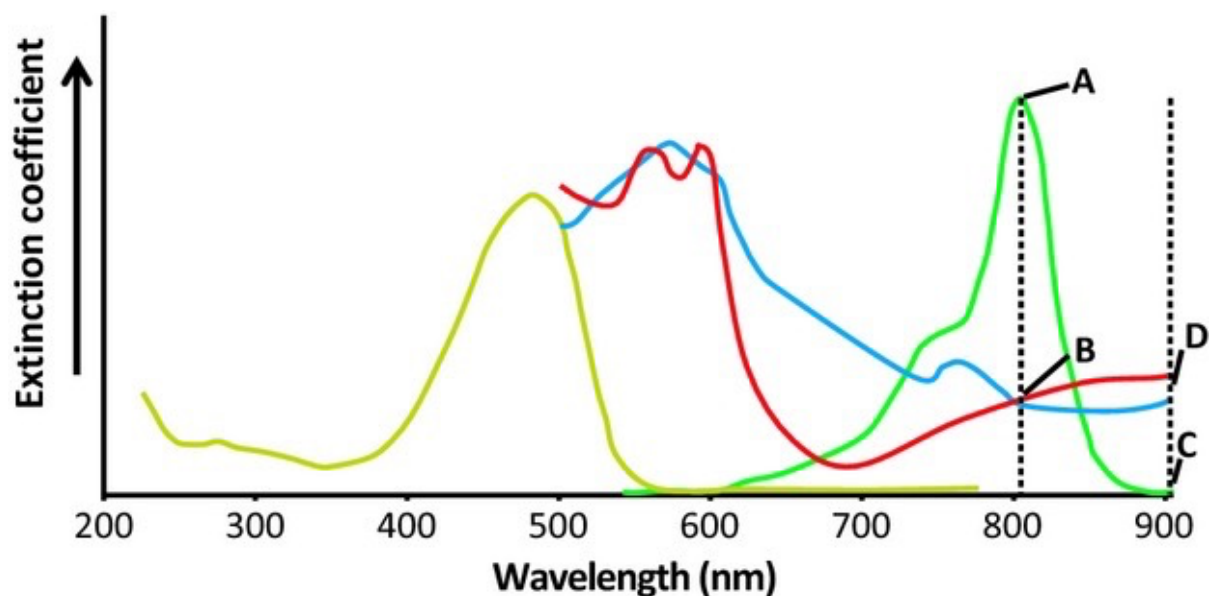
Figure 1.4:Absorption spectra of indocyanine green (ICG; green line), bilirubin (yellow line) and haemoglobin (red line for oxyhaemoglobin and blue line for reduced haemoglobin). Pulse dye densitometry measures relative ICG concentration at 805 nm (ICG peak absorption, A) and at 905 nm (no ICG absorption, C). Points B and D show independency of this measurement from arterial oxygen saturation. [142]
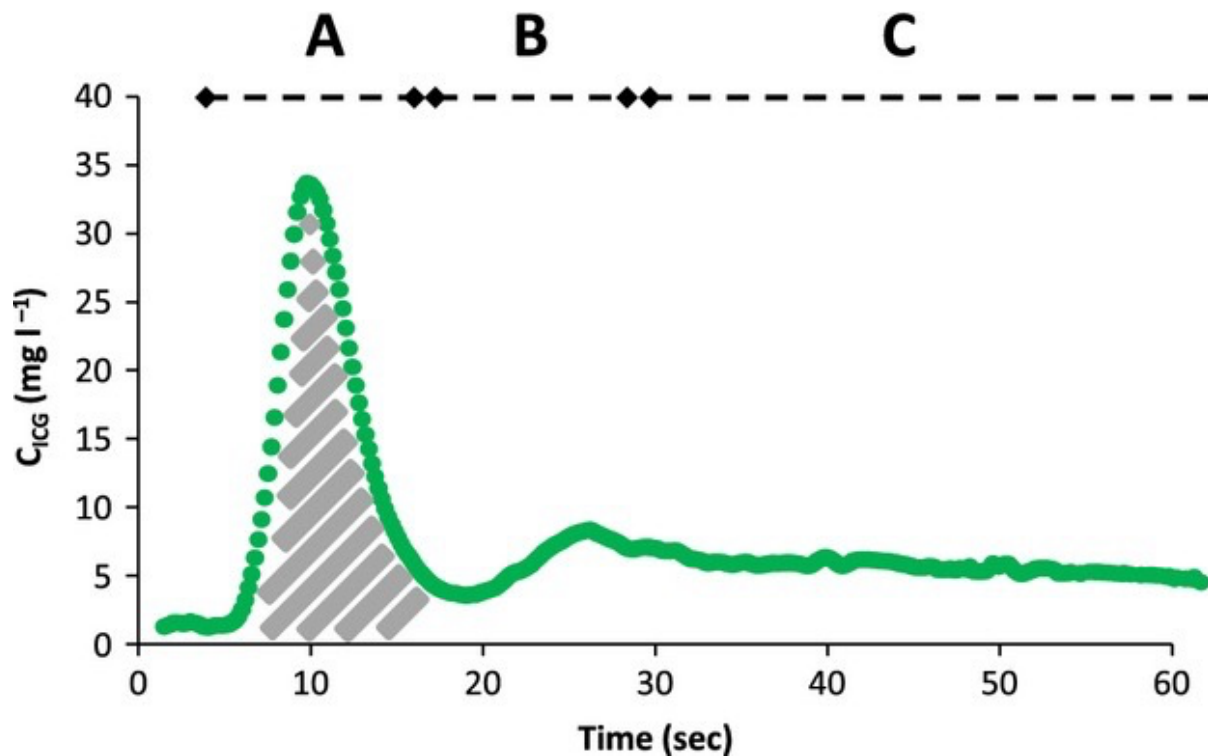
Figure 1.5: Example of typical ICG indicator-dilution curve. ICG, indocyanine green; CICG, ICG blood concentration. A: primary peak, B: secondary peak (re-circulation phase), C: (hepatic) elimination phase. Shaded grey area represents the area under the primary curve, allowing cardiac output calculations. Remainder of the elimination phase is not shown in this figure, but PDR can be calculated in this phase by curve fitting using dynamic backward extrapolation [142]

Side–effects with ICG administration are rare (1/40,000) and include urticarial or anaphylactic reactions, including patients allergic to iodine [134]. Certain drugs may alter the absorption of ICG in the liver including anti-convulsants and opioids. Due to the compound containing iodine, thyroid uptake studies should not take place within a week of the test due to interference. Its effect on pregnancy and breast milk is unknown and therefore, caution is advised.

1.2.3.4.2 Clinical usage of Indocyanine green

ICG monitoring has been used for several scenarios, cardiac output, sepsis, acute liver failure, particularly in ITU, suitability of patients for a liver transplant and prognostication of a liver to undergo hepatic resection, particularly in cirrhotic patients. The use of ICG in the

assessment of patients with cirrhosis for surgery is extensive in Asia, with Japan incorporating it into guidelines for liver damage classification [156]. Our focus shall be on the use of Indocyanine green (ICG) excretion tests in the evaluation of cirrhosis.

Early comparisons of ICG with Childs-Turcotte-Pugh (CTP) scoring suggested a good correlation between the two systems but did not show any difference in the prognosis information gathered by ICG over CTP [157-159]. Mukherjee et al. took 102 liver biopsy patients and juxtaposed them with CTP score and PDR, again finding good correlation with CTP with variability indicated by albumin and prothrombin time as well as histological activity [160]. Though, in a large study by Ikeda et al., 2215 patients were observed over 15 years and found ICGR15 >15% to be associated with progression to cirrhosis in HCV patients though not to HCC.

The model for end-stage liver disease (MELD) is used internationally to stratify patients for transplantation using creatinine, INR and bilirubin [28]. The concordance of ICG metabolism and MELD scores for all grades of cirrhosis was found to be close (r = 0.804) [148]. However, for prognostication, similar to CTP, MELD was also found to be superior to ICG [161]. Several attempts were made to improve the MELD score by including sodium for the MELD-Na or the UK version, UKELD [162]. Zipprich et al. have incorporated ICG with MELD score after finding ICG to be more accurate than galactose elimination, sorbitol clearance or lidocaine metabolism. The new model is said to be more robust than MELD or MELD-Na for patients requiring a TIPS procedure though it does not change prognostic accuracy compared to these, instead allowing better discrimination between intermediate and advanced cirrhosis [163].

The use of ICG clearance in portal hypertension has been proven in CTP-A patients it was found by Lisotti et al. to be very sensitive with it delineating clinically significant portal hypertension (AUROC=0.808), significant portal hypertension (AUROC=0.821) and the presence of oesophageal varices (AUROC=0.859) with cut-off values for ICGR15 as <6.7% (sensitivity - 95.9%), <6.9% (sensitivity - 96.6%) and <10% (sensitivity - 97.8%) respectively. Also, the cut-off of >22.9% showed a specificity of 90% for the diagnosis of oesophageal varices [164]. This high level of accuracy for the diagnosis of portal hypertension was confirmed and extended by Pind et al., though it is interesting that they found a reduced accuracy for CTP classes B and C [165, 166].

1.2.3.4.3 <u>Breath tests</u>

Comparisons of different functional liver tests have been completed in a variety of different disorders. A long-term study was carried out by the HALT-C trial group over the course of 5.5 years, comparing caffeine elimination rate, aminopyrine clearance, MEGX concentration, methionine breath test, galactose elimination capacity, dual cholate clearances and shunt, perfused hepatic mass, and liver and spleen volumes (by SPECT), finding that they all correlated with fibrosis stage, cirrhosis risk, presence of varices and size of varices as well as predicting future deterioration [167-170]. Other studies looked at the use of MEGX tests with or without GEC and compared them to CTP and MELD to find algorithmic tests continue to be superior [171, 172].

In a study by Forestier et al., 296 patients of different aetiologies and grades of liver disease underwent a comparison of algorithmic tests, TE, ICG and aminopyrine breath tests. APRI was found to be poorly correlated with all other tests except TE, though the median value was

distinctive between cirrhotic and non-cirrhotic patients. All tests otherwise correlated well together. For cirrhotic patients, most accurate tests were found for CTP, followed by ICG, MELD and TE, with the best combinations being CTP + APRI and CTP and aminopyrine [173].

Functional liver tests have shown the superiority of ICG excretion compared to other tests though similar in performance to CTP grade. The question of how much improvement this shows following treatment and possible reversal of cirrhosis remains to be seen.

1.3 Pathology

1.3.1 Introduction to Hepatitis C Virus

In this thesis, I will describe my work looking at dynamic liver tests in patients with chronic hepatitis C virus infection and here, I briefly summarise the virology and pathology of HCV infection.

1.3.1.1 Epidemiology

The Hepatitis C virus is a small ribonucleic acid (RNA) virus discovered in 1989 in the United States after increasing recognition of hepatitis associated with blood transfusion but distinct from known viral agents such as hepatitis A or B. [174].

There are seven genotypes of HCV which can be further sub-typed. There are geographical differences in the distribution of HCV, with genotype 1 being most prevalent in Northern America and Europe, followed by genotype 3 which is most widespread in South and Southeast Asia [175]. Genotypic differences are relevant for the treatment of HCV as different sustained virological response (SVR) and medication regimens are effective for each type.

In 2016, an estimated 130 to 150 million people were infected with HCV worldwide – around 3% of the population, with approximately 700 000 dying from the disease annually [176].

HCV transmission is via blood–blood contact. Previously, this was mainly through unscreened blood products and blood transfusions, although since screening began in 1992,

this route has become less frequent. An Egyptian schistosomiasis eradication programme in the 1960s resulted in the country having one of the highest prevalence's of HCV at 40% [177].

Worldwide, unsafe healthcare practices continue to contribute to the disease burden in developing countries. HCV is prevalent in Africa, Central and East Asia and Eastern Europe. Sharing needles or injecting equipment for drug use, and less frequently for tattoos and piercings, is the major source of transmission in developing countries [178]. A minority of transmissions occur vertically from mother to child or through sexual contact. The exception is among men who have sex with men, where cases of acute hepatitis C have risen recently [179]. [180]

1.3.1.2 <u>Manifestations, Diagnosis and Assessment of HCV Disease</u>

HCV primarily infects the liver and causes inflammation of the liver, termed hepatitis. Acute infection is usually asymptomatic, aiding in its transmission. Presenting symptoms can be non-specific such as lethargy or occasionally more specific such as jaundice. Diagnosis is made with detection of antibodies (anti-HCV IgG antibodies) in serum, accompanied by raised liver enzymes (alanine transaminase (ALT) and aspartate transaminase (AST)). Very occasionally, acute HCV leads to fulminant hepatitis and liver failure, necessitating liver transplantation [181]. HCV infection is confirmed by the presence of viral RNA in serum. It is possible to detect HCV antibodies in saliva or a finger-prick dried blood spot (DBS) test and utilised for screening.

About 15–25% of acute infections resolve, rendering the patient HCV RNA negative but anti-HCV positive. Thus, the majority of individuals develop chronic HCV infection, defined as the persistence of HCV RNA for more than 6 months [182].

As chronic HCV infection is mostly asymptomatic, screening at-risk groups is vital. Testing for HCV should be performed in incidental findings of persistent transaminitis even if no history of exposure or risk behaviour is apparent. Once a diagnosis of chronic HCV is made, liver fibrosis stage is established with imaging being performed with both an US scan and some form of elastography and/or other non-invasive tests or liver biopsy. Around 10–20% of patients progress to cirrhosis within 20 years. Staging and genotyping help to prognosticate both progression of disease and success of HCV treatment.

Liver cirrhosis (and arguably advanced fibrosis) is associated with an increased risk of HCC; this risk has not conclusively been shown to be eliminated following SVR. Thus, there is ongoing debate as to the best management following treatment [19] and all patients with cirrhosis enter a surveillance programme with most international guidelines recommending a six-monthly ultrasound [20, 21]. Development of liver cancer and/or end-stage liver disease may fulfil criteria for liver transplantation. HCV is one of the leading aetiologies for liver transplantation in the western world though there are signs this is beginning to change as effective HCV treatment becomes available [183]. [180]

1.3.1.3 Treatment including Direct acting antiviral

1.3.1.3.1 Goals of HCV Therapy

Successful antiviral therapy leads to a virological cure, defined by the achievement of SVR – undetectable HCV RNA in blood – at 12 weeks (SVR12) and/or 24 weeks (SVR24) after the end of therapy [184]. Where sensitive HCV assays are unavailable, undetectable HCV core antigen is an alternative goal of therapy [20, 21].

The goal of achieving SVR is to prevent the progression to cirrhosis, decompensated liver disease, development of HCC, severe extrahepatic manifestations, patient-reported outcomes such as physical and mental well-being and death [185-187]. Importantly, SVR reduces but does not abolish the risk of HCC [188] – a study of 1824 patients followed for on average 5 years post-SVR showed a standardised mortality ratio of 23 for deaths due to HCC compared to the general population [189]. Therefore, guidelines recommend continued surveillance for HCC after SVR [20, 21].

Treatment failure is usually by viral relapse before reaching 12 weeks post-treatment [185, 186]. With modern antivirals, treatment failure by viral breakthrough (HCV clears but returns during treatment) or nonresponse (HCV negativity is not achieved during treatment) are rare and likely represent nonadherence to medication. Late treatment failure, with RNA detectable after SVR12, is uncommon [190] and may be due to reinfection.

It is essential to bear in mind the cofactors for the progression of liver disease, such as obesity and excess alcohol intake, which may continue to cause liver damage after HCV clearance; thus, patients should be counselled on other aspects of liver health. Studies

investigating excess mortality post SVR have attributed this to ongoing drug and alcohol use, whilst for individuals without these behavioural markers, survival was equivalent to the general population [189]. Despite the improvement in survival from any patient with HCV described on their death certificate in 2016, there continued to be a significantly higher mortality from non-hepatic causes than the rest of the population [191]. Thus, the management of HCV infection must be multifaceted. [180]

1.3.1.3.2 <u>Who Should Be Treated?</u>

Guidelines advocate for the treatment of all patients where DAAs are available with the prioritisation of patients with advanced fibrosis or cirrhosis [20, 21, 184]. Treatment failure is usually by viral relapse before reaching 12 weeks post-treatment [185, 186].

Importantly, SVR reduces but does not abolish the risk of HCC [188] – a study of 1824 patients followed for on average 5 years post-SVR showed a standardised mortality ratio of 23 for deaths due to HCC compared to the general population [189] therefore, surveillance for HCC after SVR continues to be recommended [20, 21]. [180]

1.3.1.3.3 <u>Direct-acting Antivirals</u>

The common targets for DAAs are the NS3 gene which encodes a protease which cleaves the polyprotein produced from the viral RNA into smaller proteins, the NS5B protein that encodes the viral RNA polymerase and the NS5A molecule that has no inherent enzyme activity but interacts with other viral and host proteins involved in the viral replication cycle (Figure 1.6)[192].

Structural proteins

| Core | E1 | E2 | p7 | NS2 | NS3 | NS4A | NS4B | NS5A | NS5B |

Non-structural proteins

Cleavage by cellular signal peptidase   Autoprotease cleavage   Cleavage by HCV NS3
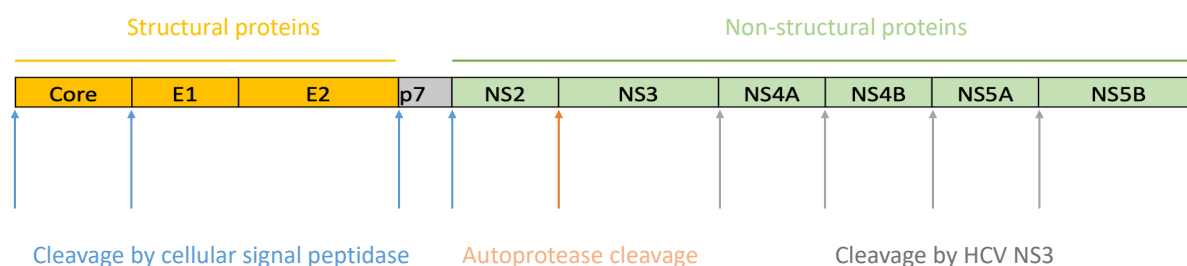
Figure 1.6: Polyprotein produced from HCV RNA with the illustration of structural and non-structural sections. The production of these individual proteins are inhibited by DAA therapy [193]

In 2011, the first DAAs in the form of NS3/4A protease inhibitors – telaprevir and boceprevir – were used with pegylated interferon and ribavirin. This significantly improved efficacy to approximately 75% with shortened treatment to 24–48 weeks [194, 195].

The first all-oral, interferon-free drug regimen in the form of sofosbuvir plus ribavirin was a turning point in treating hepatitis C. The NS5B protein was characterised in 1999 [196]; however, sofosbuvir, which targets this protein, was only approved in 2013.

Several DAA drugs are now approved and used in combination, targeting different points of HCV replication and combined pills are patient-friendly. The most frequent adverse events with DAAs include tiredness, insomnia, headache and gastrointestinal upset, which are usually mild and manageable.

The primary benefit of all oral DAA treatments is the much higher rates of SVR attained. This is shown across clinical trials and in real-world data to be above 90% in almost all patient groups. The potency of combination DAAs allows most regimens to have short durations with most regimens being 12 weeks [190, 197-202].

The current drive is for pan-genotypic all-oral DAA therapy, with two options being available. Sofosbuvir and velpatasvir can be used for all genotypes with the addition of ribavirin if tolerated for 12 weeks otherwise, without ribavirin for 24 weeks in patients with particularly difficult to manage infection [20, 197, 198]. In addition to sofosbuvir-based therapies, pan-genotypic treatments involving the protease inhibitor glecaprevir with the NS5A pibrentasvir are available [203]. For patients who fail therapy, a 'rescue' treatment including the protease inhibitor voxilaprevir (sofosbuvir/velpatasvir/voxilaprevir) is available [204].

Overall, DAA therapy, particularly when used without ribavirin, is far more tolerable for patients than interferon-based care [187, 205]. This has meant that patients are more likely to complete their course of treatments and many can continue full-time work. [180]

1.3.1.3.4 Antiviral Therapy for Patients with Decompensated Cirrhosis

One of the main pitfalls with interferon was the inability to treat the sickest patients with decompensated cirrhosis due to potentially worsening already suppressed bone marrow; risking further decompensation and death [206].

All-oral DAAs allowed therapy for patients with decompensated cirrhosis. As there is no history of antiviral treatment for these patients, there is a lack of long-term data on the impact of viral clearance and whether there may be a 'point of no return'. Multiple studies have demonstrated early improvement in the liver function in many, but not all, treated patients [190, 207]. A UK study following patients to a year post-treatment completion suggested continued improvement with reduced incidence of decompensations and other adverse

outcomes, compared with treated patients who did not achieve SVR or with a retrospective control group of untreated patients. These patients were further investigated with model for end-stage liver disease (MELD) scoring to ascertain their suitability for liver transplant following treatment and found that while 40% showed an early improvement in liver function following HCV therapy, unfortunately, the remainder showed minimal improvement and 10% showed worsening liver function (Figure 1.7) [190]. In a study looking specifically at HVPG improvement with 37 patients having a paired measurement before and following treatment with a baseline reading of above 12mm Hg, a mean reduction of 1.2mm Hg was found with 24% having a decrease of more than 20% [208].
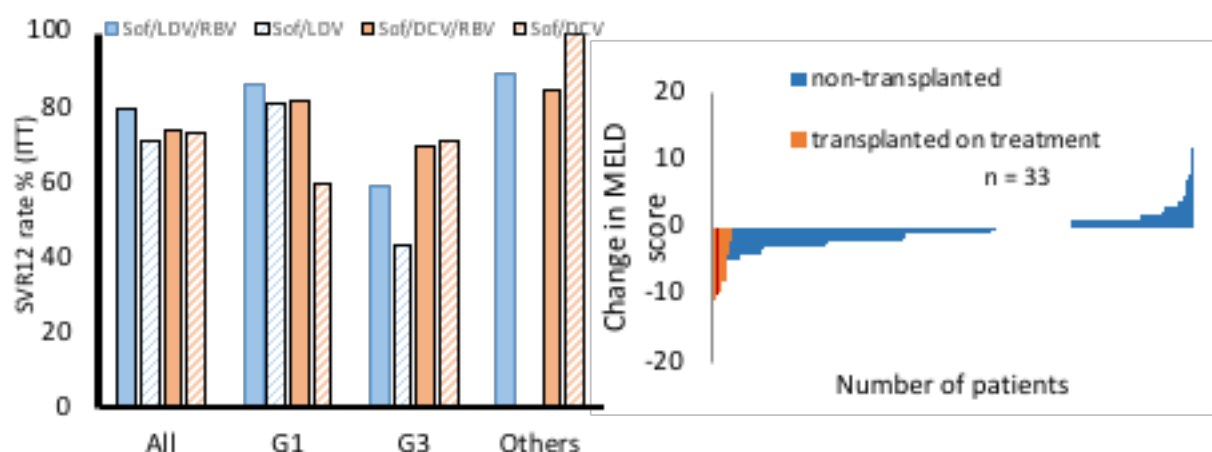
Figure 1.7: The effect of new medication regimes on cure rate and MELD scores 4 weeks post-treatment. (SVR12 defined as HCV RNA at 12 weeks post-treatment < 30 IU/ml) Genotype 3 shows 70% viral cure rate. MELD scores show functional improvement in 40% (defined as a decrease of >2) though static/worsening in 60%. [190]

For those that do not regain liver function following effective antiviral therapy, liver transplantation is required with the timing of DAA treatment dictated by a MELD cut-off of 18-20, with those below this cut-off receiving immediate treatment and those above having transplant prioritised over treatment [209]. Those with a significant improvement in MELD may be delisted, thereby reallocating a limited resource. Delisting from transplant wait list was not practiced in England, despite this, in 2017 there had been a fall in the number of

registrations for transplants for the first time following the start of DAA treatments. Indeed, in England, a fall in the number of registrations for transplants has been observed for the first time over the last two years following the start of DAA treatments [210]. A report from the European Liver and Intestine Association (ELITA) described 21 patients who were delisted from liver transplant due to clinical improvement with an overall improvement in mean MELD from 15.5 to 14, 24 weeks after starting treatment. Of the 103 original cases, 63 patients underwent or were still waiting for liver transplantation [211]. The difficulty is patients around the cut-off values with a slight improvement in MELD, which makes them ineligible for liver transplant though they have ongoing morbidity due to their liver disease with the ability to diagnose these patients early, preferably before treatment commences, being imperative. Such patients have been referred to as in a state of 'MELD purgatory'.

1.3.1.3.5 Use of TE and functional tests

Fibrosis regression following SVR has been demonstrated since the mid-90s with IFN treatment [212] however, with DAAs now drastically increasing the volume of patients and more importantly, allowing treatment for patients with cirrhosis, this is more relevant than ever. We have tackled the basic science above and will concentrate here on non-invasive measures used to assess this change.

Testing began as early as 2002 by contrasting MEGX and GEC with liver biopsy in patients treated with IFN [213]. Several studies which investigated IFN treatment noted the improvement in markers particularly following SVR at up to 3 years post-treatment [124, 212-219]. Arima et al. found that 49/67 patients treated with IFN, regardless of the virological outcome, had a reduction in fibrosis level from F3 or F4 down by at least two

grades, with 6 out of 13 with biopsy-proven cirrhosis dropping to F0 or F1 on TE [218].

Poynard et al. showed the effect of SVR with 24/43 patients downgraded from cirrhosis to fibrosis, but unfortunately, there were 15 new cases of cirrhosis from the overall cohort of 128 as diagnosed by Fibrotest at ten years post-treatment. TE was also used in this study but performed worse than Fibrotest [124]. In a comparison of liver biopsies with serological markers, 38 patients were observed with pre and post-treatment liver biopsies (48-104 months post-SVR), with the biopsies revealing cirrhosis regression in 23 patients, mostly down to F3. A range of algorithmic tests were examined, with most showing a decrease in scores between time points, however, only predicted liver fibrosis (PLF) (which combines TE, King's score, Forns tests and APRI score) indicated regression vs continued cirrhosis, though of note, the median values were the same for both groups [220].

An Egyptian study of 337 patients found a reduction of TE from 23.5kPa to 18.1kPa in at SVR12 with correlated reductions in FIB-4 and APRI scores [221]. Sporea et al. looked at 170 patients with genotype 1b cirrhosis at baseline, end of treatment and 12 weeks post to find a significant decrease from 27.4kPa to 21.3kPa though 13% remained stable with 11.8% showing a worsening TE with Fibrotest used as a guide to necro-inflammation and was found to be higher in those with a drop in TE vs those with stable or increased TE, thus indicated the possibility these results are secondary to a reduction of inflammation at the end of treatment [222]. Olveira et al. showed a significant drop in TE values for cirrhosis but also interestingly for values above 21kPa which is the usual cut-off for clinically significant portal hypertension though around half of these cases were HIV co-infected patients [223].

An emerging consensus is that the so-called "point of no return", where a patient with cirrhosis will not show signs of regression, is approximately at the region where hepatic

venous pressure gradient (HVPG) is increased above 10mmHg, also known as clinically significant portal hypertension (CSPH). Several studies have looked at this possibility with these patients newly in a position to be treated. Mandorfer et al. investigated 60 patients with HVPG with the majority CTP A and 41/60 CSPH. All groups showed an improvement in measurements following treatment, with the improvement reducing with advanced disease, with three patients having complete resolution. This was associated with an improvement of TE though still within the cirrhotic range. The only predictor for improvement proved to be CTP A; patients with CTP B were less likely to improve [224]. Lens et al. looked at 226 patients with 21% as CTP B and 75% with oesophageal varices and all patients had CSPH. Although there was an overall improvement in the overall score at SVR 24 ($p<0.05$), CSPH persisted in 78%, with 25% still over 16mmHg. They found the presence of varices and low albumin to be associated with the persistence of CSPH as well as smaller reductions in TE. For TE, the correlation of value with HVPG was weak ($r = 0.16$) this is shown as those with TE >21kPa still had CSPH, and 16 patients with TE <13.6kPa persisted with CSPH [225].

In conclusion, the ability to treat HCV, especially in the most advanced cases, allows us the exciting possibility of treating more patients, but the responsible allocation of resources is imperative and will allow better treatment for all patients. One of the important gaps in our current knowledge is the lack of an established recommendation regarding which patients will improve following treatment and thereby avoid transplantations and which patients will not improve following therapy and may still require liver transplantation or may enter a 'virus-negative- non progressive phase' with significant impairment in their quality of life but not reach the criteria for liver transplantation.

1.3.2   <u>Introduction to Hepatocellular carcinoma following DAA treatment</u>

In this thesis, I will describe my work looking at liver tests in patients with hepatocellular carcinoma following DAA treatment for chronic HCV infection and here I briefly summarise the clinical need and methods for HCC assessment.

1.3.2.1   <u>Controversies</u>

HCV is a known leading cause of liver cirrhosis and HCC, particularly once cirrhosis and end-stage liver disease have set in. HCC is the second most frequent malignant cause of death worldwide [226] and so the introduction of DAA therapy was seen as a monumental advance for long-term patient prognosis. Their introduction facilitated the treatment and cure of patients with advanced liver disease who remain at risk of HCC [186] and would require lifelong surveillance [20, 21].

Unfortunately, soon after the introduction of DAA treatment, reports surfaced of an unexpectedly high rate of HCC occurrence and recurrences following treatment with DAA [227-230]. Conti et al. observed 285 patients without a history of HCC for 24 weeks following treatment completion and found that 3.16% (95% CI 1.45-5.90) developed de novo HCC with no indicating factors at baseline that these patients would develop cancer [228]. Supporting this, Ravi et al. found 9.1% (6/66) patients developed de novo HCC within nine months of starting treatment, with all bar one being less than 3cm [231]. Conversely, multiple studies have shown no increase in HCC occurrence. Cheung et al. showed, of all patients without a history of HCC (n=377), 25 had a new HCC (6.6%) over the follow-up period of 15 months from treatment start, with 15/299 having achieved SVR24 [190]. A Belgian study of 490 DAA-treated patients with 490 IFN-treated patients to find similar HCC occurrence

rates of 1.7% and 1.9%, respectively [232]. Waziry et al. performed a meta-analysis and found 26 studies for patients treated with DAA or IFN, which incorporated 6,002 and 5,521 patients, respectively, with per year risks of 2.96/100 (95% CI: 1.76–4.96) and 1.14/100 (95% CI: 0.86–1.52) (RR: 0.68; 95% CI: 0.18–2.55; p=0.55) [233]. A massive American cohort of 62,354 patients with and without cirrhosis showed that although patients with cirrhosis who had cleared virus with DAA therapy did develop malignancy, the frequency was not increased, 0.74 per 100 patient-years vs 1.4 per 100 patient-years (HR: 1.04 (95% CI: 0.83–1.30)) [234]. A large European study of 3,917 patients with 2,710 cirrhotics found that 51/2,710 (1.88%) developed HCC, which showed an incidence of 0.97/100 patients years (95% CI: 0.73–1.26), with the majority occurring within the first year and 22 occurring whilst on treatment. Of the 55 patients developing HCC (Inc. F3 fibrosis), 39 had a single tumour or 2-3 tumours <3cm. They compared the incidence with untreated historical controls for each CTP stage within a similar geographical region to find a lower incidence following DAA treatment [235]. A Sicilian study recently showed a rate of 3.4% out of 2,249 cirrhotic patients with the majority as CTP-A with 69.6% having an US within 6 months of starting treatment and 73% within BCLC stage A and showed albumin, platelets and SVR to be associated with HCC development [236]. A Danish study observed 1075 cirrhotic patients with an incidence rate of 2.85 per 100 patient-years, an increase from 0.8 per 100 patient-years. With 40.2% being within Milan criteria with survival at 43.6% and 15.2% for one and five-year survival, respectively though interestingly, they did not find an increased survival for patients within Milan criteria [237]. Masetti et al. found de novo HCC occurrence in 54/943 patients, median follow-up (17.3 months), with the majority (83%) within Milan criteria with the suggestion that a persistently raised AFP (>6ng/ml) at the end of treatment indicated the production of HCC, though there was no difference between cohorts at the start of treatment [238].

These studies have looked at occurrences of HCC in the DAA era, but there are three unmet challenges for clinicians (i) are there any baseline features predictive of HCC development, (ii) are patients who are diagnosed with HCC during treatment less likely to achieve SVR and (iii) whether there are clinical differences in HCC developing in a non-viraemic versus viraemic patients.

Van der Meer et al. followed up 530 patients for 8.4 years and found older age, male sex, genotype 3, advanced fibrosis, alcohol consumption, and diabetes mellitus to be associated with an increased HCC occurrence in IFN-treated patients [239]. Romano et al. also found HBV co-infection and CTP stage to be indicative of HCC production (HR: 2.55; 95% CI: 1.36–4.78; p=0.004) for patients treated with DAAs [235]. Other studies have advocated lower platelet count, high HCV titre and higher aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio to be associated with increased however, many of these studies use SVR status within their analysis, which would not be known at baseline as well and none of them looked at baseline ultrasound scans to find abnormalities [240]. The role of non-malignant baseline nodules has also been investigated as a possible risk for HCC development. Toyoda et al. recently found that the emergence of non-hypervascular hypointense nodules (NHHNs) increased with worsening fibrosis, defined by Fib-4 score, as found by MRI [241].

In addition to the impact of HCV clearance on HCC development, there is controversy regarding the impact of HCC on HCV treatment outcomes. Prenner et al. showed a substantially increased treatment failure rate of 42% for patients with an HCC present on treatment initiation, falling to 3% in patients with a previous history of treated HCC prior to DAA commencement. This implies hepatocellular carcinoma decreases the chance of

successful hepatitis C virus therapy with direct-acting antivirals though this study included 130 patients who were post or peri-transplant during DAA treatment out of a total cohort of 421 [242]. A recent meta-analysis pooled 1,665 cirrhotic patients with current or prior HCC and compared them to 21,025 non-HCC patients to find a reduction in SVR for those with HCC (OR: 0.68, p=0.087 (significance was set to 0.10)) [243].

The prognosis following the diagnosis of HCC in patients with HCV and cirrhosis is poor, with survival as low as 0.7-0.9 years [244]. In the SHARP trial of Sorafenib in patients with advanced HCC of any aetiology and cirrhosis of CTP grade A with no previous systemic treatment, the time to progression on imaging regardless of the initial cause was 2.8 months in the placebo group [245]. It is not known whether clearance of HCV impacts tumour progression, but anecdotal evidence has suggested that it may do so.

To address these questions, we examined the NHS England early expanded access programme (EAP), which provided access to all-oral DAA therapy for 12 weeks for patients with advanced liver disease with ongoing follow-up.

1.3.2.2   Diagnostic guidelines, RECIST scoring, Barcelona clinic liver cancer staging (BCLC) and Milan criteria staging and treatment

There are multiple scoring systems and guidelines for the care of HCC, with each guideline having its positives and negatives. Suspicious lesions in cirrhotic livers are investigated for radiological hallmarks of hypervascularity in the arterial phase and washout of the contrast in the portal phase or delayed phase and are most useful in lesions of 1-2cm in diameter. This ties in with the use of the Li-RADS classification of tumours which aims to image acquisition

and interpretation by using the above hallmarks to stratify lesions according to the risk of malignancy. This gives eight classes ranging from non-categorisable (LR-NC), definitely benign (LR-1), up to definitely HCC (LR-5). There are two additional classifications for non-HCC malignancy (e.g. cholangiocarcinoma) and tumour in vein [246, 247]. Problematic lesions are those falling into the LR-3 category of an intermediate probability of malignancy and are avoided as much as possible though they come in various guises though usually below 2cm. In these cases, a specialist review of imaging in a multidisciplinary environment is suggested. If this proves inconclusive, two options are available: an interval scan or biopsy. Biopsies may cause seeding of HCC cells along the needle route and thus causing multiple tumour sites.

Once a tumour is diagnosed, prognostication and treatment algorithms are implemented. Albumin-bilirubin (ALBI) score was developed in a cohort of 1,313 patients to avoid the more subjective parts of the CTP score, namely grading of ascites and encephalopathy. Following this, it was validated in a cohort of 5,097 patients with HCC and 501 non-HCC patients to find albumin and bilirubin to be as useful as CTP as well as further splitting CTP grade A into two separate groups with survival in the validation cohort for group 1 being 86.6 months, group 2 45.8 months and group 3 13.8 months [248].

Treatment must then be determined, with the most widely used algorithm being the Barcelona-Clinic Liver cancer (BCLC) staging criteria [249, 250] and takes into account the size and multiplicity of tumours and combines these with grade of cirrhosis by way of CTP grade and activity according to the Eastern cooperative oncology group (ECOG) grades. A grade is then awarded based upon these and a suggestion for treatment modality is given. These may be curative with regard to ablation, resection or transplantation, semi-curative

with chemo-embolisation, or advise systemic therapy or best supportive care. The curative treatments project a half-life of >5 years, with this reducing to three months for those with best supportive care [250].

If transplantation is advised, the Milan criteria for transplantation is affected, which was developed in 1996 to identify which cirrhotic patients would benefit from liver transplantation [251]. This looks solely at the characteristics of the tumour with regards to size and number of tumours as well as vascular involvement and metastases to give a 4-point score and binary outcome.

If it is decided that either loco-regional therapy or supportive measures are put into place, i.e. non-surgical, then the ongoing assessment by non-invasive means is undertaken. The most widely used criteria for this is the response evaluation criteria in solid tumours (RECIST) criteria [252, 253]. This defines progression or regression of tumour size, considering the primary tumour as well as secondary tumours and assessing them against size at the time of diagnosis to delineate them into one of four groups, complete or partial resolution, stable disease or progressive disease with increases of 20% or decreases of 30% being relevant.

1.3.3   <u>Non-hepatitis C related studies on liver fibrosis.</u>

In addition to my work on the identification and significance of liver fibrosis in hepatitis C associated liver disease, I conducted pilot studies on liver fibrosis in patients with sickle cell anaemia and cystic fibrosis. Full details of these conditions and the studies I performed are outlined in the introduction to the appropriate chapters and here, I will briefly review current knowledge of fibrosis and its detection in these disorders.

1.3.4   <u>Sickle cell anaemia</u>

We shall detail sickle cell disease and go on to the damage this may cause to the liver as well as detail the work already performed on non-invasive testing and treatment options.

1.3.4.1   <u>Epidemiology</u>

Sickle cell disease (SCD) is an autosomal recessive disease, first described in the west in 1920 [254] and mainly affects people with ancestry from areas with a high prevalence of malaria, such as sub-Saharan Africa, India and the Mediterranean with worldwide estimates of 305,800 babies born per year [255] and 14,000 sufferers in the UK [256]. Survival is increasing at a remarkable rate with estimates of up to 67 years old with survival to adulthood in London at 99% [257-259].

Adult haemoglobin (HbA) replaces foetal haemoglobin (HbF) within the first year of life. HbA comprises an Alpha-globin and a β-globin, with the β-globin gene (HBB) being affected in sickle cell anaemia. The sickle mutation (HbSS) is the substitution of thymine to adenine on the 17th nucleotide on the HBB gene [260, 261].

The impact of this substitution is that the Hb molecule carries oxygen normally but, in the deoxygenated state, is more likely to aggregate with other HbSS molecules to form a polymer which leads to the red blood cell becoming misshapen [262]. The outcome is the reduction of RBC lifespan due to increased phagocytosis by macrophages resulting in anaemia. Also, the sickle shape leads to inefficient flow of cells through small blood vessels leading to blockages, lactic acidosis, tissue necrosis and pain [7, 260, 263, 264].

Symptoms of SCD are generally secondary to this, with painful crises, particularly at joints, being the most frequently seen. Treatment for acute attacks include oxygen, fluids and analgesia. Chronically, renal complications, pulmonary hypertension, and cardiac or liver disease may occur. [260, 264]

Long-term treatment is based upon avoidance of precipitating events. Hydroxyurea/carbamide is an oral chemotherapeutic medication given to increase HbF production and thereby reduce the need for the body to produce defective adult haemoglobin [265, 266]. A Cochrane study verified a reduction of symptoms but was unable to confirm the effect of other organ damage or long-term risks associated [267]. Chronic erythrocyte transfusion is a mainstay of therapy with the chief goals being stroke prevention and avoidance of anaemia and may be given as a simple transfusion or exchange transfusion with the goal of reducing HbS [268]. Iron overload is almost inevitable with transfused patients with haemosiderosis mainly occurring in the liver; thus, chelation therapy is imperative. Bone marrow transplant is available to a minority of patients, though gene therapy is the most exciting future treatment. [260, 264]

1.3.4.2   <u>Liver disease</u>

Liver disease in sickle cell disease continues to be poorly defined, especially in adults. The phrase is used as an umbrella term covering acute and chronic disease of both hepatic and biliary origin. An in-depth review of acute and biliary disease is out of the scope of this thesis but may be found in the following references [269-272]. Chronic viral hepatitis should also be ruled out as a cause of liver dysfunction as this may cause fibrosis and cirrhosis independently and used to be prevalent in this group prior to transfusion screening.

Most patients with SCD are not diagnosed with liver disease during their lifetime, but multiple biopsies and particularly autopsy studies report a prevalence of around 7-10% cirrhosis with no other cause found [273-275] though the major difficulty is trying to separate those with pure cirrhosis due to SCD and those with iron overload [276, 277]. In a study by Bauer et al., they found 10% to have unexplained cirrhosis following the removal of patients with haemosiderosis though it should be noted that this was prior to the discovery of hepatitis C [278]. Indeed, a study looking at sickle disease in children (median age 11.2 years) did not find any cases of fibrosis [279]. Berry et al. found 3/38 patients in London to have died from cirrhosis without another factor being identified and a further 8 showing evidence of chronic hepatic sequestration [271].

The clinical course of sickle hepatopathy is also difficult to determine with a study looking at mortality in England and France from liver cirrhosis to be mainly due to viral hepatitis and varying amounts of haemosiderosis and a single death out of 67 due solely to liver disease [280]. Feld et al. found a varying effect of iron deposition on the amount of fibrosis as well as on death, though increased serum ferritin and direct bilirubin were found to be associated with higher mortality [281]. Another study found that deaths from liver disease in the USA between 1999 and 2009 were 6.9% [282].

The current hypothesis for pathophysiology is repeated microvascular occlusion, particularly within the sinusoids of the liver, causing patchy necrosis and thus initiating a fibrosis process which progresses to cirrhosis with another being bile states with engorgement of Kupffer cells with sickled red blood cells [271, 283, 284]. This process is similar to acute intrahepatic obstruction but in a less extreme and transient manner.

1.3.4.3 <u>Diagnosis</u>

With SCD hepatopathy not being fully elucidated, the ability to diagnose this condition is hampered. The use of liver serum tests are not correlated very well with liver. Bilirubin and γGT are usually raised in SCD due to the haemolysis and therefore are less useful, though a transient hyperbilirubinaemia may indicate sickle hepatopathy. There is no difference between upper values for serum tests between hepatopathy and non-hepatopathy [285]. The use of ferritin is controversial, with studies showing a correlation with liver deposition [286-289]and others showing the opposite [290-292] but this issue is also complicated by the fact that ferritin levels escalate with inflammation which then may render it less useful [293]. Cross-sectional imaging has been utilised, but if there is iron deposition, this may be difficult for MRI images. MRI R2* imaging such as FerriScan may be used for exact quantification, though this does not inform us about fibrosis levels within the liver [294]. Normal values for FerriScan can be seen in Table 1.3. An alternative is an MRI spin echo. Liver biopsy is avoided in SCD patients due to the bleeding risks though a transjugular approach may be safer. While this is helpful, the amount of fibrosis and damage to the liver overall is still not determined and may not occur in a linear fashion [295].

Table 1.3: Liver iron concentration cut-offs, paired with their clinical relevance and sensitivity and specificity [294]

LIC threshold, mg Fe/g dry weight (µmol Fe/g dry weight)	Clinical relevance	Sensitivity, %, (95% C.I)	Specificity, %, (95% C.I)
1.8	Upper 95% of normal	94 (86-97)	100 (88-100)
3.2	Suggested lower limit of optimal range for LICs for chelation therapy in transfusional iron loading	94 (85-98)	100 (91-100)
7	Suggested upper limit of optimal range for LICs for transfusional loading and threshold for increased risk of iron-induced complications	89 (79-95)	96 (86-99)
15	Threshold for greatly increased risk for cardiac disease and early death in patients with transfusional iron overload	85 (70-94)	92 (83-96)

LIC: Liver iron concentration, mg: Milligram, g: Grams, µmol: Micromolar, Fe: Iron, %: Percentage, 95% C.I: 95% Confidence interval

1.3.4.3.1 <u>Use of TE</u>

The need for a cheaper, more accessible as well as accurate test is required in these circumstances. The use of TE in SCD was examined by Draser et al., who looked at 193 patients of all genotypes and grouped HbSS and Hb Sβ0 thalassaemia to compare with HbSC patients. They found TE increased with age and serum liver tests, as well as weak correlations for ferritin values obtained from serum and Ferriscan for the HbSS group with a liver iron concentration (LIC) correlated at r=0.18 and p=0.04, while these were absent for HbSC. 6 patients were known to have abnormal findings upon cross-sectional scanning, either due to chronic sickle cell intrahepatic cholestasis or iron deposition, with these being corroborated on TE with values ranging from 13.6-21.3kPa (mean 16.2kPa). The authors also applied ELF score to the same patients and only found a weak correlation with serum ferritin but nothing else of significance [296]. In a smaller study correlating TE over time in a set of

patients who are not very well defined, described only as adults suffering from SCD, they found TE to be correlated with serum tests, though with only five patients having an MRI R2*, they did not find a correlation with LIC [297]. The correlation of TE with serum blood results was confirmed in a study from Greece looking at 100 patients with HbSS and HbSβ thal (no further breakdown given), with this, also showing a correlation for LIC for all patients and even stronger for 22 HbSS patients (r=0.770, p=0.001) with higher TE for patients having transfusions [298]. It has also been noted that liver stiffness increases in the first 48 hours from veno-occlusive crises though normalises within two weeks with a doubling of median stiffness score from 6.2kPa to 12.3kPa. This study correlated 15 patients' TE scores paired with liver biopsy to find a good correlation between Ishak fibrosis score and liver stiffness, though there was only a single severe fibrotic patient and no cirrhotics. The authors also showed no correlation between TE and histological liver iron deposition [299].

The question of whether TE correlates with LIC is essential. A small study of miscellaneous aetiology found TE not to be correlated with liver iron concentration by biopsy with a weak correlation on MRI [300], with these findings being corroborated by several larger studies in other haematological conditions [301-303]. This has been refuted by several smaller studies that only looked at MRI data without histology [304, 305]. The use of MRI was reviewed in a meta-analysis of 20 studies and found it to be accurate for ruling out iron overload though less consistent in positive diagnosis (NPV=0.05, PPV=4.86), although this was again covering multiple diseases, with most studies concentrating on thalassaemia [306]. Finally, a recent study examined the effect of a year of iron chelation in SCD patients and found a significant reduction in both MRI R2 score and TE, though it should be noted that the effect of removing the causative agent, i.e. iron, may allow the regression of fibrosis as described previously [307].

1.3.4.4 <u>Treatment</u>

Treatment options are limited to supportive care with the avoidance of sickling being the mainstay and thus the treatment for SCD as detailed above. Special attention must be paid to avoid iron overload; thus, chelation is considered appropriate when the threshold of 7 mgFe/g dry weight within the liver is reached. The avoidance of biliary congestion, which may exacerbate damage, is prudent with cholecystectomy for symptomatic gall stones and Ursodeoxycholic acid (UDCA) may be used if sludge is found within the gall bladder. The use of transplants is controversial, with early outcomes being poor, but increasingly the success of these are improving, with less than 50 successful cases being reported [308].

1.3.5 Cystic fibrosis

Another genetic disease with consequences for the liver is cystic fibrosis. We shall review the disease, pathophysiology and its effect on the liver with diagnosis and treatment for this chronic disorder.

1.3.5.1 Epidemiology

Cystic Fibrosis is an autosomal recessive disease with the most common genotype mutation affecting the nucleotide c.1521_1523delCTT. The protein p.Phe508del is affected by this mutation, widely known as F508del and is found in 89.5% of CF patients in the UK [309]. Over two thousand mutations have been identified, with all mutations eventually disrupting the function of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), which is a transmembranous channel found on epithelial cells [310, 311]. The various genetic mutations lead to an increased viscosity of secretions, which inhibits the normal function of various systems. The respiratory system is most significantly inhibited with the production of hyper viscous mucous with microorganisms that cannot be cleared, leading to a pro-inflammatory environment [310]. There were 10,469 patients in the UK in 2017, with 214 new diagnoses and 132 deaths and a median age at death being 31 [309].

1.3.5.2 Liver disease

Cystic fibrosis-related liver disease (CFLD) has been used as an umbrella term for all liver diseases for patients with CF, including biliary diseases such as focal biliary cirrhosis and micro gallbladder though here we shall only use the term CFLD to define fibrosis and multi lobar cirrhosis of the liver. CFLD is a difficult disease to quantify and diagnose due to the

quiescent nature of symptoms and the fact that most patients will have at least one episode of raised liver function tests, though not necessarily in correlation with liver disease [312]. A recent long-term study followed 298 patients over a 21-year period to find at least one episode of raised ALT in 93% and γGT in 39% and persistently raised for more than six months in 85% and 18%, respectively. The authors found AST and γGT increases of 1.5 times normal to be associated with the raised likelihood of cirrhosis, although the confidence intervals are rather wide [313].

This study perfectly illustrates the other difficulty found, that despite elevated serum liver tests, only 20-30% develop focal biliary cirrhosis and 4-10% of all CF patients eventually progress to cirrhosis [314-316]. Most studies investigate paediatric disease as it has historically been thought that CF liver disease has peak prevalence in the first decade of life at 2.5% per 100 patient-years (95% CI: 1.8-3.3) with a sharp decline following this [316]. However, as treatment for CF continues to improve and life expectancy increases, a second adult-onset liver disease is becoming apparent [317, 318]. In a UK study of 154 patients who developed adult-onset liver disease, they found 28 patients developing advanced fibrosis or cirrhosis, with 19 of these with portal hypertension and 9 showing varices on endoscopy [318]. A large French study of 3,328 pancreatic insufficient patients studied over a period of 32 years found an overall prevalence of 18% for CFLD and 5% for cirrhotic disease. In addition, they found an increasing incidence of 1%/year, reaching 32% by 25 years old for CFLD and a more moderate increase of 0.4%/ year from the age of 5 to 25, reaching a maximum of 10.2% for severe CFLD and concluded this may be due to longer life expectancy. It must also be noted that the authors excluded patients born before 1985 and those with pancreatic sufficiency, assuming that these patients had less severe disease [317]. Koh et al. investigated patients known to not have CFLD as adolescents, for a period of up to

38 years. Of the 36 patients, they found that between 22 and 47% of patients developed CFLD with a median age of 34.8 years old [319].

In the UK prevalence of adult CFLD overall reduced from 2016 to 2017 but for both cirrhotic with portal hypertension and those without increased from 1.6% and 2.1% to 1.8% and 2.3%, respectively [309]. Overall, risk factors for the production of multi lobular cirrhosis of the liver have included male sex, CF class I, II or III, meconium ileus, pancreatic insufficiency, CF-related diabetes, alpha-1 anti-trypsin deficiency and the genetic modifier SERPINA1 [320-326].

1.3.5.2.1 <u>Use of TE</u>

Early studies found TE and pSWE were only able to identify patients with portal hypertension and not earlier disease, whilst recommending a cut-off of 7.95 ± 5.88kPa [327]. A longitudinal follow-up to this study did show significant differences between CFLD (4.9kPa) and non-CFLD (4.3kPa) (p=0.012), however, the differences in the score is minimal [328]. An Australian study investigated 50 patients, showed a significant difference between CFLD and non-CFLD patients (p<0.001) with a cut-off for CFLD as ≥6.8kPa, with a sensitivity of 76.0%, specificity 92.0% and an AUROC of 0.87. Portal hypertension placed at ≥8.9kPa (sensitivity of 87.5%, specificity 90.5% and an AUROC of 0.96), with this value also indicating the presence of varices. They found APRI to be useful, though TE was the only diagnostic [329]. Another study found the optimal cut-off for CFLD to be 5.3kPa, though a greater specificity was gained above 6kPa [330]. Interestingly, in long-term studies, the finding of patients with significant portal hypertension and raised TE and APRI scores were found histologically not to have cirrhosis [319, 331-333].

Overall a mixed age, pooled meta-analysis looked at 644 patients with a median age of 24 and found a cut-off of ≥5.95kPa to be optimum for CFLD with a sensitivity of 55% and specificity of 87% [334].

1.3.5.3 <u>Diagnosis</u>

Diagnosis of CFLD has proven difficult leading to difficulty in prevalence statistics [335]. Indeed, Lewindon et al. found that outcomes of 40 patients with a follow-up of 12 years were only predicted by fibrosis level following dual pass liver biopsy and indicated development of portal hypertension [336]. The CF foundation criteria split cohorts into three groups; CF-related liver disease with cirrhosis/portal hypertension, liver involvement without cirrhosis/portal hypertension and preclinical [323]. These criteria are further detailed in Table 1.4.

Table 1.4: Several diagnostic criteria applied to cystic fibrosis liver disease (CFLD)

Author	Year	Criteria
Debray et al. [321]	2011	At least two of the following to be present: - Hepatomegaly, 2cm below the costal margin (confirmed by ultrasound) and/or splenomaegaly - Abnormalities of ALT, AST or γGT at 3 consecutive occasions within 12 months - US evidence of liver involvement (heterogeneous echogenicity, irregular margins or nodularity), portal hypertension (splenomegaly, increased thickness of the lesser omentum, spontaneous splenorenal anastomosis, large collateral veins, or ascites), or biliary abnormalities (bile duct dilatation).
Koh et al. [319]	2017	Diagnosis should be considered if one of the following is present - Liver biopsy demonstrating pathology - radiologic evidence demonstrating diffuse liver disease or cirrhosis Diagnosis of CFLD should be considered if two or more categories are present: - At least two persistently abnormal values on multiple dates over at least 2 consecutive years : ALT, AST, γGT, or ALP - Evidence of hepatomegaly, splenomegaly, or portal hypertension by imaging - Abnormal Fibroscan® (>6.8 kPa) at any time - Persistently abnormal values on multiple dates over at least 2 consecutive years: APRI (≤0.50), FIB-4 (≥3.25), or AAR (≥1)

Flass et al. [323]	2012	1. CF related liver disease with cirrhosis/portal hypertension - clinical exam/imaging, histology, laparoscopy
		2. Liver involvement without cirrhosis/portal hypertension consisting of at least one of the following:
		  - Persistent AST, ALT, γGT >2 times upper limit of normal
		  - Intermittent elevations of the above laboratory values
		  - Steatosis (histologic determination)
		  - Fibrosis (histologic determination)
		  - Cholangiopathy (based on ultrasound, MRI, CT, ERCP)
		  - Ultrasound abnormalities not consistent with cirrhosis
		3. Preclinical: No evidence of liver disease on exam, imaging or laboratory values

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, γGT: Gamma-glutamyl transpeptidase, ALP: Alkaline phosphatase, US: Ultrasonographic, CFLD: Cystic fibrosis liver disease, APRI: AST to Platelet Ratio Index, FIB-4: Fibrosis-4, AAR: Aspartate aminotransferase and alanine aminotransferase ratio, MRI: Magnetic resonance imaging, CT: Computerised tomography, ERCP: Endoscopic retrograde cholangiopancreatography

1.3.5.4 **Treatment**

Treatment options for CFLD are limited, with centres using the unlicensed secondary bile acid UDCA in the hope that it increases biliary flow and decreases toxic bile acid build-up, However, as this is the only current treatment, NICE continues to recommend its usage while blood tests indicate liver damage, with the discontinuation when these normalise [337]. The advent of protein modifying drugs for CF has transformed the management of the associated chest disease but their impact on liver disease has not yet been assessed. This issue is described in more detail in the discussion.

Treatment for cirrhosis complications is similar to those described above with ongoing surveillance and treatment for complications. The thresholds used for liver transplantation as synthetic failure is a relatively late phenomenon in CFLD though evidence of cirrhosis, decompensation, portal hypertension or uncontrollable variceal bleeding are important indications with the timing preferably before the decline in lung function, though a paired lung and liver transplant may be performed with some advocating for the addition of the pancreas if CF diabetes is present [321, 323, 324, 335, 338].

1.4 **Hypothesis and aims**

This study focuses on hepatitis C associated liver fibrosis with secondary studies on liver fibrosis associated with sickle cell disease and cystic fibrosis.

Hypothesis:-

1) Evaluation of liver function with indocyanine green clearance and/or transient elastography will allow classification of patients into those who may, or may not, respond to antiviral therapy with an improvement in liver function.

2) Risk factors for the development of liver cell cancer following antiviral therapy for hepatitis C can be determined from an analysis of case records of a large population at risk

Pilot studies

The frequency of liver disease in a cohort of patients with cystic fibrosis and sickle cell anaemia has been studied along with investigations to identify characteristics of patients at greatest risk.

1.5 <u>Statement</u>

This project initially began as a joint project between QMUL, LapResearch UK and HCA international to utilise the strengths of each contributor to provide a platform for basic science research. Unfortunately, this arrangement was not finalised in time for my induction and therefore, I proceeded outside of this program but with the same broad outline for a basic science project. However, it became clear that the intervention I was due to investigate would not be available, so we pivoted to another basic science project. This, in turn, was abandoned due to logistical issues and we restarted with a clinically based strategy that initially investigated hepatitis C with markers of liver function following DAA treatment. This was then expanded following the reports of increased HCC production following DAA treatment. We then added different types of liver disease in the forms of adult-onset of SCD and CF. Towards the end of the data collection, I began higher surgical training and was involved in the COVID response within Kent, which further lengthened the project submission. However, I was able to continue to submit most chapters for presentation at EASL and published two papers from this work.

2   Methods

2.1   Transient Elastography - Fibroscan®

Fibroscan® mini 430 from Echosens (France) was used to gain transient elastography (TE) readings. All operators attended a half day training with a Fibroscan® representative ,including directly observed measurements.

Patients fasted for 3 hours prior to TE being performed. They were lain flat with their head on a pillow and right arm placed above or behind their head, allowing the opening of the rib spaces for the probe placement. If further space was required then the right leg was placed over the left. The operator was seated to the patients' right. Once the machine was started, patient name, date of birth and hospital number were entered.

The patient's xiphisternum was then palpated and a perpendicular line followed to the mid-axillary line, this equated to the 5th/6th rib space. The probe was placed perpendicular to the skin, in an intercostal space, with a water-based transducer gel used as a medium and the pressure applied is checked to be within acceptable limits. An ultrasound reading given on the screen, the A-mode and TM-mode (time motion), which is the A-line displayed over a period of time see Figure 2.1. The A-line was then inspected to check it was straight and the TM-mode should not show any heterogeneity. This indicated the probe was over the liver and not placed over lung or colon, and that there isn't a large distance between the skin and liver which, would indicate a layer of fat and therefore an XL probe should be employed. Once adequately positioned, the button on the probe is pressed sending a shear wave into the tissue.
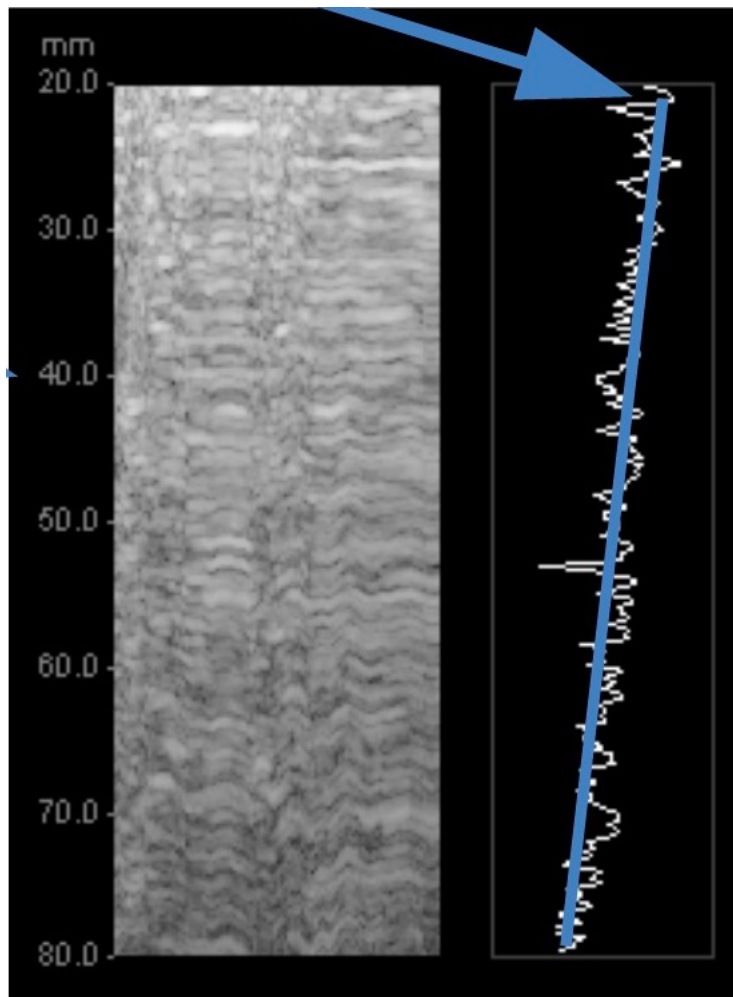
Figure 2.1: The ultrasound reading on a Fibroscan® machine. The right side displays the A mode which is the immediate reading for a single US beam, the blue line indicates this is overall straight and therefore acceptable. The left side displays the time motion (TM) mode which is the A line displayed over time.

The shear wave propagation is represented on the elastogram (Figure 2.2) along with the TE measurement. The elastogram is then inspected to ensure an accurate measurement is attained. The lines of the shear wave should reach at least 60mm depth and be straight, showing no impedance to the wave such as a vessel. The lines should be parallel and not diverging as this would indicate the wave is hitting something such as a rib which is causing it to diverge and thus the signal is weakened. Finally, to check the depth is correct for the measurement there should not be an exaggerated angle at the top which would cause an over estimation due to the liver being too deep, this should have been checked on the original A-line view.

91
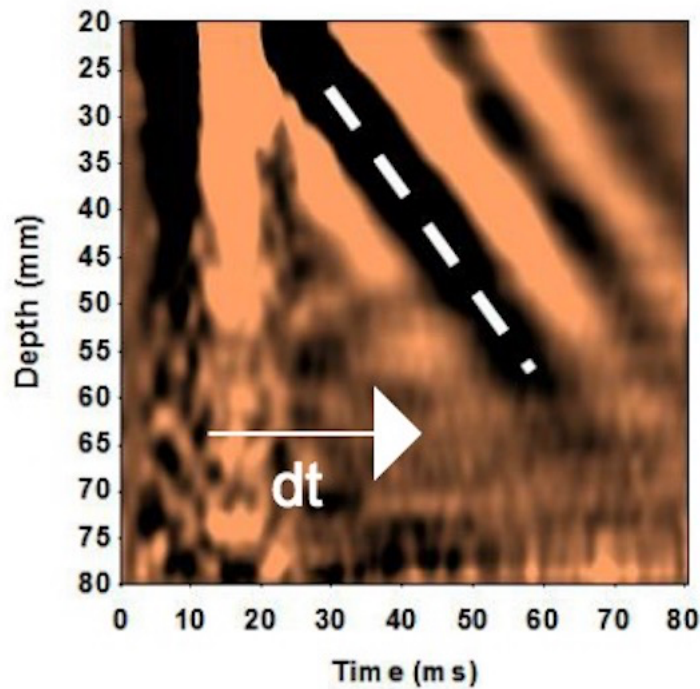
Figure 2.2: Elastogram obtained following the introduction of a shear wave with the disruption to tissues presented over time. White dashed line shows the passage of the wave is straight and the black lines are parallel. The white arrow (dt) shows the wave has reached the required depth of 60mm, therefore this reading is acceptable.

If all of these conditions are satisfied, the reading is accepted and then repeated to produce ten validated measurements. Inaccuracy of 30% is allowed to get a maximum of 13 measurements and the IQR/median ratio should be less than 30%. The median measurement is taken by the machine as being the overall reading. The probe is disinfected with a non-alcohol wipe in preparation for the next patient.

2.2 **Indocyanine green excretion test**

Measurements for ICG were taken at the same appointment as the Fibroscan®, so patients were fasted for three hours prior to the investigation. The patient had been at rest for at least 5 mins prior to the investigation to insure they had reached their resting heart rate thus not factoring in the measurements taken. They were cannulated within the anti-cubital fossa with at least a 20 gauge cannula under sterile technique with the cannula flushed and made sure to be working prior to the investigation starting.

ICG is supplied in powder form and is reconstituted with water only and is administered at a dose of 0.5mg/kg. It should be stored at less than +25˚C and out of direct sunlight. It shows little decomposition once reconstituted for the first few hours.

The measurements for ICG clearance were taken using the PulsioFlex® monitor (Getting AB, Germany) and connected to the LiMON® (Getting AB, Germany) near infrared module and sensor. The machine is started and the patients name, date of birth and hospital number as well as weight are entered. The sensor is placed upon the patients finger and a stable reading of the background levels attained. The indocyanine green powdered dye (Verdye, Diagnostic Green) was diluted to a concentration of 0.5mg/kg with sterile water and mixed vigorously for 30 seconds. The calculation is performed by the machine, which is then instructed to begin reading and the ICG injected as a bolus via the cannula in a sterile manner and followed by a 10 ml flush of sterile water. It will then detect an increase in ICG has the first pass through the liver and the measurements will take place. The sensor is to stay in place on the patient to allow measurement of ICGR15 with the timing being dictated by the machine and a visible countdown being shown. At the end of the measurement a reading will

be given detailing the PDR and ICGR15 values. The cannula will finally be removed from

the patient and haemostasis assured.

2.3 The association between hepatocellular carcinoma and direct acting anti-viral treatment in patients with decompensated cirrhosis

2.3.1 Study Design

A longitudinal, case-controlled observational study was performed of patients who received antiviral treatment under the NHS England early access programme (EAP). All patients developing HCC within the database were extracted and frequency matched with patients not developing HCC.

2.3.2 Study setting

2.3.2.1 NHS England early access programme (EAP)

The NHS England early access programme (EAP) provided use of direct-acting antiviral (DAA) treatment prior to EU licensing approval (June 2014-September 2015). Entry to the English early access programme specified that patients had to have either a diagnosis of hepatic decompensation in the past or have current evidence of CTP score B or C. Additional patients with HCV associated complications (chiefly vasculitis) who were deemed at high risk of early demise were also offered. The program was later replaced by the NHS England HCV policy to treat patients with compensated cirrhosis.

Treatment initiation was conducted by 20 HCV centres in England. This was run as a hub and spoke model

2.3.2.2   <u>EAP treatment regimens</u>

Funding for the EAP came from NHS England and provided 12 weeks therapy with either sofosbuvir/ledipasvir or sofosbuvir plus daclatasvir, with or without ribavirin. The specific treatment regimens prescribed were decided by local multidisciplinary team meetings (MDT).

2.3.2.3   <u>HCV Research UK</u>

A biobank and clinical database were set up in 2012 to facilitate HCV research, namely, HCV research UK (HCVRUK). At the inception of the EAP, all sites agreed to participate in data collection for HCVRUK and following this; all patients were encouraged to enrol in HCV Research UK (HCVRUK) with written informed consent. Entry into the database was not a prerequisite for treatment and patients were reassured they would receive the same antiviral treatment whether or not they agreed to share data. However, all centres offering antiviral therapy had to be members of HCV Research UK ensuring that all patients had the opportunity to contribute to the national database.

2.3.3 Study participants

2.3.3.1 Inclusion criteria

The following inclusion criteria were used:

- De novo HCC following EAP treatment

- The patient was treated within the EAP;

- Indication for treatment was advanced liver disease

- Have either a diagnosis of hepatic decompensation in the past or have current evidence of

CTP score B or C

2.3.3.2 Exclusion criteria

The following exclusion criteria were used:

- Those treated for extrahepatic disease

- Prior history of HCC diagnosis

- Liver transplant prior to entry into the EAP

2.3.3.3 Controls

Controls were collected as (two controls per case) with no history of HCC diagnosis. These

were frequency matched for:

- Age

- Gender

- Child-Turcotte-Pugh score

- Length of follow-up

2.3.3.4 Rationale for patient selection

Therefore, the HCV Research UK database was interrogated for all cases of de novo HCC diagnosed from the start of the early access programme until 15 June 2017, regardless of diagnostic modality to conduct a more comprehensive study of HCC in EAP patients.

2.3.3.5 Method of follow up

Data collection was performed prospectively from baseline with patient follow-ups every two weeks while on treatment and at four and 12 weeks post. Antiviral treatment, clinical status and blood tests were performed as appropriate. Patients then went back to their usual six-monthly follow-ups and continued the imaging schedule they had from prior to enrolling in the EAP.

2.3.4 Variables

2.3.4.1 Primary Outcome

To determine if there any baseline characteristics which may predict which patients will go on to develop de novo HCC.

2.3.4.2 Secondary Outcome

To determine if there is any impact of HCC development on sustained virological response. To determine the effect on prognosis and progression of HCC in viraemic and non-viraemic patients.

2.3.4.3   Primary endpoint

Primary endpoints were the development of HCC, sustained virological response and overall survival.

2.3.4.4   Secondary endpoint

Secondary endpoints were progression of non-malignant liver lesions to HCC and the further progression of HCC.

2.3.5   Data Sources

Patient selection was conducted centrally at the HCV Research UK offices. Following initial quality checks any missing data was sent via requests to the sites including the up to date patient status. This was important for prospective control patients to confirm they had not developed HCC following their last update. All scan and MDT results were also collected. Once this was returned centrally, quality checks were performed and the results forwarded.

2.3.6   Mitigating bias

2.3.6.1   Selection bias

All patients entering the EAP were offered the opportunity to enter the HCV Research UK database to allow the broadest possible patient cohort; however, certain selection biases such as non-participation bias undoubtedly remain. Specific measures were put into place, to mitigate these. These included: -

- Inconvenience – Data collection by each site to allow the least inconvenience to patients as much as possible.

- Central data collection - this was particularly important for control selection as the research team was blinded to the patient outcome and thus reducing unintentional bias.

- Matching criteria - this was kept as succinct as possible to allow any potential factors to come to the fore without causing statistical errors due to follow up time or initial disease severity.

2.3.6.2 Recall and reporting bias

The study was carried out prospectively, over multiple sites, logged in real-time and designed to keep data collection as objective as possible throughout.

2.3.6.3 Potential confounders

Baseline assessment of alcohol excess, diabetes mellitus, metformin use, HIV co-infection and previous hepatic disease was noted. These were not be re-assessed during follow-up though they may confound recovery of liver function. However, as this is a real-world study, it was felt, to exclude these patients would not allow the maximum transferability of the results and it was thought unlikely that significant changes in lifestyle would occur following therapy.

2.3.7 <u>Study size</u>

There was no formal power calculation as we wanted to study as many patients who developed HCC as possible. We thus included all HCC patients plus twice the number of controls.

2.3.8 <u>Quantitative variables</u>

Baseline data included age, gender, ethnicity, alcohol usage, smoking status, cannabis use, diabetes mellitus, HIV status, non-HCC cancer history, psychiatric history including suicide attempts, COPD and cardiac status and baseline medications including use of proton pump inhibitors or statins. Data were also available for HCV (route of infection, genotype), date of cirrhosis diagnosis and decompensation diagnosis, previous HCV treatment and Child-Turcotte-Pugh score within the year preceding treatment. The Child-Turcotte- Pugh score was converted to a stage centrally for interpretation purposes (stage A—score 5-6, stage B—7-9, stage C—10-15). Local accredited laboratory measurements for the preceding year were collected with the highest serum HCV RNA, lowest serum sodium, lowest creatinine, highest alanine aminotransferase (ALT), aspartate transaminase (AST), highest bilirubin, lowest albumin, highest alpha-foetoprotein (AFP), highest clotting studies and lowest full blood count measurements used. The model for end-stage liver disease (MELD) score, AST to platelet ratio index (APRI) score and albumin to bilirubin (ALBI) grade were calculated centrally. Length of follow-up was defined as the date of onset of DAA treatment until the date of death, date of transplantation or date of survey, whichever occurred first.

DAA treatment type and commencement date were noted. Sustained virological response (SVR) was defined as negative for serum HCV RNA at 12 weeks (SVR12) following the

completion of treatment. Patients with incomplete HCV treatment outcome data either due to death prior to SVR12 tests or those lost to follow-up were removed from the analysis.

All patients were subject to national guidelines recommending an ultrasound scan every six months with further cross-sectional imaging if indicated. All local imaging and multidisciplinary team (MDT) reports were collected centrally by the study team, for the year prior to therapy and following therapy, up until the study endpoint. Tumour size measurements were taken from radiological reports and Barcelona clinic liver cancer (BCLC) scores [339], Liver Reporting & Data System (Li-RADS) grading [246], Milan criteria [251] and response evaluation criteria in solid tumours (RECIST) criteria [253] were applied and appropriate scores/grades generated by the study team. RECIST criteria, which take into account the size and progression of the primary lesion, secondary lesions, nodal, vascular and metastatic disease to give an overall definition for complete resolution, partial resolution, stable or progressive disease, were used to assess tumour progression with the date of cross-sectional imaging being used to define the observation period. The frequency of surveillance scans and the presence of pre-existing lesions were evaluated using six-monthly reporting windows with the date of DAA commencement being day 0. Patients with positive scans or those transplanted or died were censored at that point.

The date of HCC diagnosis was the date of the first cross-sectional imaging, satisfying European Association for the Study of the Liver (EASL) HCC diagnosis guidelines, as determined following local multidisciplinary team meeting or, for cases with tissue diagnosis on explant histology, as the date of surgery. Dates and types of HCC treatment were obtained from sites as well as the date of transplant and date of death.

Given the probability that cancers diagnosed within six months of treatment initiation may have been present at treatment onset, we analysed data for 'early' cancer (within six months of DAA initiation) and late cancers—diagnosed after this time point.

2.3.9   Statistics

Statistical analysis was performed in conjunction with Polychronis Kemos (statistician, Queen Mary University of London).

Data were analysed using IBM SPSS version 25 (Armonk, NY) and $p<0.05$ was considered statistically significant.

2.3.9.1   Primary outcome

Baseline characteristic data are presented as the median and interquartile range (IQR) for continuous variables, as data were non-parametric, or as frequencies and percentages for categorical ones. Mann-Whitney U and chi-square tests were used for baseline characteristics and subsequent comparisons.

Count data for two-group comparisons were analysed with two proportions tests using the normal approximation method to calculate the P-values, thus accounting for missing values. Odds ratio analyses were performed using the z-score calculated as $\ln(OR)/SE\{\ln(OR)\}$. The odds ratio (OR), standard error and 95% confidence intervals were calculated according to Altman, 1991[340].

To analyse the association of HCC development with several variables in our dataset and investigate potential confounding factors, we used multiple logistic and Cox regression models. Initial univariate analysis with respect to both deviance and Hosmer-Lemeshow goodness-of-fit tests was initially performed while maintaining the variance inflation factor to the minimum. Important factors were then added to a binomial logistic model, which was built to explain the HCC status (Yes/No) and potential interactions that were included as interaction terms in the model. To understand a time-dependent outcome (time to develop HCC), a Cox proportional hazards regression analysis was used and produced hazard rates allowing the quantification of the effect (risk) per group or unit change depending on the nature of each predictor. The effect of each variable is presented with hazard rates and 95% confidence intervals. The hazard rate was calculated for a clinically meaningful increment of change for continuous variables.

2.3.9.2 Secondary outcome

To analyse the effect of HCC on SVR achievement, count data analysis was again employed to calculate p-values. Odds ratios were calculated with standard error and 95% confidence intervals as described above.

To determine the effect on prognosis and progression of HCC in viraemic and non-viraemic patients, time-to-event analyses were performed using the non-parametric Kaplan-Meier method [341]. The survival distributions were compared for equality for two groups at each comparison. All 'lost to follow-up' cases were censored up to the most recent time point with available information. The log-rank test results are presented for each comparison, but the Breslow and Tarone-Ware tests were also considered.

2.4 Changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy

2.4.1 Study design

This was a prospective, longitudinal, observational trial, looking at a single cohort of patients with hepatic C cirrhosis. Recruitment was from a single NHS trust following a recommendation by the responsible clinician. Consenting patients were planned for review up to one year after completion of therapy with data captured at baseline, four weeks (+/- 2 weeks) following treatment and again at one calendar year.

2.4.2 Study setting

All patients were recruited from Barts Health NHS trust. The Hepatology department initially required all patients to be treated at The Royal London Hospital, however, part way through the study this was extending to include clinics at all other sites operating in the Barts Operational Delivery Network, namely, Whipps Cross and Newham University hospitals.

2.4.3   Study participants

2.4.3.1   Inclusion criteria

The inclusion criteria were:

• Patients attending Bart's Health NHS trust with cirrhosis (defined as transient

elastography score >11.5 or APRI score >2 or liver biopsy or imaging report of cirrhosis)

who are planning to commence antiviral therapy for chronic hepatitis C.

• Age 18 or above

• Willing and able to provide informed consent

2.4.3.2   Exclusion criteria

Exclusion criteria were defined as below and set to include as wide a patient base as possible.

• Any inclusion criteria not met

• Pregnancy or breastfeeding, confirmed with pregnancy test if needed

• Known allergy to ICG

2.4.3.3   Rationale for selection of study population

The selection criteria were left broad to attempt to reflect a real-world setting. Patients with

compensated liver cirrhosis with chronic HCV infection were considered for the study.

Previously decompensated patients were deemed appropriate, but those with ongoing

decompensation were not, as the aim of the study was to identify factors associated with

future liver deterioration as those with on-going liver failure were likely to undergo either

transplantation or serious sequelae of infection. The criteria used to determine cirrhosis

matched those use in the NHS England HCV early access scheme. It should be noted that the cut-off used for TE score by NHSE was 11.5kPa and is slightly lower than other guidelines which use 12.5-13kPa to define cirrhosis.

To maintain the transferability of the study to everyday practice most co-morbidities, including previous malignancy, liver transplant and HIV co-infection were permitted. Patients with prior exposure to treatment and all genotypes are also included.

2.4.3.4 <u>Source of patients</u>

Potential participants were seen by their clinical team and those felt to be appropriate were put forward to the weekly multi-disciplinary team (MDT) meeting for the approval of treatment as well as the best treatment available, in accordance with NHS England (NHSE) protocol. All treatment regimens were acceptable for the purposes of this study as the aim was to monitor the effect on the liver and not individual treatments. Those participants who would potentially be suitable for the study were flagged at this point.

They were then seen in clinic by their clinical team, consisting of doctors and specialist nurses, and treatment regimes offered and explained as well as their usual clinical schedules and team contact details. Subsequently, they were offered the opportunity to partake in research with the understanding that this would not affect their treatment in any way. They were then referred to the research team for informed consent to be taken. This included the issuing of a patient information leaflet as well as consent form.

As per their normal treatment pathway, the patients returned for an agreed appointment date (usually within 1 to 2 weeks though could be up to 3 months) at which point they would start their DAA treatment. At any point between or at this appointment patients baseline measurements were taken.

2.4.3.5 Contacting patients

Once patients were enrolled into the study, follow ups were organised with the clinical team whenever possible. Otherwise a separate appointment was organised following contact via telephone, and if this was not possible a letter was sent to their home to contact the research team. Several patients were either uncontactable or would not be available within the set protocol time and therefore this appointment was recorded as missed.

2.4.3.6 Method of follow up

It was proposed that the patient's normal clinical team would see potential participants and those felt to be appropriate, would be put forward to the weekly multi-disciplinary team (MDT) meeting for approval of treatment as well as selection of appropriate therapy. This is in accordance with NHSE guidelines. All treatment regimens were acceptable for this study as the aim was to monitor the effect on the liver and not individual treatments. Those participants who would potentially be suitable for the study were pre-identified at this meeting.

Suitable participants were then seen in clinic by their clinical team, which consisted of doctors and specialist nurses and the selected treatment regimen offered and discussed as well as their usual clinical follow-up schedules and team contact details. Subsequently, they were

offered the opportunity to partake in this research project with the understanding that failure to participate would not affect their treatment in any way. They were then referred to the research team for informed consent to be taken. This included issuing a patient information leaflet as well as a consent form.

At their first appointment, patients were given an opportunity to (re-)review the patient information sheet and have any remaining related questions answered (up to 15 minutes). Specifically, it was highlighted, that their participation was voluntary. Written consent was then taken (5 minutes) and patients underwent a Fibroscan® if not already performed (10-15 minutes) and finally intravenous cannulation followed by the Indocyanine green excretion test (15-20 minutes).

One month (+/- 2 weeks) following completion of treatment a repeat appointment was organised for post-treatment tests. Patients were invited to return 12 months after starting their treatment. Please see the flowchart in Figure 2.3 to see a representation of this.

```
┌─────────────────────────┐
│  Patients eligible for   │
│  treatment               │
│  identified by MDT       │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  Appointment with        │
│  clinical team.          │
│  Patient's treatment     │
│  plan is agreed          │
│  and offer to            │
│  participate in research │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  Informed consent.       │
│  Indocyanine             │
│  green and Fibroscan®    │
│  performed               │
│  at single appointment   │
└─────────────────────────┘
            │
            │        Within 3 months
            ▼
┌─────────────────────────┐
│  Patient undergoes       │
│  treatment.              │
└─────────────────────────┘
            │
            │        4 Weeks (+/- 2 Weeks)
            ▼
┌─────────────────────────┐
│  Repeat Indocyanine      │
│  green and               │
│  Fibroscan®  performed   │
└─────────────────────────┘
            │
            │        52 Weeks  (+/- 2 Weeks)
            ▼
┌─────────────────────────┐
│  Repeat Indocyanine      │
│  green and               │
│  Fibroscan®  performed   │
└─────────────────────────┘
```

Figure 2.3: Flowchart displaying the patient pathway following identification for enrolment to changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy study.

2.4.4   Variables

2.4.4.1   Primary Outcome

To determine the changes in liver fibrosis and function in patients with cirrhosis, undergoing antiviral therapy for chronic HCV.

2.4.4.2   Secondary Outcome

To determine whether pre-treatment TE and ICG testing allowed stratification of patients into those likely to recover liver function following treatment for chronic HCV and those that will have a worsening or static liver function and therefore require liver transplantation.

2.4.4.3   Primary endpoint

The primary endpoint was a change in TE, as measured by Fibroscan®, and a change in ICG clearance, after initiating antiviral therapy in patients with viral hepatitis induced cirrhosis undergoing antiviral treatment.

2.4.4.4   Secondary endpoint

To determine whether a cut-off value for baseline TE and ICG testing predicts which patients will have a statistic or worsening liver disease and would thus require transplantation.

2.4.5   Data sources

The initial referral was made via MDT outcomes and included the treatment regime as well as the length of the proposed treatment. Data was collected from patient consultations and examination, medical notes, electronic medical notes, electronic pathology results and results of individual testing for TE and ICG testing as performed by the study team. These were entered into electronic clinical research forms on the hospital computing system.

2.4.6   Mitigating bias

2.4.6.1   Selection bias

The inclusion and exclusion criteria were selected to allow as many patients as possible to be offered the opportunity to enter the study; however, certain selection biases such as non-participation bias undoubtedly remain. Specific measures were put into place, to mitigate these. These included: -

- Interpreters - The local population has a high immigrant community with several languages spoken. In-room translation services were provided, either physically or telephonically with the subject and where appropriate, their families to try to overcome this.

- Inability to fund access - Upon increasing the service to a wider geographical area, not originally part of the study, an amendment was submitted to allow travel expenses to be paid to enable as many patients as possible to attend.

- Inconvenience – to reduce withdrawal due to the inconvenience of participating in the study the time for each appointment was kept to a minimum. Wherever possible, interventions were coordinated with the medical team to reduce the disruption to

patients. The interventions they required (e.g. have a cannula placed for blood sampling and ICG test rather than separate venepuncture and cannulation) were minimised in this way, thus allowing better attendance to the appointments.

2.4.6.2   **Recall and reporting bias**

The study was carried out prospectively, logged in real-time and designed to keep data collection as objective as possible throughout. Patients were seen throughout the day and on different days to avoid systematic bias.

All machines were calibrated and serviced according to manufacturer guidelines to avoid measurement errors.

2.4.6.3   **Potential confounders**

Baseline assessment of alcohol excess, diabetes mellitus, metformin use, HIV co-infection and previous hepatic disease was noted. These were not be re-assessed during follow-up though they may confound recovery of liver function. However, as this is a real-world study, it was felt, to exclude these patients would not allow the maximum transferability of the results and it was thought unlikely that significant changes in lifestyle would occur following therapy.

2.4.7   Study size

As this was initially a proof-of-concept study, we minimised the number of variables studied (five were selected - biochemical liver function tests, MELD score, Childs Pugh score, Indocyanine green and TE). In line with standard recommendations that for each variable a minimum of 10 patients should be studied we aimed to recruit 50 patients and, allowing for a 15% drop out (8), we aimed to recruit 58 participants. The early data from this study may then be used to calculate the effect size and thus allow for a larger, more definitive study to be performed. Data were planned to be presented with a median level to a 95% confidence interval.

2.4.8   Quantitative variables

Baseline demographics (age, ethnicity and gender), medical history (diabetic status, metformin use, HIV status, alcohol use currently, previous high alcohol intake (>6units/day), previous HCC and HCV treatment history), examination, full blood count, liver function tests, clotting, HCV genotype and investigations looking for decompensation (i.e. ultrasound abdomen and oesophageo-gastro-duodenoscopy) were assessed. The local accredited NHS lab performed all laboratory tests. Childs-Turcotte-Pugh score was taken from the medical notes and if this was not stated, this was calculated centrally. MELD and APRI scores were calculated centrally. A TE and ICG excretion test were performed at baseline.

At the subsequent two visits, the latest blood tests as dictated by the clinical team were noted as well as a repeat of the TE and ICG excretion test. Any other changes in liver health were noted at this point, e.g. HCC recurrence. A diagrammatic representation of the schedule is detailed in Table 2.1.

Table 2.1: Diagrammatic schedule for data noted for each appointment

Assessment	Details of requirements	1st visit within 3 months of starting treatment	2nd visit following completion of treatment (+/- 2 weeks)	3rd visit after 52 weeks after treatment initiation
Informed consent		✓		
Recording demographics	Age	✓		
	Ethnicity	✓		
	Sex	✓		
Record past medical history		✓		
Recording clinical examination		✓	✓	✓
Recording Routine tests (NB no additional tests will be performed and tests not performed will not be repeated for the study)	FBC	✓	✓	✓
	LFT, AST	✓	✓	✓
	Clotting screen	✓	✓	✓
	Genotype if available	✓		
	US	✓		
	OGD	✓		
Recording scores	Model for end stage liver disease (MELD)	✓	✓	✓
	Childs-Pugh	✓	✓	✓
Transient elastography		✓	✓	✓
Indocyanine green testing		✓	✓	✓

FBC: Full blood count, LFT: Liver function tests, AST: Aspartate aminotransferase, US: Ultrasound scan, OGD: Oesophago-Gastro-Duodenoscopy

2.4.8.1 Withdrawal or discontinuation

2.4.8.1.1 Criteria for Discontinuation

The patient's best interests always take precedence over this study. If the patients' health was deemed to be affected by the study, the patient was to be withdrawn and reasons clearly documented in the registry. If this was due to an adverse event, then this was to be disclosed on an Adverse Event Form and patient followed up to the satisfaction of the Chief Investigator.

2.4.8.1.2 Subject withdrawal (including data collection / retention for withdrawn participants)

If patients have any concerns or following giving consent, these were to be discussed with the research team and patients reminded that they were free to withdraw consent at any time and for any reason. Any data already collected was to be analysed, but no further information was to be collected unless the patient specifically states otherwise.

2.4.9 Ethics application

The project was registered to ClinicalTrials.gov (NCT02782247) and the initial application to the ethics committee occurred in February 2016 with the study designated reference 16/WA/0082. A proportional review was held at Wales Research Ethics committee seven occurring on 9/3/16. Further clarification on several points was sought following this and final approval achieved on 16/3/16. All participants provided written informed consent before undertaking any study-related procedures. The study was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice Guidelines and the Declaration of Helsinki.

2.4.10 Ethics amendment

At the study commencement standard of treatment was twelve weeks and this was stated in the initial application however, following commencement patients who did not achieve SVR were given treatment extensions to between 16 and 24 weeks. A major amendment was submitted to allow these patients to be included. A slight increase for the follow up appointment was also instigated to bring this to +/- 2 weeks of the allocated follow up appointment date, thus allowing more patients to fulfil these commitments without

interruption to their normal lives. Also included in this amendment was the proviso to progress from paper electronic case reporting forms to electronic as per Barts Health trust guidelines. Finally, the expansion of the Hepatology department offering of DAA therapy meant clinics would be delivered at two new sites within Bart's Health NHS trust. To allow for these patients to attend The Royal London site for their investigations travelling expenses were included. Final approval was given for protocol v5 (see appendix X) on 20/3/17.

2.4.11 <u>Statistics</u>

Statistical analysis was performed in conjunction with Polychronis Kemos (statistician, Queen Mary University of London).

Data were analysed using IBM SPSS version 28 (Armonk, NY) and $P < 0.05$ was considered statistically significant.

Baseline characteristic data are presented as the median and interquartile range (IQR) for continuous variables, as data were non-parametric, or as frequencies and percentages for categorical ones.

2.4.11.1 <u>Primary Outcome</u>

To investigate liver fibrosis and liver function changes, initial median values were produced with their associated 95% confidence interval and plotted to review an overall trend. Following this, a repeated measures analysis was performed, allowing us to investigate the variance within each subject, thereby understanding how the scores change over time and the changes occurring between each patient's visit. Furthermore, the observations coming from

the same subject at different times are highly correlated and therefore, the observations cease to be independent. We introduce the random effects in our model for each subject for these reasons. This adjustment factors in the differences between the subjects in the TE reading at baseline and returns the effects of the change over time as a result (fixed effects). Overall, this improves the fit of the model produced. A Spearman's correlation test was then performed to assess the coordination of the two readings from indocyanine green excretion.

2.4.11.2 Secondary Outcome

To investigate the impact of treatment for hepatitis C on other variables, we evaluated blood tests and relevant scoring systems by visual inspection both individually, as a median, as well as Wilcoxon Signed-Rank test between baseline, four months and 12-month results.

We then generated a receiver-operating characteristic (ROC) curve for each time point, from which the area under the curve (AUROC) was calculated for ICGR15. The corresponding significance was stated and a cut-off value was identified with accuracy assessments performed, namely sensitivity, specificity, positive and negative predictive values, and the odds ratio for the cut-off value calculated. Fixed effects models were then applied to produce an equation utilising baseline ICGR15 to predict ICGR15 at either time point.

The cut-off value was then utilised to divide the cohort and each was compared via a univariate model. A Mann-Whitney U test was run to test differences in continuous variables. Distributions of the values were assessed by visual inspection and depending upon this, either a comparison of distribution (stated as mean rank) for those not similar or a comparison of

the median for those with similar distributions is stated. A Chi-square analysis was run for categorical data.

2.4.11.3 <u>Missing data</u>

Missing values and patients who missed appointments had these values omitted from the analysis for that time. However, if they re-engaged with subsequent appointments, all further data were included for analysis.

2.5 What is the distribution of transient elastography results in patients deemed at risk of Sickle cell liver disease

2.5.1 Design

As a hepatology service, we offer a quarterly joint clinic with the haematology team to review all patients suffering from, or at risk of, sickle cell liver disease (SCLD). At the time of commencing this service laboratory blood tests and liver biopsies were the only means of monitoring for liver disease that were available. The introduction of TE measurements, as a non-invasive means of assessing liver fibrosis, provided an alternative means to assess liver damage in patients with haemoglobinopathies. The service was introduced in 2017, when it was planned to perform a TE measurement on all patients with abnormal liver function tests and offer a liver biopsy to patients with abnormal scores (arbitrarily defined as >7.2kPa). This project was an audit of this on-going service and was not regarded as a separate research trial.

2.5.2 Ethics application

The project was submitted to the online health research authority and medical research council ethics decision portal (http://www.hradecisiontools.org.uk) on 1/06/2017 and was found to not require NHS REC approval as this was not research due to the study being part of regular patient care.

2.5.3 Patients

All patients under the care of the haematological service at the Royal London Hospital are reviewed yearly, at least. This may take place in their outpatient clinic; otherwise, those taking hydroxyurea have contact via the nurse specialist every three months to monitor the medication effect. Those receiving blood transfusions are regularly seen in the designated haematology unit within the hospital.

2.5.3.1 Inclusion criteria

This was a clinical audit and therefore there were no prespecified inclusion criteria. Patients seen in the joint hepatology/haematology clinic were referred for a TE measurement. To minimise observer variation, I performed most of these investigations but the trained TE technician was available to perform scans in my absence. Patients referred were attending clinics at The Royal London Hospital with abnormal liver function tests who, in the opinion of the referring clinician, were at risk of liver disease. Specific liver function tests investigated were, elevated transaminases or a bilirubin over 100μmol/L, this higher cut off was used as patients with sickle cell disease have a higher turnover of red blood cells and therefore have a chronically elevated bilirubin consequently 100μmol/L is deemed as an abnormal result.

2.5.3.2 Exclusion criteria

Patients were excluded from this review if they had any of the following criteria:-

• HbSC genotype

• Pregnancy or breastfeeding, checked with pregnancy test if needed

- Any patient without a relevant blood test within the preceding 18 months

- Elastography performed less than 10 days following admission for sickle crisis

2.5.3.3 Rationale for selection of study population

SCLD has been investigated in the paediatric population quite considerably, but less is known about those patients having adult onset of SCLD. Overall prevalence, early warning signs and the effect of long-term treatment have been poorly investigated partly due to the paucity of patients reaching advanced ages.

Patients were reviewed irrespective of treatment modality and length of treatment for sickle cell. TE was performed at least 10 days following admission for sickle cell crises and any patient undergoing a scan during this time was excluded from the audit. This criterion was introduced due to reports of an immediate increase in elastography post crises with this settling within forty-eight hours [299].

2.5.3.4 Method of follow up

In this population with multiple hospital attendances, we tried to minimise unnecessary appointments by linking the TE appointment to another hospital visit. Patients were offered an appointment at their convenience - either during their upcoming routine appointments with their medical team or within the transfusion unit. When patients attend their appointment, a TE was performed and arrangements made to review the result in their next clinic appointment.

2.5.4   Outcome Measures

To determine the prevalence of sickle cell liver disease in terms of both severe fibrosis and cirrhosis in a single tertiary centre and to identify factors associated with a deterioration in liver health. An important secondary aim was to determine the need for a Fibroscan®/liver service embedded within the haematology department.

2.5.5   Data sources

Data was collected from physical and electronic medical notes, electronic pathology results, specific haematology database (Microsoft Access) and results of individual testing for TE as performed by the study team.

2.5.6   Quantitative variables

At baseline, demographics (age, ethnicity and gender) and medical history were noted, as was sickle cell status including genotype and treatment history, , the frequency of transfusions and acute admissions over the preceding 18 months. Average baseline blood tests were calculated by taking the lowest haemoglobin, platelet count, mean corpuscular volume (MCV), serum sodium, potassium, urea and creatinine noted as well as the highest bilirubin, alanine aminotransferase (ALT) and aspartate transaminase (AST). The average serum ferritin was also used. Ultrasound findings of coarsened echotexture, irregular margins, nodularity, hepatomegaly or cirrhosis were deemed as indicative ultrasound changes.

As patients on transfusion are required to eat regularly, the length of fasting prior to the test was reduced to 2 hours. For the purposes of analysis, severe fibrosis was taken as a TE of more than 7.2kPa and cirrhosis as more than 12kPa.

2.5.6.1 **Potential confounders**

Baseline assessment of previous hepatic disease will be noted including cholecystectomies and gallstone disease which is increased within this population. Increased liver iron concentration may affect the TE reading and thus the closest reading to the elastography date will be taken to correlate this and investigate whether this may indeed be the case.

2.5.7 **Mitigating bias**

2.5.7.1 **Selection bias**

The selection criteria were set to allow as many patients as possible the opportunity to enter the study, however non-participation bias will undoubtedly remain. To try and mitigate these certain measures are put into place:

- The appointment time will be kept to the minimum and wherever possible, co-ordinated with the medical team to reduce the disruption to patients and thus allow better attendance to appointments.
- Flexibility when the Fibroscan® occurred was possible, as many patients attended on several occasions during the study period.
- The length of fasting prior to the test is waived to improve attendance as well as not affect their other treatments as patients on transfusions are required to eat regularly.

2.5.7.2   Recall and reporting bias

The study was carried out in a prospective manner, logged in real time and designed to keep data collection as objective as possible throughout. Patients were seen throughout the day and on different days to avoid systematic bias. Patients on transfusion had tests before starting transfusion to avoid inconsistency of results.

Machines will be calibrated and serviced according to manufacturer guidelines to avoid measurement errors.

2.5.8   Statistics

Statistical analysis was performed in conjunction with Polychronis Kemos (statistician, Queen Mary University of London).

Data were analysed using IBM SPSS version 28 (Armonk, NY) and $p<0.05$ was considered statistically significant.

Baseline characteristic data are presented as the median and interquartile range (IQR) for continuous variables, as data were non-parametric, or as frequencies and percentages for categorical ones.

A Mann-Whitney U test was run to test differences in continuous variables. A Mann-Whitney U test was run to test differences in continuous variables. Distributions of the values were assessed by visual inspection and depending upon this, either a comparison of distribution (stated as mean rank) for those not similar or a comparison of the median for those with

similar distributions is stated. A Chi-square analysis was run for categorical data. Spearman's rho test for correlation was applied. The test of significance was two-tailed with confidence intervals stated to a level of 95%.

Univariate variables which were found to be significant or deemed clinically important were assessed within an independent samples Kruskal-Wallis multivariate analysis to account for the ordinal progression of fibrosis.

2.6 What is the distribution of transient elastography results of adult patients with cystic fibrosis

2.6.1 Design

As a hepatology service, we offer a quarterly joint clinic with the respiratory team to review all patients suffering from, or at risk of, cystic fibrosis liver disease (CFLD). At the time of commencing this service laboratory blood tests and liver biopsies were the only means of monitoring for liver disease that were available. The introduction of TE measurements, as a non-invasive means of assessing liver fibrosis, provided an alternative means to assess liver damage in patients with cystic fibrosis. The service was introduced in 2017, when it was planned to perform a TE measurement on all patients seen by the respiratory service. This project was an audit of this on-going service and was not regarded as a separate research trial.

2.6.2 Patients

All patients under the care of the respiratory service at the Barts Health NHS Trust are reviewed yearly, at least. This may take place in their outpatient clinic; otherwise, for those requiring frequent admissions, on the ward.

2.6.2.1 Inclusion criteria

This was a clinical audit and therefore there were no prespecified inclusion criteria. All patients seen within the respiratory clinic with a diagnosis of cystic fibrosis were referred for a TE measurement. To minimise observer variation, I performed most of these investigations

but the trained TE technician was available to perform scans in my absence. Specific liver function tests investigated were, elevated transaminases or bilirubin.

2.6.2.2   Exclusion criteria

Patients were excluded from this review if they had any of the following criteria:-

• Pregnancy or breastfeeding, checked with pregnancy test if needed

• Any patient without a relevant blood test within the preceding 18 months

2.6.2.3   Rationale for selection of study population

CFLD has been investigated in the paediatric population quite considerably, but less is known about those patients having adult onset of CFLD. Overall prevalence, early warning signs and the effect of long term treatment have been poorly investigated partly due to the paucity of patients reaching advanced ages. Patients were reviewed irrespective of treatment modality and length of treatment for cystic fibrosis.

2.6.2.4   Method of follow up

Following advice from the respiratory team, patients were strategically invited for appointments to avoid cross contamination of infections. This is in accordance with CF guidelines and local protocols and are as follows:

- For Non-Pseudomonas Aeruginosa (NPA), Pseudomonas Aeruginosa (PA), non-Pseudomonas Aeruginosa-Non-tuberculous mycobacteria (NPA-NTM), Pseudomonas Aeruginosa-Non-tuberculous mycobacteria (PA-NTM) patients,

if they are booked sequentially, the patients are not to be sat next to each other in the waiting room - patients to be sat alternatively in separate waiting rooms

- Need to leave ~5-10minutes between NPA, PA, NPA-NTM, PA-NTM patients being in the room.

- Multidrug resistant Pseudomonas Aeruginosa (MRPA-LES) and Multidrug resistant Pseudomonas Aeruginosa-Non-tuberculous mycobacteria (MRPA-NTM) patients - leave a 3 hour gap between these patient.

- M.abscessus/Burkholderia cepacia complex (BCC) and M.chelonae - only one patient to be booked on each clinic list, no other CF patients to be booked onto these lists.

- Wipe down couch, machine and probe with red wipes between each patient (they take about 2-3minutes to work after they are dry).

When patients attend their appointment, a TE was performed and arrangements made to review the result in their next clinic appointment.

2.6.3   Outcome Measures

2.6.3.1   Primary Outcome of the audit

To determine the prevalence of cystic fibrosis liver disease in terms of both severe fibrosis and cirrhosis in a single tertiary centre and to identify factors indicating a deterioration in liver health. An important secondary aim was to determine the need for a TE/liver service embedded within the respiratory department.

2.6.4 Ethics application

The project was submitted to the online health research authority and medical research council ethics decision portal (http://www.hradecisiontools.org.uk) on 1/06/2017 and was found not to require NHS REC approval as this was not research due to the study being part of the regular patient care.

2.6.5 Source of patients

All patients treated for cystic fibrosis within the Royal London were considered for this study. The respiratory team carried out a database investigation for 2016 and all patients identified as suffering from cystic fibrosis were offered a TE reading. The last routine blood test results prior to the TE were taken; this was usually within the preceding one year.

Patients were either called to organise appointment times by a dedicated administrator or an appointment letter was sent. Before the appointment, they were called or sent a reminder text message of the appointment, in accordance with the trust policy. On the first round of appointments, 69 patients attended their appointment with seven declining investigation and 15 cancellations. For those that did not attend their first appointment, a second appointment was offered. In total, 79 patients had a TE reading.

2.6.6 Data sources

Data was collected from medical notes, electronic medical notes, electronic pathology results and results of individual testing for TE as performed by the study team.

2.6.7 **Potential confounders**

Baseline assessment of previous hepatic disease will be noted including cholecystectomies and gallstone disease which is increased within this population. CF patients are noted to have colonisation of various bacteria which has been implicated in the production of CFLD, we shall note these and review whether any association is found within our cohort.

2.6.8 **Mitigating bias**

2.6.8.1 **Selection bias**

The selection criteria were set to allow as many patient as possible the opportunity to enter the study, however non-participation bias will undoubtedly remain. To try and mitigate these certain measures are put into place:

- The appointment time will be kept to the minimum and wherever possible, co-ordinated with the medical team to reduce the disruption to patients and thus allow better attendance to appointments.
- We offered appointments on three different occasions to allow as many patients as possible to attend.
- The risk for cross contamination was kept to a minimum and this was conveyed to patients where required.

2.6.8.2 <u>Recall and reporting bias</u>

The study was carried out in a prospective manner, logged in real time and designed to keep data collection as objective as possible throughout. Patients were seen throughout the day and on different days to avoid systematic bias.

Machines will be calibrated and serviced according to manufacturer guidelines to avoid measurement errors.

2.6.9 <u>Quantitative variables</u>

At baseline, demographics (age, ethnicity, current and lowest BMI and gender) and medical history were noted, as was, cystic fibrosis status including genotype, meconium ileus, pancreatic insufficiency, UDCA use, severe alcohol intake, diabetes mellitus, metformin use, respiratory tract organisms, viral screening positivity, hepatomegaly on examination, variceal presence, spirometry results, latest ultrasound liver results and liver biopsy results. Average baseline blood tests were calculated by taking the lowest haemoglobin, platelet count, mean corpuscular volume (MCV), serum sodium, potassium, urea and creatinine noted as well as the highest bilirubin, alanine aminotransferase (ALT) and aspartate transaminase (AST). APRI, Fib-4 and AAR scores were calculated centrally. Ultrasound findings of coarsened echotexture, irregular margins, nodularity, hepatomegaly or cirrhosis were deemed as indicative ultrasound changes. scoring criteria from Debray et. al [321], Koh et al. [319] and Flass et al [323] were applied to the data. Patients were scored according to the available data with any missing values omitted from the criteria.

TE was usually performed by myself to minimise observer bias but the trained TE technician was available to perform scans in my absence. For the purposes of analysis, fibrosis was taken as a TE of more than 6kPa and severe fibrosis/cirrhosis as more than 9kPa.

2.6.10 <u>Statistics</u>

Statistical analysis was performed in conjunction with Polychronis Kemos (statistician, Queen Mary University of London).

Data were analysed using IBM SPSS version 28 (Armonk, NY) and $p<0.05$ was considered statistically significant.

Baseline characteristic data are presented as the mean and standard deviation for continuous variables, as data were parametric, or as frequencies and percentages for categorical ones.

Upon sub-group analysis, distribution was non-parametric and therefore, a Mann-Whitney U test was run to test differences in continuous variables. Distributions of the values were assessed by visual inspection and depending upon this, either a comparison of distribution (stated as mean rank) for those not similar or a comparison of the median for those with similar distributions is stated. A Chi-square analysis was run for categorical data. Following this, Spearman's rho test for correlation was applied. The test of significance was two-tailed with confidence intervals stated to a level of 95%.

Univariate variables found to be significant or deemed clinically important were assessed within a multivariate regression analysis. The model was then validated for variance and AUROC.

3   Results

3.1   The association between hepatocellular carcinoma and direct acting anti-viral treatment in patients with decompensated cirrhosis

3.1.1   Chapter summary

This chapter will investigate the relationship between DAA therapy in decompensated cirrhotic patients and the likelihood to develop de novo HCC. We used the HCVresearch UK registry, which prospectively gathered information about patients enrolled in the extended access program within the UK. This work has been peer-reviewed and published in the journal Alimentary Pharmacology and Therapeutics (DOI: 10.1111/apt.15296).

3.1.2   Hypotheses

We hypothesise that:

• There any baseline features which predict HCC development,

• Patients who are diagnosed with HCC during treatment less likely to achieve SVR,

• HCCs developing in an uninfected liver (i.e. post-SVR) are not more aggressive than cancers that develop in an HCV-infected liver

3.1.3   Results

3.1.3.1   Initial studies

Following the initial reports of increased HCC development following DAA treatment, we performed an audit of cases within the Royal London hospital to check the feasibility and

need for this study. We found 35 patients over eight years developing HCC following decompensated liver disease due to HCV. We, therefore, approached HCV Research UK with these initial findings and the need to interrogate a larger database for patients treated with DAAs.

3.1.3.2 Ethics

HCV Research UK gained ethical approval by the National Research Ethics Service (NRES) committee East Midlands—Derby 1 (Research Ethics Committee reference 11/EM/0314). Subsequent ethical applications were made to HCVRUK's Tissue and Data Access Committee with approval for this study (TR000424) being given on 21/3/2017. The study was performed in accordance with the 1975 Declaration of Helsinki guidelines on ethics as reflected in a priori approval by the institution's human research committee.

3.1.3.3 Requests for extra data

The HCVRUK team collated initial data and quality checked before imparting this to the research team. This including baseline characteristics and blood tests but also imaging for the year prior to therapy and MDT outcomes. These were reviewed and where extra information and imaging was required, a follow-up data request was requested from all sites, including clarification and further imaging requests. In particular, two patients were found to have cholangiocarcinoma rather than HCC and were thus excluded from further analysis.

3.1.3.4 **Baseline characteristics**

We identified 81 patients in the early access programme within the HCV Research UK database treated with DAA therapy between June 2014 and September 2015 who developed HCC subsequent to the onset of therapy. These were frequency matched with 178 early access programme patients who were treated with DAAs but did not develop HCC within the follow-up period. We excluded patients lost to follow up or who died before SVR outcome became known (1 HCC patient, 13 non-HCC patients).

HCC was diagnosed by MRI in 45 patients, CT scan in 26, while eight patients had incidental HCC diagnosed within their explanted liver. One patient had a date of diagnosis, but no mode of diagnosis was available. The demographics of the cohort are shown in Table 3.1. Frequency matching provided groups with similar age, Child-Turcotte-Pugh stage and gender distributions.

Table 3.1: Baseline characteristics of HCC and non-HCC patients

Characteristic	Non-HCC (n=165)	All HCC (n=80)
Median age, (IQR), yrs. †	57 (52.9-61.9)	57 (51.8-60.9)
Male sex, n (%) †	123 (75)	61 (76)
CTP grade (%) †	B (62)	B (65)
Mean MELD score (IQR)	11 (9-14)	11 (9-14)
Median length of follow up, (IQR), mths. †	33.5 (29.8-34.5)	22.4, (13.3-32.2)
Ethnicity, n (%)		
White-British	100 (61)	53 (66)
Asian	27 (16)	10 (13)
Other	38 (23)	17 (21)
Alcohol, n (%)		
Never	36 (22)	15 (19)
Current	29 (17)	8 (10)
Past/Former	94 (57)	57 (71)
Unavailable	6 (4)	0
Smoking status, n (%)		
Never	42 (25)	15 (19)
Currently	62 (38)	36 (45)
Past/Former	48 (29)	23 (29)
Unavailable	13 (8)	6 (7)
Genotype, n (%)		
Genotype 1	83 (50)	34 (42)
Genotype 3	65 (40)	42 (53)
Other	17 (10)	4 (5)
Diabetes, n (%)		
Yes	31 (19)	27 (34)
No	99 (60)	41 (51)
Unavailable	35 (21)	12 (15)
Past history of Non-HCC Ca, n	17	5
Previous treatment failure, n (%)	102 (62)	50 (63)
Treatment regimen, n (%)		
Sof/Led	6 (3)	1 (1)
Sof/Led/Riba	115 (70)	59 (74)
Sof/Dac	3 (2)	0
Sof/Dac/Riba	41 (25)	20 (25)
SVR achieved, n (%)	143 (87)	54 (68)
Median albumin, (IQR), g/L	29 (26-34)	27 (23-32)
Median alpha-fetoprotein, (IQR), ng/ml	7.0 (5-15.1)	7.0 (4-16.5)
Median alkaline Phosphatase, (IQR), U/L	148 (108-202)	121 (101-186)
Median bilirubin, (IQR), μmol/L	34 (22-49)	38 (23-52.75)
Median INR, (IQR),	1.3 (1.2-1.4)	1.3 (1.2-1.5)
Median platelet, (IQR), x10^9/L	74 (53-98)	63 (44-85.5)
Median sodium, (IQR), mmol/L	136.0 (134-139)	136.0 (132-138)
Median BMI, (IQR), kg/m^2	27.6 (24.6-32.3)	27.0 (24.7-31.4)

†Frequency matching criteria. Unknown values were excluded where unknown values existed. n: number, IQR: Inter-quartile range, yrs.: years, mths.: months, HCC: hepatocellular carcinoma, CTP: Childs-Turcotte-Pugh, SVR: Sustained viral response, Sof: Sofosbuvir, Led: Ledipasvir, Dac: Daclatasvir, Riba: Ribavirin, INR: international normalised ratio, BMI: body mass index. Standardised units supplied where appropriate

The cohort was predominately male (75%) and white (62%). Staging of cirrhosis according to Child-Turcotte-Pugh, following conversion from raw scores to stages, showed most patients were Child-Turcotte-Pugh stage B (63%) followed by A (22%) and C (15%) and for model for end-stage liver disease (MELD) score, a median of 11 (7-35). In line with the inclusion criteria for the early access programme, all patients with a Child-Turcotte-Pugh score of A had a previous history of decompensation and these 37 controls and 17 HCC patients had decompensating events of ascites (22), encephalopathy (7), variceal bleeding (6) and unknown (19). This is a representative mix of patients who were treated within the EAP program. Median follow-up was 32.4 months (22.5-34.2 months). Median follow up was longer in the non-HCC (33.5 months) than the HCC cohort despite this being a matching criterion although the upper quartile for both was similar (34.5 months and 32.2 months respectively).

Outside of the matching criteria, there were similar proportions of patients who never drank alcohol between the non-HCC (22%) and HCC groups (19%). There was a slightly higher proportion of patients who admitted to continuing to drink alcohol in the non-HCC (17%) vs HCC groups (10%). HCV genotypes 1 and 3 were the most prevalent with their being a slightly higher preponderance within the HCC patients for genotype 3, while genotype one was seen more in the non-HCC group. There are slightly fewer current smokers within the non-HCC group (38%) than the HCC group (45%).

Of the treatments prescribed following MDT discussion, most patients received ribavirin-containing anti-viral therapy (95.9%) with most having previous interferon exposure (HCC = 62.5%, non-HCC = 62%). The most common treatment regimen was sofosbuvir + ledipasvir + ribavirin (65.7%) spread equally between the groups with sofosbuvir + daclatasvir +

Ribavirin being the next most common regime. Fifty-four (67.5%) of the HCC patients (n = 80) achieved SVR12, as did 143 of 165 (86.6%) controls.

Of the blood tests, median alpha-fetoprotein (AFP) was equal at 7ng/ml for both groups at baseline. This is an important test, used routinely to screen and aid in the diagnosis of HCC and this being equal is an interesting finding. A raised median alkaline phosphatase within the non-HCC group (148U/L) compared to the non-HCC patients (121U/L). The international normalised ratio was equal at 1.3 for both categories, as was sodium at 136mmol/L. These indicate a similar group of patients at the baseline of the study.

Detailing the tumour characteristics, the median time from onset of anti-viral treatment to HCC diagnosis for patients treated with DAAs was 8.74 months (3.43-16.8 months). Overall, primary tumour size ranged from 9.5 to 120 mm with no lymph nodes, vascular involvement or metastases being found though two did not have information on size. We assessed the cancer stage using the Milan criteria which determine suitability for liver transplantation in patients with cirrhosis and HCC. The proportion of patients with HCC at the point of diagnosis who fell within the Milan criteria (i.e. circumscribed) was 61/72, following exclusion of those diagnosed on explant. Similar assessment, according to Li-RADS criteria, showed one category three tumour, 33 category 4 and 36 category 5 cancers with two unable to be categorised. Analogous assessment according to Barcelona clinic liver cancer (BCLC) scores showed 10 grade 0, 38 grade A, 3 grade B, 9 grade C, 7 grade D cancers with five unable to be categorised overall. (See Table 3.2)

Table 3.2: Description of tumours, excluding those found on explant

Parameter	All (n=72)
Size of primary lesion, mm	9.5-120
More than 1 lesion, n (%)	20 (28)
Fits within Milan criteria, n (%)	61 (85)
Li-RADS criteria, n (%)	
LR-3	1 (1)
LR-4	33 (46)
LR-5	36 (50)
Unavailable	2 (3)
BCLC Grade, n (%)	
0	10 (14)
A	38 (53)
B	3 (4)
C	9 (13)
D	7 (9)
Unavailable	5 (7)

Li-RADS: Liver Imaging Reporting and Data System, BCLC: Barcelona clinic liver cancer, n: number, Standardised units supplied where appropriate

3.1.3.5 Scan frequency

To rule out the possibility that patients who developed HCC were not receiving regular surveillance imaging, we interrogated imaging data in the year prior to the early access programme onset. There were scans available for 130 of the controls and 63 of the HCC cases. 35/165 (21%) controls compared to 17/80 (21%) HCC cases did not have a surveillance scan in this period. There were 44 non-HCC and 25 HCC patients with a single scan in the year prior. This was partly due to a mismatch in timing with some of these patients receiving scans during their treatment. Several patients received three or more scans within the year prior with the maximum being seven of different modalities, usually for surveillance of different lesions thought to be non-malignant. See Table 3.3 for the frequency of scans in the year prior.

Table 3.3: Frequency of scans performed in the year prior to starting DAA treatment for all patients split into non-HCC and HCC

Number of scans performed	Non - HCC (n=165)	All HCC (n=80)
0 scans, n (%)	35 (21)	17 (21)
1 scan, n (%)	44 (27)	25 (31)
2 scans, n (%)	56 (34)	16 (20)
3+ scans, n (%)	30 (18)	22 (28)

n: number, HCC: Hepatocellular carcinoma

3.1.3.6 <u>Non-malignant lesions</u>

Lesions found on screening imaging instil a level of diagnostic uncertainty for clinicians due to the difficulty in attributing a correct level of significance to these lesions. We thus investigated our cohort on the background of initial reports of increased HCC following DAA treatment. We interrogated the scans performed in the year prior to treatment to ascertain their prevalence as well as importance in developing HCC.

Non-malignant lesions were seen on scans performed within 12 months of DAA onset in 23/130 (18%) of the control patients, compared to 24/63 (38%) HCC cases (p=0.002, OR: 2.15, 95% CI:1.1-4.1) (Figure 3.1).
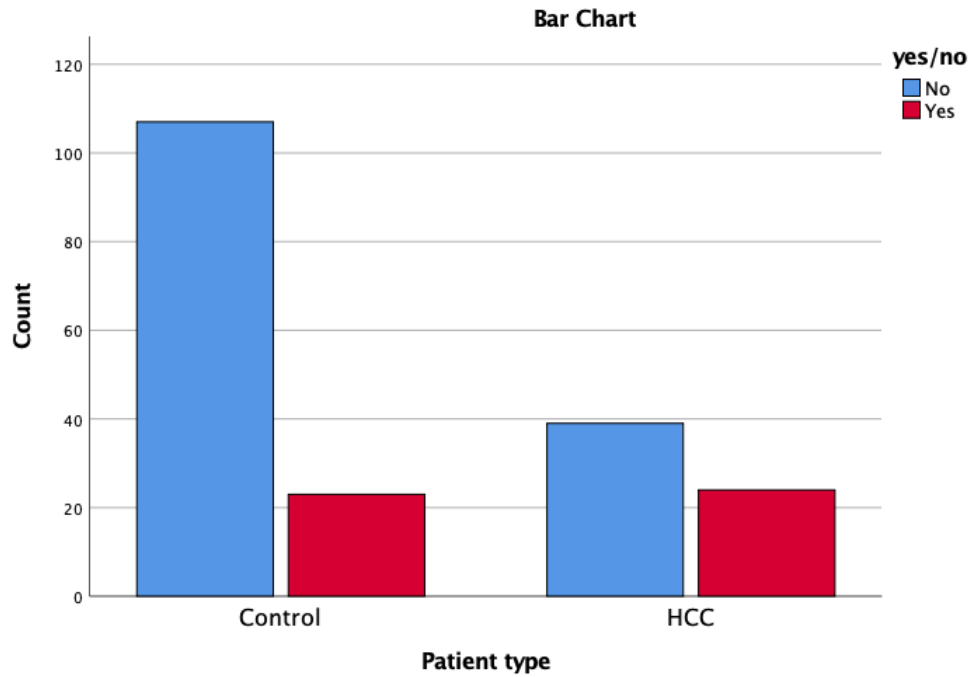
Figure 3.1: Clustered bar chart showing presence of nonmalignant lesions on pre-treatment scans (p=0.002, OR: 2.15, 95% CI:1.1-4.1)

To standardise the reporting as much as possible we used the nomenclature from the radiology reports and found, 12 of the control patients had cysts, five had nodules, three had haemangiomas and three had 'non-descript lesions', with seven patients having more than one of the described lesions (but always of the same type). The corresponding data for the HCC patients were six cysts, nine nodules, one haemangioma and eight 'nondescript lesions' with nine patients having more than one of the described lesions (but again, always of the same type) (Table 3.4).

Table 3.4: Breakdown of non-malignant lesion types according to HCC vs Non-HCC

Type of lesion identified	Non-HCC	HCC
Single abnormality, n		
Cyst	9	3
Nodule	2	7
Haemangioma	2	1
Lesion	3	4
Multiple abnormalities, n		
Cyst	3	3
Nodule	3	2
Haemangioma	1	0
Lesion	0	4

n: number, HCC: Hepatocellular carcinoma

Further interrogation of subsequent radiology reports and MDT outcomes was performed to investigate the malignant potential of each lesion for the HCC cases. Based on the radiologist stating if a lesion had either progressed or if an HCC was diagnosed in the same anatomical region, 15 of the 24 (63%) non-malignant lesions were considered to have progressed to HCC, with six of these patients presenting with an early HCC and the remaining nine developing a late malignancy. The breakdown for these baseline lesions is shown in Figure 3.2.
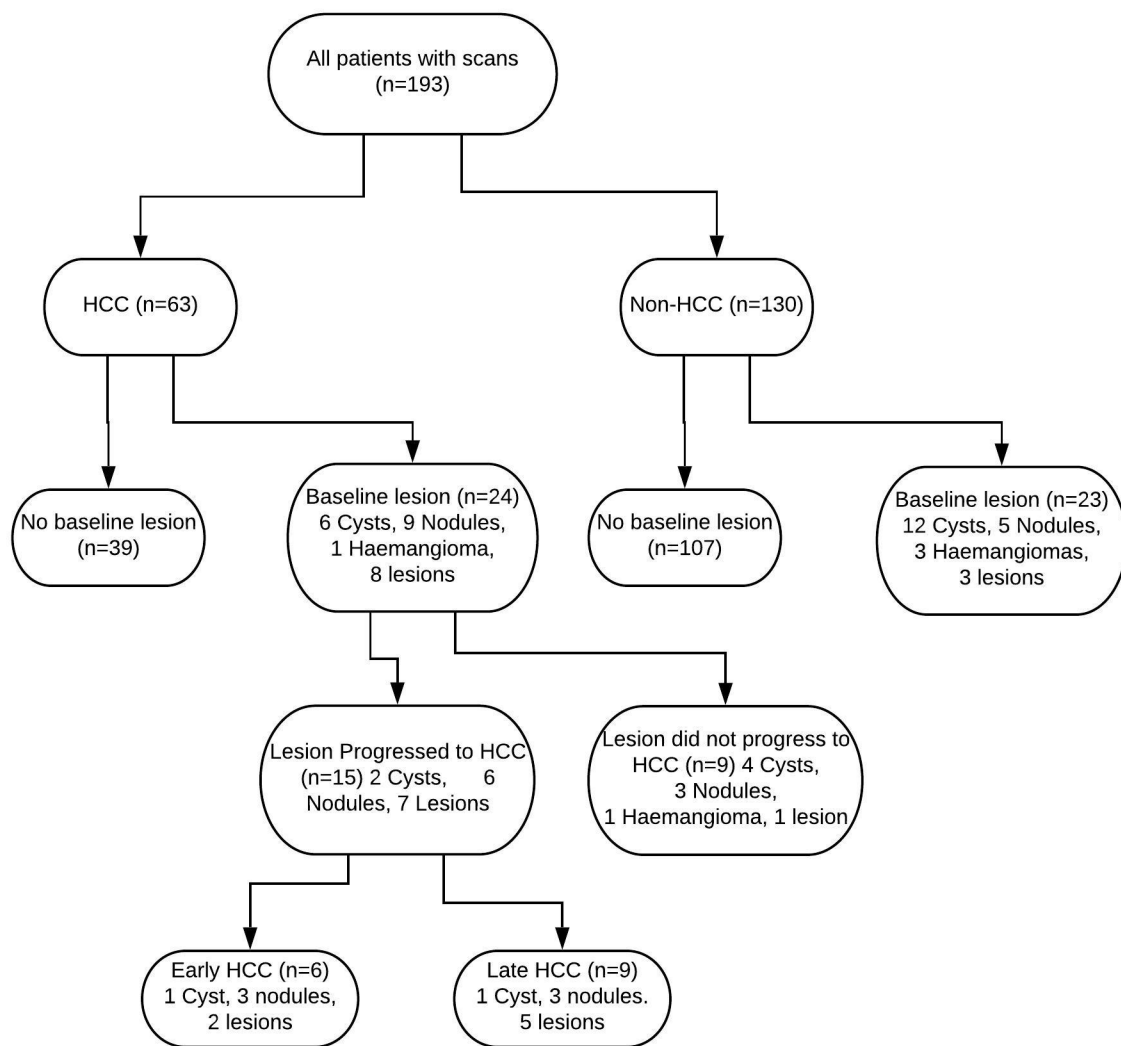
Figure 3.2: Flowchart for baseline nonmalignant lesions from screening scans from the year prior to starting EAP treatment. These are shown separated by non-HCC and HCC and then early vs late HCC.

3.1.3.7 Predictors for HCC development

All characteristics, which were not used for matching, were evaluated in univariate analysis to ascertain their potential as indicators for HCC development. Ethnicity, smoking status and cannabis usage were found not to be indicative. Usual risk factors for liver disease such as heavy alcohol intake and genotype were not associated with HCC development either. This was also the case for HCV viral load, which may be due to the fact that viral load reduces as liver cirrhosis worsens and hepatocyte volume decreases. Long-term proton pump inhibitor

use has been flagged as a potential risk factor for HCC development, but we did not substantiate this within our cohort (p=0.49). For blood markers, neither bilirubin, γGT, INR or sodium were significant. We did find significance for HIV co-infection which would be clinically valid, however, on closer inspection of the data, there were only 16 cases overall and we thus excluded this variable as power was limited. A breakdown of the univariate investigations may be seen in Table 3.5.

Table 3.5: Results of univariate analysis, presenting the predictors that have an effect on the development of HCC

Characteristic	p-value
Ethnicity	0.15
Heavy Alcohol intake (>6units/day)	0.31
Smoking status	0.26
Cannabis Usage	0.1
Genotype	0.1
Diabetes	0.02
HIV	0.02
Concurrent PPI use	0.49
Albumin, med	0.02
Alkaline phosphatase, med	0.14
Bilirubin, med	0.22
γGT, med	0.16
HCV Viral Load (iu/ml), med	0.96
INR, med	0.07
Platelet, med	0.02
Sodium, med	0.15

p-value significant <0.05, HIV: human immunodeficiency viruses, PPI: Proton pump inhibitor, γGT: Gamma-glutamyl transferase, HCV: Hepatitis C virus, INR: International normalised ratio

Factors associated with the development of HCC were diabetes (p=0.021), low albumin (p=0.016), non-malignant lesion seen on pre-treatment scans (p=0.005) and a low platelet count (p=0.018). These variables were entered into both logistic and Cox regression models for multivariate analysis, with both models returning all but albumin as statistically significant predictors. The effect size of albumin was reduced in the multivariate models due to its strong correlation with platelets (Spearman rho p-value=0.007). In Table 3.6, we present the individual univariate effects and the results from the Cox regression analysis. We

used this regression in order to fully incorporate the time-dependent nature of the outcome (time from the start of treatment to HCC development). The strongest indicator we found was non-malignant lesions at baseline (p=0.014), followed by platelets (p=0.016) and finally, diabetes mellitus (p=0.036).

Table 3.6: Results of multivariate analysis, presenting the predictors that have an effect on the development of HCC

Variable	Univariate Effect	Univariate p-value	Cox-regression multivariate effect	Cox-regression multivariate p-value
Platelets	Mean difference: 10.0, 95% CI: 2.0 - 19	0.018	HR: 1.59, 95% CI: 1.09 – 2.29 (Change of 50×10^9/L)	0.016
Diabetes	OR: 2.1, 95% CI: 1.1 – 3.4	0.021	HR: 1.68, 95% CI: 1.03 – 2.74	0.036
Non-malignant lesions at baseline	OR: 2.6, 95% CI: 1.3 – 5.1	0.005	HR: 1.99, 95% CI: 1.15 – 3.45	0.014
Albumin	Mean difference: 2.0, 95% CI: 0.4 – 3.6	0.016	n.s	n.s

p-value significant: <0.05, OR: Odds ratio, HR: Hazards ratio, CI: confidence interval, n.s: Not significant

3.1.3.8   **Virological response to DAA therapy in patients with and without HCC**

To further investigate the effect of HCC presence, we explored the effect this would have on achieving SVR12. To ascertain the difference between the whole cohort, we initially compared the non-HCC group with the HCC group. Of 165 patients, 143 (87%) of the non-HCC patients achieved SVR12, compared with 54/80 (68%) of the HCC patients (p< 0.001, OR: 3.13, 95% CI: 1.64-5.99). Following the exclusion of those with HCC diagnosed on explant, we found 48/72 (67%) achieved SVR12 with the persistence of a significant difference (p<0.001, OR: 3.25, 95% CI: 1.67-6.32). The difference in SVR12 rate is not accounted for by either Child-Turcotte-Pugh grade (p=0.68) or MELD score (p=0.95) (See Figure 3.3).
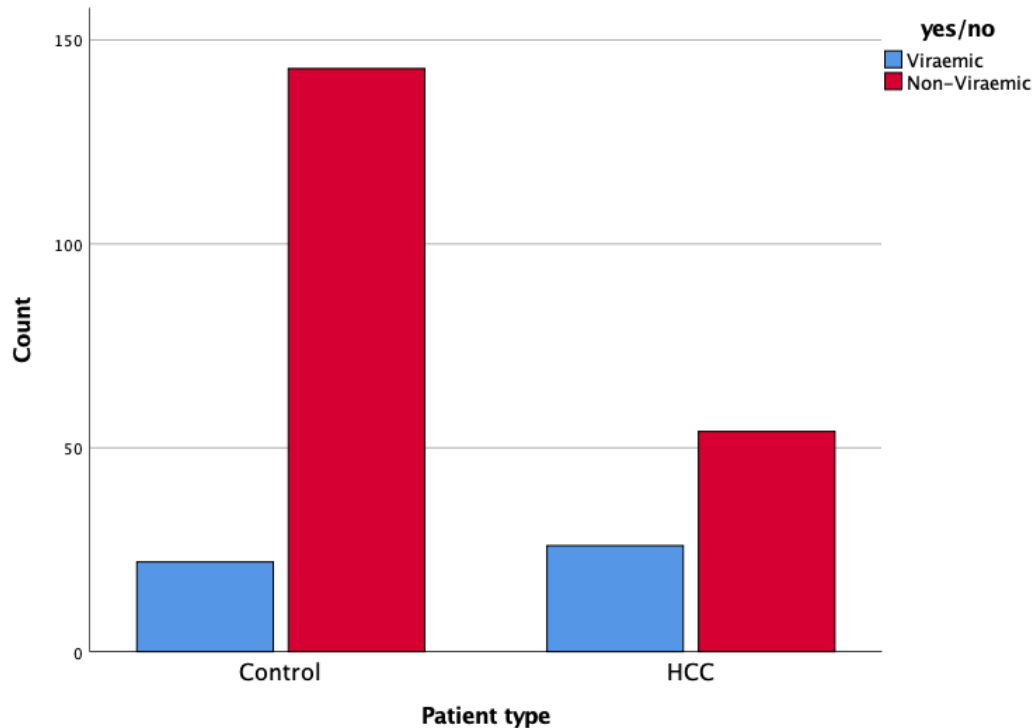
Figure 3.3: Clustered bar chart showing patients achieving SVR12 according to development of HCC (p<0.001, OR: 3.25, 95% CI: 1.67-6.32)

To delve deeper into the possibility of HCC presence diminishing SVR12, we assumed patients developing HCC early (i.e. within the time frame of 12 weeks therapy plus 12 weeks follow-up to determine treatment outcome) would have HCC cells which were not detectable at the time. For patients who developed an early HCC, 20/28 (71%) achieved an SVR (p=0.045, OR: 2.6, 95% CI: 1.02-6.62). We then compared SVR12 rates in late HCC patients to find, the response was also lower compared to the controls, 34/52 (65%) (p<0.001, OR: 8.26 95% CI: 4.43-15.38).

3.1.3.9 Survival for HCC vs control

To detail the initial effects of HCV on survival and to get an understanding of the overall dataset we plotting the survival estimation for the whole cohort from cirrhosis diagnosis date (see Figure 3.4). Although there were 24 patients with missing dates we chose this parameter to gain the best overall picture. This showed the expected reduction in survival over time with 50% survival at 200 months.
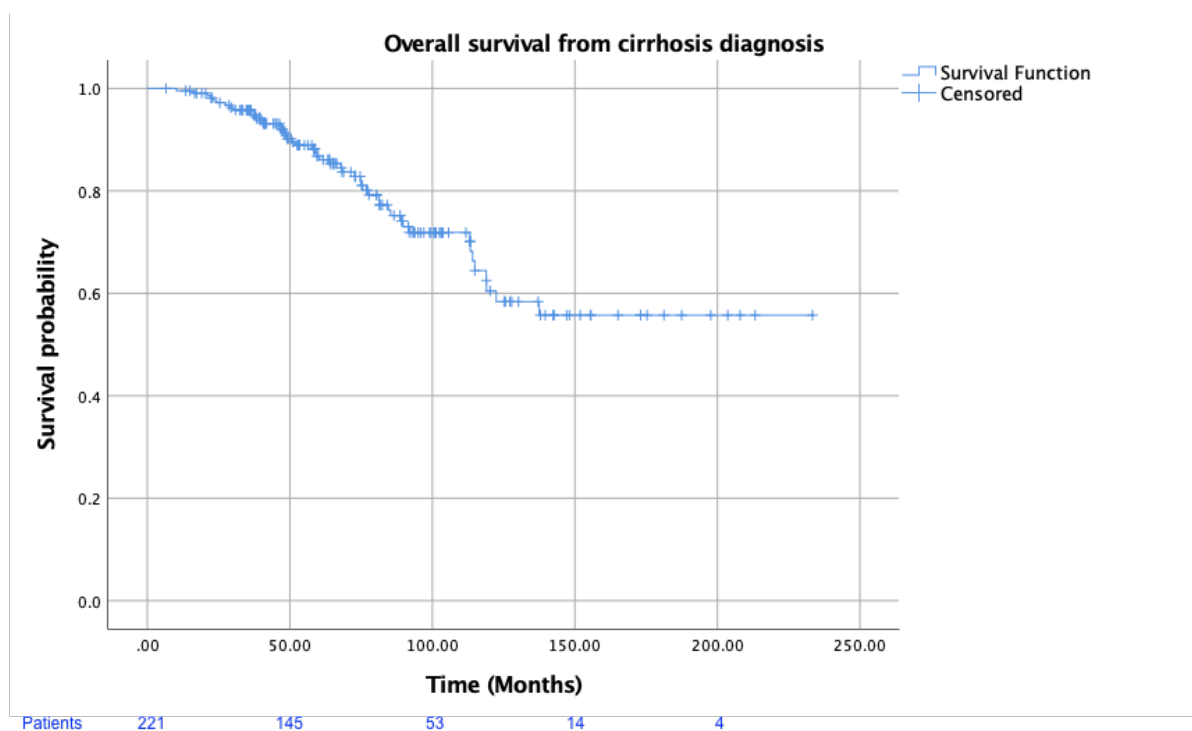
Figure 3.4: Time from HCV diagnosis to death. Kaplan-Meier estimation depicted

We then split the cohort according to controls and HCC patients with the indication that survival and found a non-significant difference of 0.067 with the HCC group showing a reduction in survival (Figure 3.5).
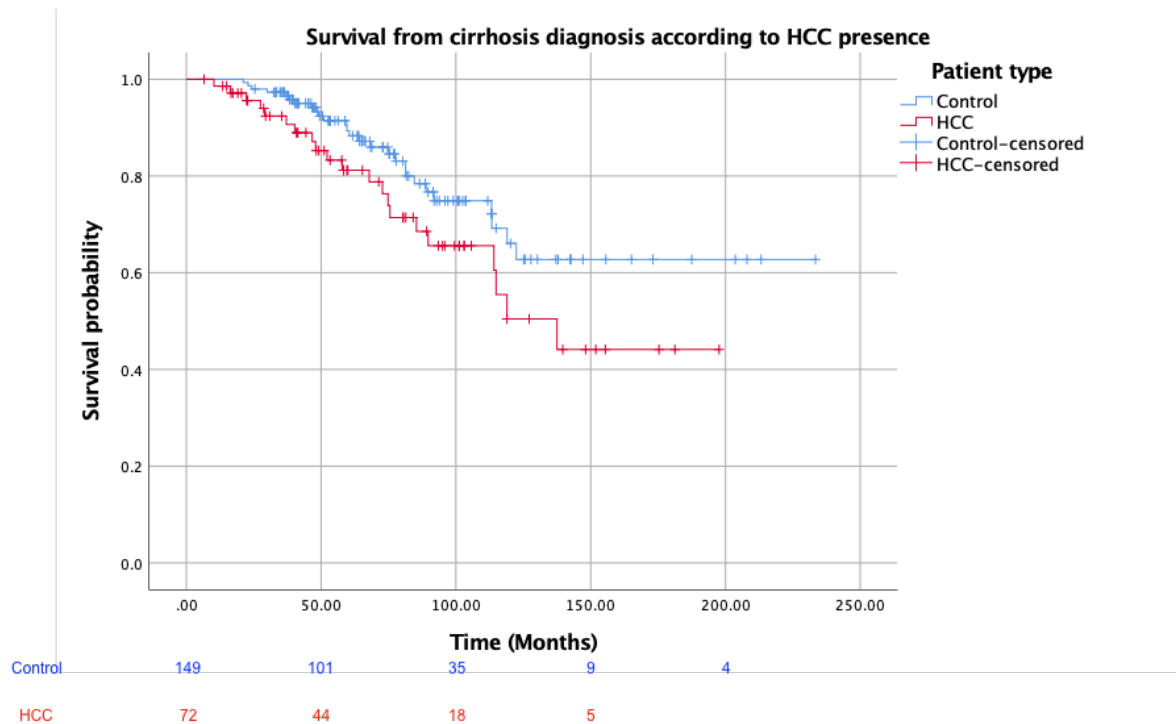
Figure 3.5: Time from HCV diagnosis to death split by non-HCC vs HCC. Kaplan-Meier estimation depicted. Log-Rank test p=0.067

3.1.3.10 <u>Sub group characteristics</u>

For prognostic analysis of HCC patients, we separated these according to the time of HCC development. Given the probability that cancers diagnosed within six months of treatment initiation may have been present at treatment onset, we analysed data for 'early' cancer (within six months of DAA initiation) and late cancers—diagnosed after this time point.

Using the above definition, twenty-eight patients were diagnosed with an HCC within the first six months of treatment (19 being diagnosed during early access programme treatment) and 52 patients as late HCC. The median age of 55 years for early HCC was similar to 57.2 year of the late HCC and males of 79% and 75% respectively and would be expected as these were matching criteria. However, the percentage of CTP stage B is slightly lower in the early HCC at 54% with the remaining 46% split equally between grade A and C. Alternatively, the late HCC had more CTP grade A (23%) than C (10%). This higher proportion of CTP grade

150

B for late HCC is also demonstrated with higher median MELD score than the early HCC. The median length of follow-up is reduced for early HCC as would be expected. There is a slight tendency towards genotype 3 over genotype 1 for early HCC over late HCC (32% vs 57% for early HCC against 48% vs 50% for late HCC). Diabetic prevalence was similar within the two cohorts with slightly more being treated with sofosbuvir, daclatasvir and Ribavirin in the late HCC (29%) as opposed to the early HCC (18%). In general, blood tests were similar with a slightly higher ALP within the late HCC (139U/L) as opposed to early HCC (111U/L). These data are presented in Table 3.7.

Table 3.7: Baseline characteristics of patients with early HCC (ie within the time frame of 12 weeks therapy plus 12 weeks follow-up to determine treatment outcome) and late HCC (all others)

Characteristic	Early HCC (<6 months) (n=28)	Late HCC (>6 months) (n=52)
Median age, (IQR), yrs.	55 (50-60.9)	57.2 (54.2-61.4)
Male sex, n (%)	22 (79)	39 (75)
CTP grade (%)	B (54)	B (67)
Mean MELD score (IQR)	10 (9-13)	12 (9-15)
Median length of follow up, (IQR), mths.	15.3 (5.3-24.1)	24.7 (17.2-32.9)
Ethnicity, n (%)		
White-British	20 (72)	33 (63)
Asian	4 (14)	6 (12)
Other	4 (14)	13 (25)
Alcohol, n (%)		
Never	5 (18)	10 (19)
Current	3 (11)	5 (10)
Past/Former	20 (71)	37 (71)
Unavailable	0	0
Smoking status, n (%)		
Never	2 (7)	13 (25)
Currently	13 (47)	23 (44)
Past/Former	11 (39)	12 (23)
Unavailable	2 (7)	4 (8)
Genotype, n (%)		
Genotype 1	9 (32)	25 (48)
Genotype 3	16 (57)	26 (50)
Other	3 (11)	1 (2)
Diabetes, n (%)		
Yes	10 (36)	17 (33)
No	15 (54)	26 (50)
Unavailable	3 (10)	9 (17)
Past history of Non-HCC Ca, n	2	3
Previous treatment failure, n (%)	19 (70)	31 (60)
Treatment regimen, n (%)		
Sof/Led	1 (3)	0
Sof/Led/Riba	22 (79)	37 (71)
Sof/Dac	0	0
Sof/Dac/Riba	5 (18)	15 (29)
SVR achieved, n (%)	20 (71)	34 (65)
Median albumin, (IQR), g/L	28 (23-32)	27 (22.5-31)
Median alpha-fetoprotein, (IQR), ng/ml	9 (5.6-25)	6.1 (3.6-12.3)
Median alkaline Phosphatase, (IQR), U/L	111 (90-154)	139 (105-189)
Median bilirubin, (IQR), umol/L	32 (20-52)	39 (25-53.5)
Median INR, (IQR),	1.3 (1.2-1.5)	1.4 (1.2-1.5)
Median platelet, (IQR), x10^9/L	68 (44-95)	59 (43.5-80)
Median sodium, (IQR), mmol/L	137.0 (133-140)	136.0 (131.5-137)
Median BMI, (IQR), kg/m^2	27.5 (24.3-33)	27.1 (25.3-30.5)

Unknown values were excluded where unknown values existed. n: Number, IQR: Inter-quartile range, yrs.: Years, mths.: Months, HCC: Hepatocellular carcinoma, CTP: Childs-Turcotte-Pugh, SVR: Sustained viral response, Sof: Sofosbuvir, Led:

Ledipasvir, Dac: Daclatasvir, Riba: Ribavirin, INR: International normalised ratio, BMI: Body mass index. Standardised units supplied where appropriate

3.1.3.11 <u>Scan frequency</u>

We compared cancers that developed soon after therapy with those developing later to test the hypothesis that the elimination of the virus-associated inflammatory response leads to a more aggressive tumour. We investigated the treatment and prognostic factors for each group.

Initial treatment for HCC is based upon several factors including the size, position and metastatic status of the tumour. These decisions are made at MDTs at the local centres with patients receiving follow up scans and treatments as required. The intent of the initial treatment will either be curative or palliative and this can be seen in Table 3.8.

Table 3.8: Frequency of screening scans in the year prior to starting DAA treatment split by early vs late HCC

Number of scans performed	Early HCC (n=28)	Late HCC (n=52)
0 scans, n (%)	6 (21)	11 (21)
1 scan, n (%)	11 (39)	14 (27)
2 scans, n (%)	3 (11)	13 (25)
3+ scans, n (%)	8 (29)	14 (27)
Scan within 6 months of starting DAA, n (%)	22 (79)	37 (71)

n: Number, HCC: Hepatocellular carcinoma, DAA: Direct acting antiviral

3.1.3.12 Progression of liver cancers arising early after starting DAA compared to later cancers

We compared cancers that developed soon after therapy with those developing later to test the hypothesis that the elimination of the virus-associated inflammatory response leads to a more aggressive tumour. We investigated the treatment and prognostic factors for each group.

Initial treatment for HCC is based upon several factors including the size, position and metastatic status of the tumour. These decisions are made at MDTs at the local centres with patients receiving follow up scans and treatments as required. The intent of the original treatment will either be curative or palliative and this can be seen in Table 3.9.

Table 3.9: The intent of initial treatment for HCC split by early vs late HCC

Treatment intent	Early HCC (n=28)	Late HCC (n=52)
Curative , n (%)	21 (75)	25 (48)
Palliative, n (%)	6 (21)	20 (38)
unknown, n (%)	1 (4)	7 (14)

n: Number, HCC: Hepatocellular carcinoma, DAA: Direct acting antiviral

We detail here the treatment that patients received. For the treatment of the HCC, for the early HCC 5 patients had ablations with 1 having two rounds of therapy, with none of these going on to have a transplant. Eleven had Transcatheter chemoembolisation (TACE) treatment, 2 with multiple attempts, with eight going on to have a liver transplant. Four patients died (one following an RFA and one following a transplant). A further eight patients had a transplant with one subsequent death. One patient did not receive treatment and no additional information is available. For late HCC 14 had ablations with 3 having multiple exposures, two went on to have TACE, three went on to a transplant and one died. Ten had

TACE with 5 having multiple exposures, three went on to transplant and one onto palliative care and 1 died. One had a resection, seven patients had a transplant, and 13 were put onto palliative care or passed away. Three patients were awaiting treatment and four patients have not had any treatment.

Liver transplantation is based on several of the above factors, but also takes into account the overall fitness of the patient to undergo a major operation outside of their liver status. We thus compared the overall proportion of patients that received a liver transplant to find that patients with early HCC were more likely to receive a graft (p=0.004, OR: 4.00, 95% CI: 1.506-10.62) as can be seen in Figure 3.6.
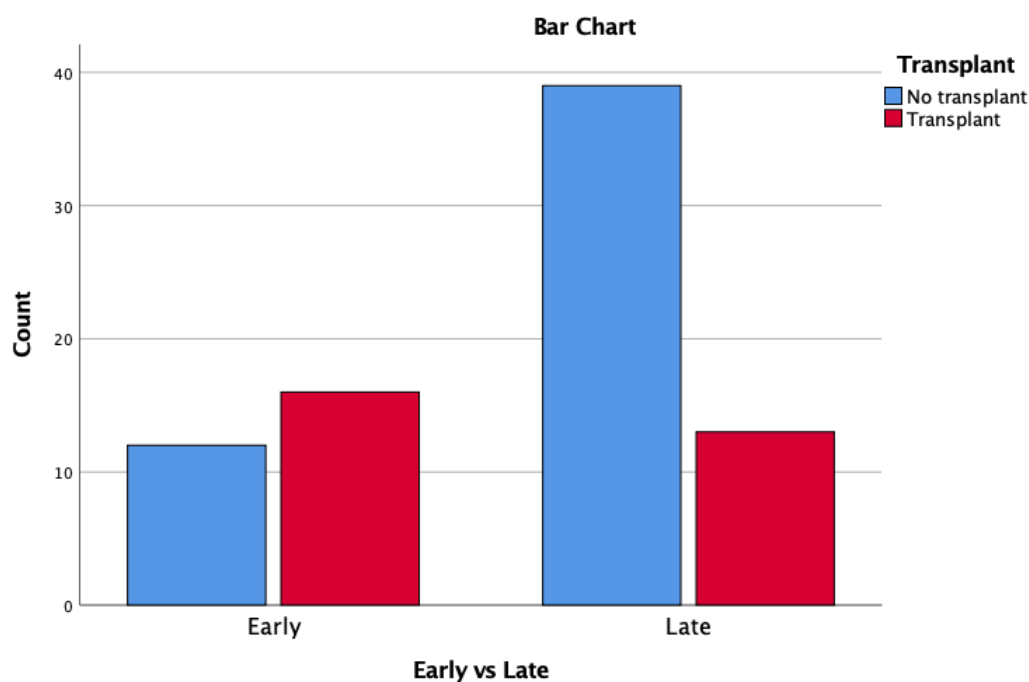
Figure 3.6: Clustered bar chart showing those receiving a transplant split by early vs late HCC, p=0.004, OR: 4.00, 95% CI: 1.506-10.62

With the investigation of the individual tumours with regards to progression as according to the RECIST criteria, with patients being censored at transplantation date, we found no

significant difference between early and late HCCs (p=0.25, HR: 1.44, p=0.292, 95%CI: 0.731-2.84) (Figure 3.7).
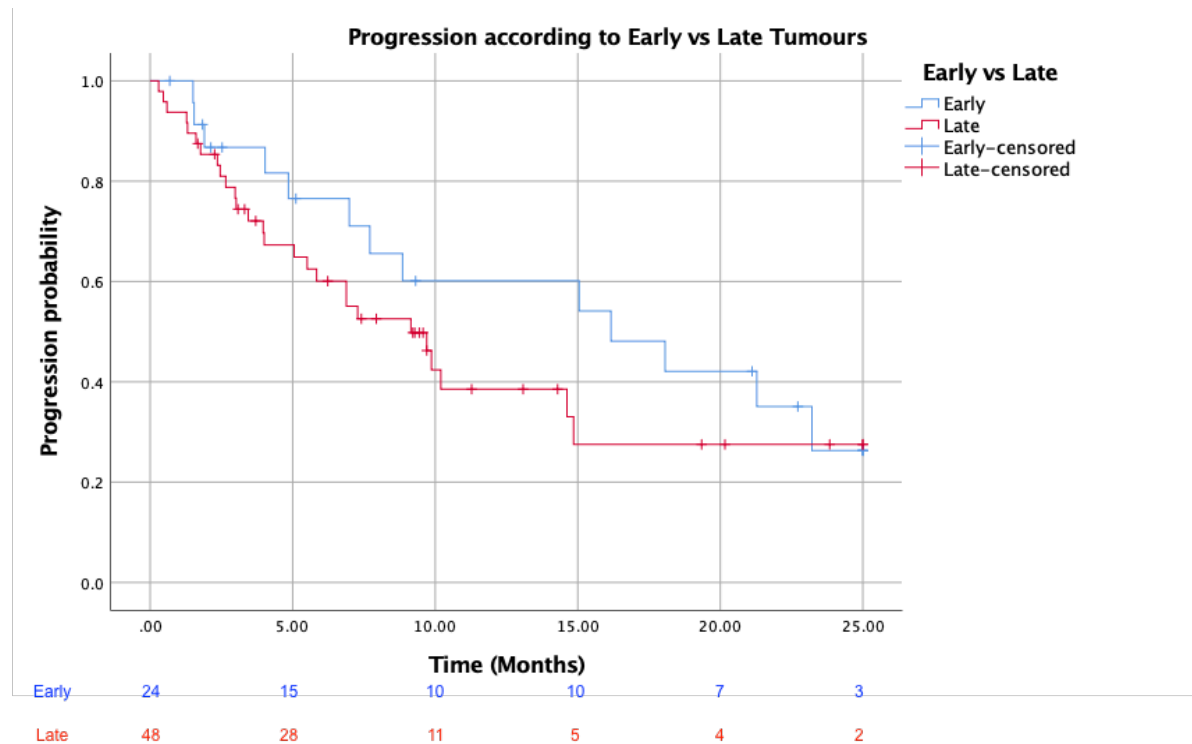
Figure 3.7: Time from HCC diagnosis to the first progression split by early vs late HCC. Kaplan-Meier estimation depicted. Mantel-Cox comparison test p=0.25.

Looking into the survival of patients divided by time of tumour we again found no significant differences between the two groups (p=0.12, HR: 2.37, p=0.134, 95%CI: 0.766-7.33) (see Figure 3.8).
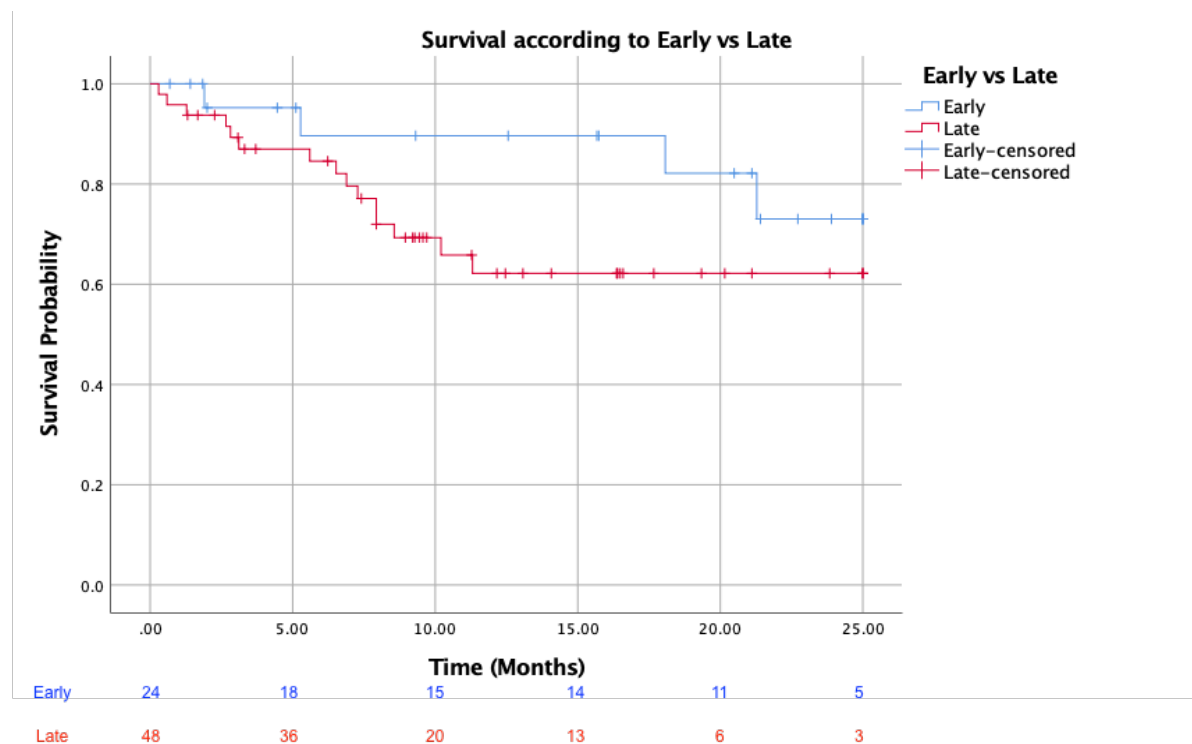
Figure 3.8: Time from HCC diagnosis to death split by early vs late HCC. Kaplan-Meier estimation depicted. Mantel-Cox comparison test p=0.12

3.1.3.13 Progression of liver cancer following viral clearance

To examine the hypothesis that malignancy developing in an uninfected liver (i.e. post-SVR) may be more aggressive than cancers that develop in an HCV-infected liver, we split the cohort by those with ongoing viraemia post-treatment.

We initially described the characteristics of the HCCs according to raw size followed by the various scoring techniques used for grading. There was no significant size difference for the primary tumour between non-viraemic (9.5-120 mm) and viraemic (14-100 mm) patients with 13 non-viraemic (28%) and seven patients with viraemia (28%) presenting with more than one tumour. We assessed the cancer stage using the Milan criteria which determine suitability for liver transplantation in patients with cirrhosis and HCC. The proportion of patients with HCC at the point of diagnosis who fell within the Milan criteria (i.e. circumscribed) was 61/72, following exclusion of those diagnosed on explant. Of 47, 39

157

(83%) patients achieving an SVR were within Milan criteria compared to 22/25 (88%, p=0.57) patients who did not achieve SVR. Similar assessment, according to Li-RADS criteria split into non-viraemic and viraemic patients, found one category 3, 23 category 4 and 22 category five cancers and ten category 4 and 14 category five tumours respectively. Analogous assessment according to Barcelona clinic liver cancer (BCLC) scores showed 7 grade 0, 25 grade A, 2 grade B, 6 grade C, 6 grade D within the non-viraemic patients and 3 grade 0, 13 grade A, 1 grade B, 3 grade C, 1 grade D viraemics. These data are presented in Table 3.10 and no statistically significant differences between viraemic and non-viraemic patients was found.

Table 3.10: Description of tumours split by viraemic and non-viraemic, excluding those found on explant

	Non-viraemic (n=47)	Viraemic (n=25)
Size of primary lesion, mm	9.5-120	14-100
More than 1 lesion, n (%)	13 (28)	7 (28)
Fits within Milan criteria (%), n (%)	39 (83)	22 (88)
Li-RADS criteria, n (%)		
LR-3	1 (2)	0
LR-4	23 (49)	10 (40)
LR-5	22 (47)	14 (56)
Unavailable	1 (2)	1 (4)
Barcelona Grade, n (%)		
0	7 (15)	3 (12)
A	25 (53)	13 (52)
B	2 (4)	1 (4)
C	6 (13)	3 (12)
D	6 (13)	1 (4)
Unavailable	1 (2)	4 (16)

Li-RADS: Liver Imaging Reporting and Data System, BCLC: Barcelona clinic liver cancer, n: Number, Standardised units supplied where appropriate

We then looked into the progression of these tumours and found no difference between tumours developing in a viraemic and non-viraemic liver (p=0.170, HR: 1.476, p=0.232, 95%CI: 0.779-2.80) (Figure 3.9).
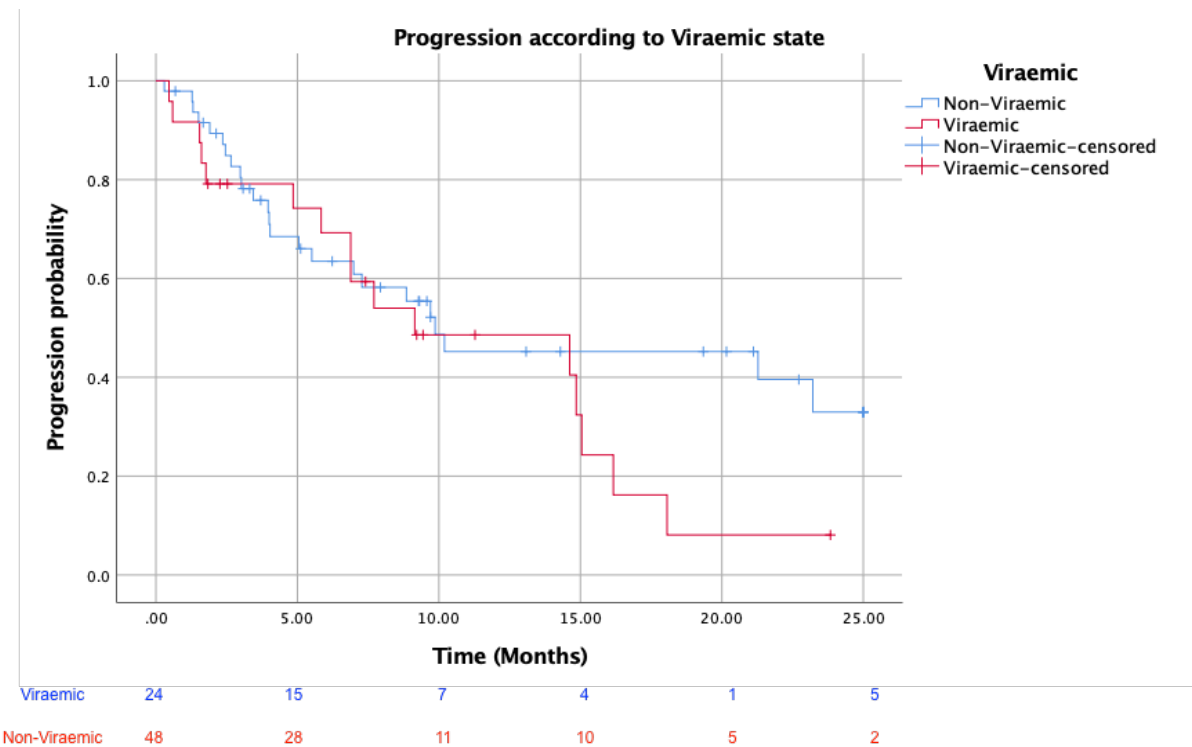
Figure 3.9: Time from HCC diagnosis to the first progression split by ongoing viraemia vs viral clearance. Kaplan-Meier estimation depicted. Mantel-Cox comparison test p=0.170.

Survival was also found to be similar for both cohorts (p=0.700, HR: 0.719, p=0.526, 95%CI: 0.259-2.00) (Figure 3.10).
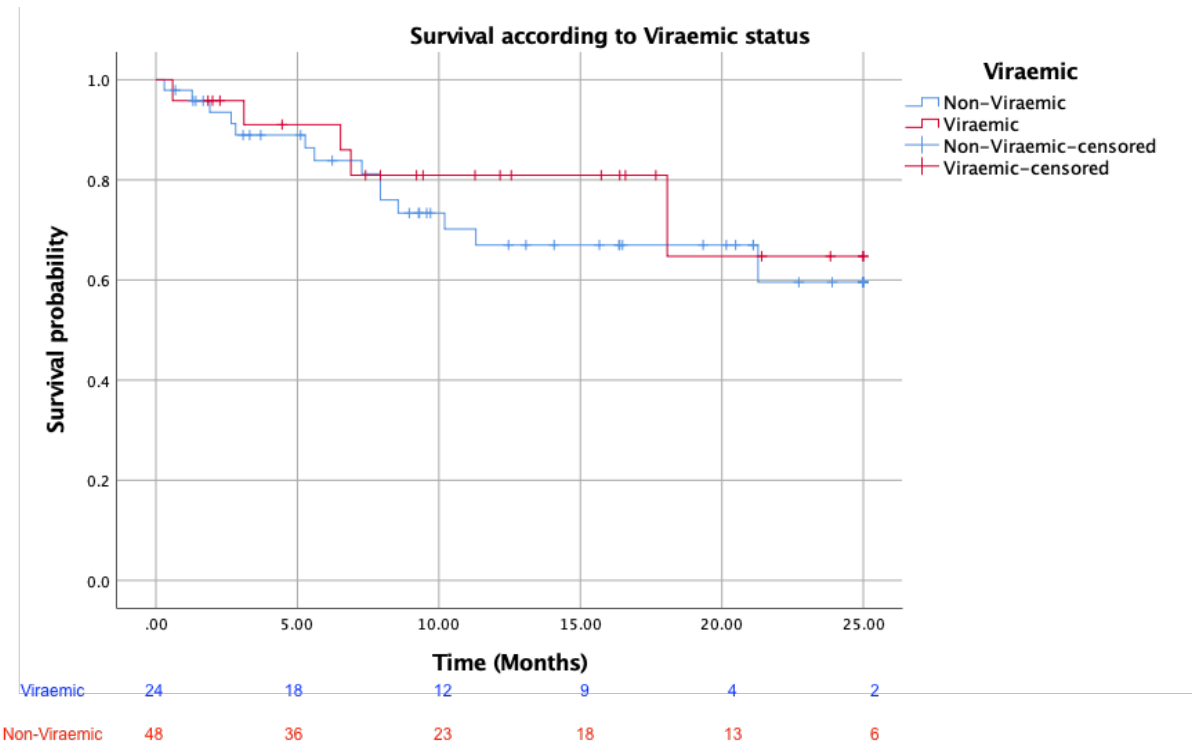
Figure 3.10: Time from HCC diagnosis to death split by ongoing viraemia vs viral clearance inclusive of only EAP patients. Kaplan-Meier estimation depicted. Mantel-Cox comparison test p=0.700

3.1.3.14 Early vs late vs viraemia

To delineate any interactions between these two factors, we performed an exploratory

investigation which showed no significant differences as expected due to the small numbers.

However, there may be a better prognosis for patients who are non-viraemic with an early

HCC with regards to progression but not survival (see Figure 3.11 & Figure 3.12).
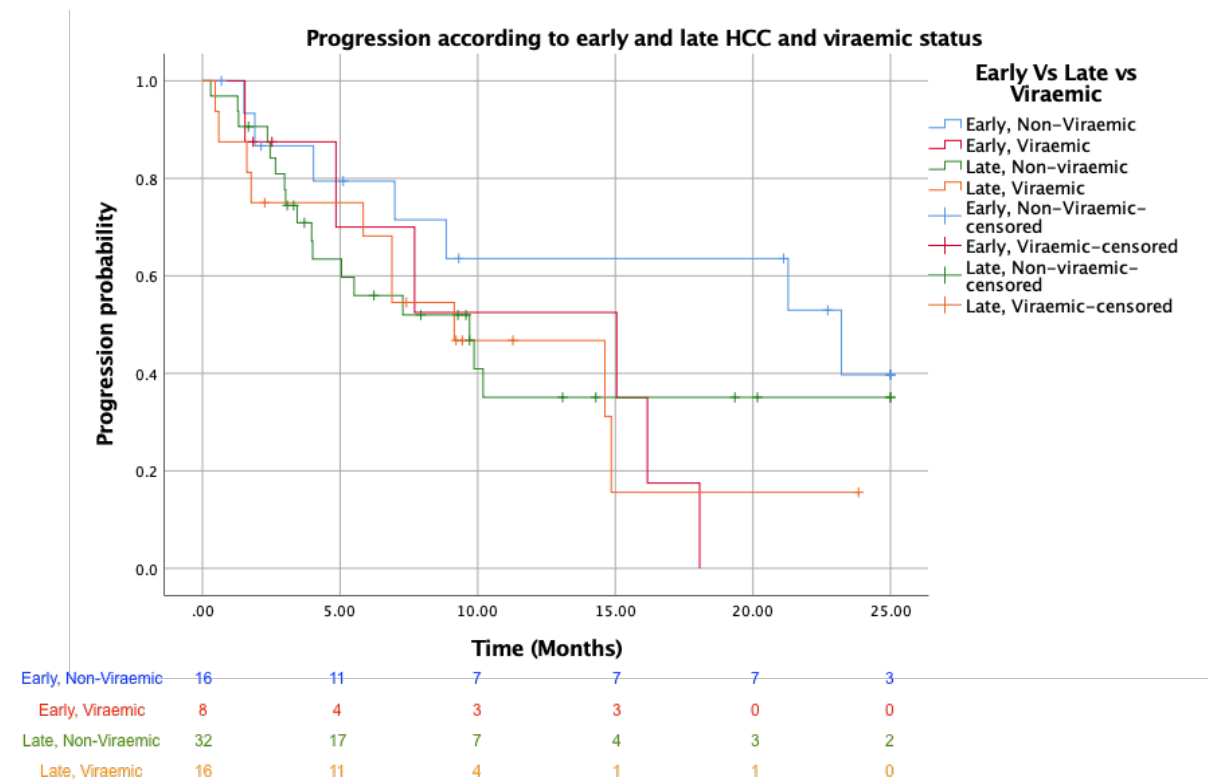
Figure 3.11: Time from HCC diagnosis to progression split by early and late HCC and viraemic status. Kaplan-Meier estimation depicted. Log-Rank test p=0.354.
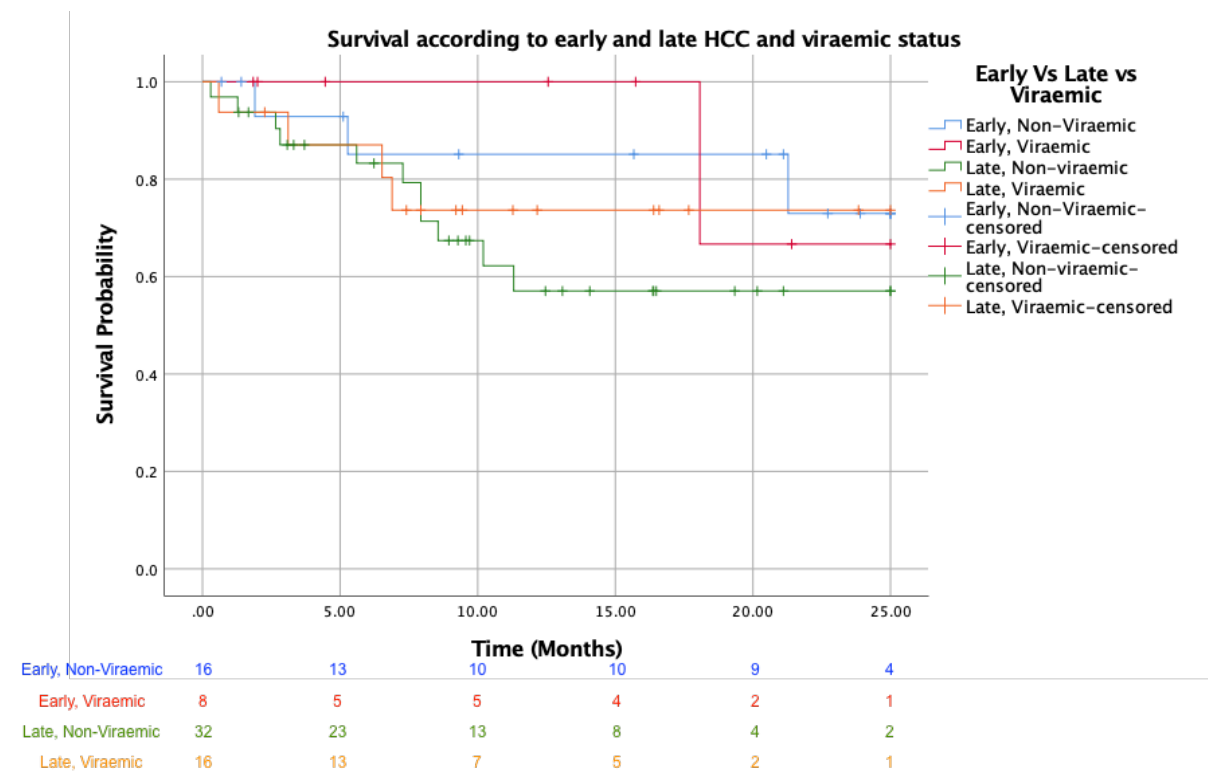
Figure 3.12: Time from HCC diagnosis to death split by early and late HCC and viraemic status. Kaplan-Meier estimation depicted. Log-Rank test p=0.398.

3.1.3.15 Other factors affecting progression and survival

To further investigate other factors which may indicate worsening survival or progression we interrogated data on the different baseline characteristics which indicated increased HCC risk. We looked at progression (HR: 0.998, p=0.751, 95%CI: 0.987-1.01) and survival for low platelets progression (HR: 0.991, p=0.301, 95%CI: 0.975-1.01) with neither showing an effect. We then looked at diabetes mellitus and found no impact on progression (p=0.635, HR: 0.843, p=0.646, 95%CI: 0.416-1.71) (see Figure 3.13), or survival (p=0.530, HR: 0.714, p=0.533, 95%CI: 0.248-2.06) (see Figure 3.14).

Figure 3.13: Time from HCC diagnosis to the first progression split by diabetes status. Kaplan-Meier estimation depicted. Log-Rank test p=0.635.

Figure 3.14: Time from HCC diagnosis to death split by diabetes status. Kaplan-Meier estimation depicted. Log-Rank test p=0.530

With regard to baseline non-malignant lesions, a significant difference was found for those patients who did have a lesion being more likely to have progression of their HCC (p=0.036, HR: 0.459, p=0.041, 95%CI: 0.218-0.969) (see Figure 3.15). Although this effect is also indicated for survival this did not reach significance (p=0.072, HR: 0.339, p=0.087, 95%CI: 0.098-1.17) (see Figure 3.16).

Progression according to the presence of baseline lesions

No Lesions	50	25	13	9	6	1
Lesions present	22	18	8	6	5	4
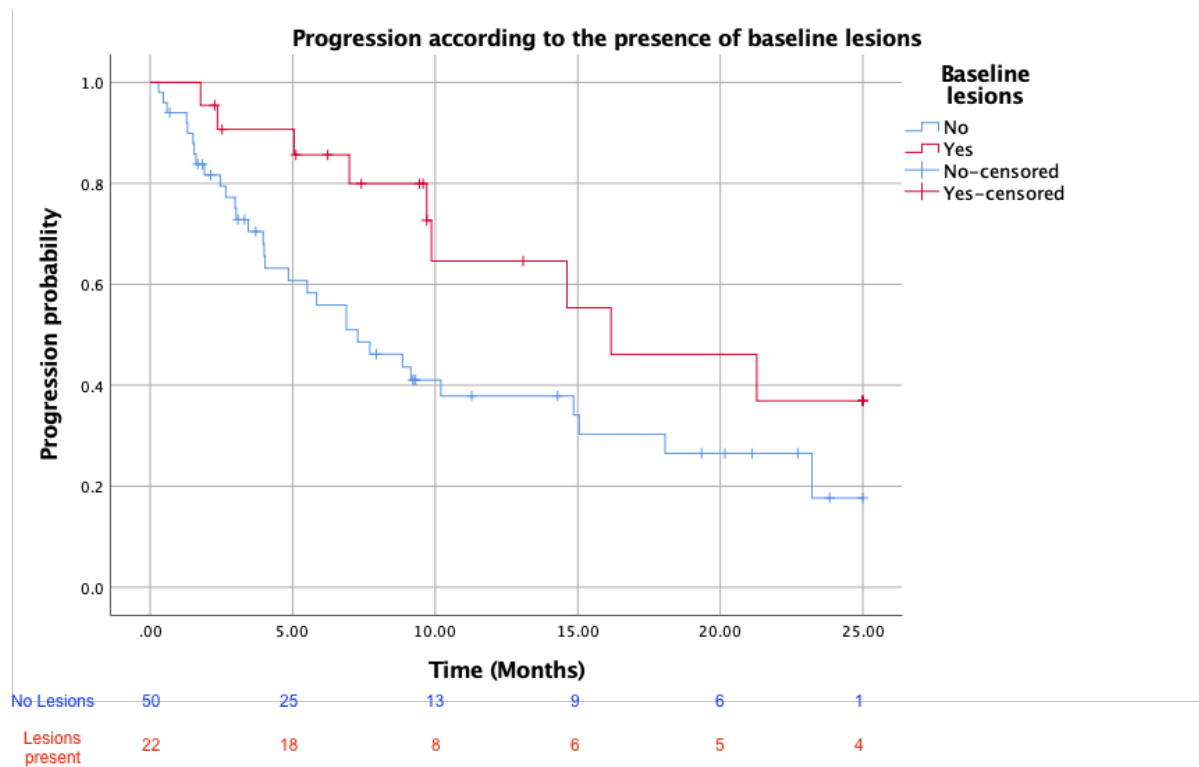
Figure 3.15: Time from HCC diagnosis to the first progression split by presence of baseline lesions. Kaplan-Meier estimation depicted. Log-Rank test p=0.036.

Survival according to the presence of baseline lesions

No Lesions	50	35	23	16	9	3
Lesions present	22	19	12	11	8	5
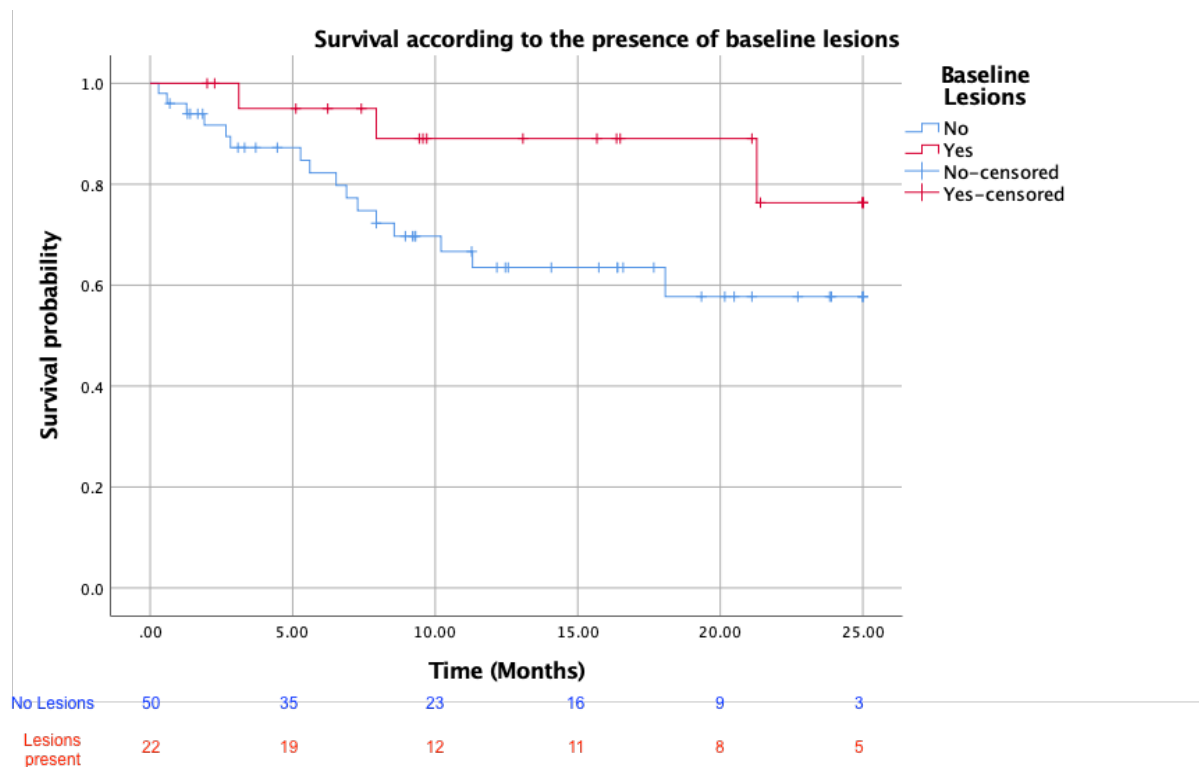
Figure 3.16: Time from HCC diagnosis to death split by the presence of baseline lesions. Kaplan-Meier estimation depicted. Log-Rank test p=0.072.

164

3.1.3.15.1 Genotype

Outside of the above factors we also investigated the importance of genotype on the aggressiveness of HCC on the background of a decompensated cirrhotic liver and found no effect on progression (p=0.464) (Figure 3.17) or survival (p=0.358) (Figure 3.18).
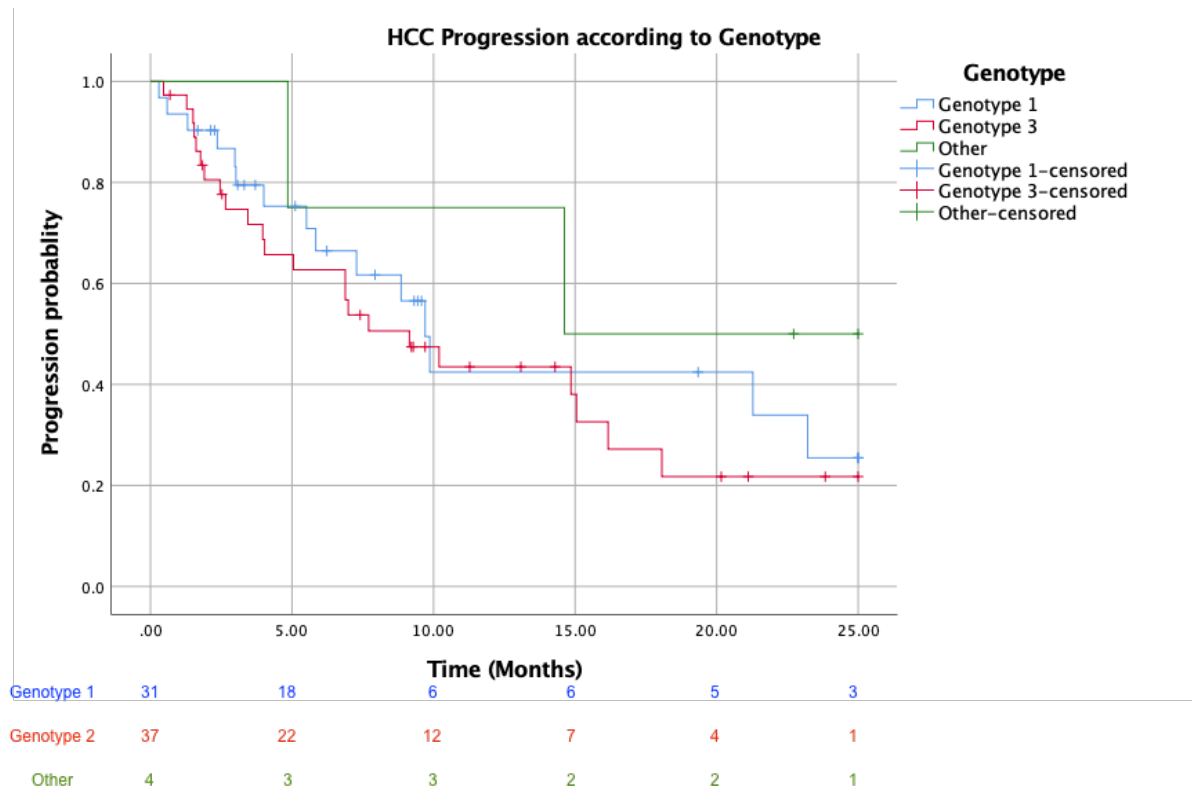
Figure 3.17: Time from HCC diagnosis to the first progression split by genotype. Kaplan-Meier estimation depicted. Log-Rank test p=0.464.
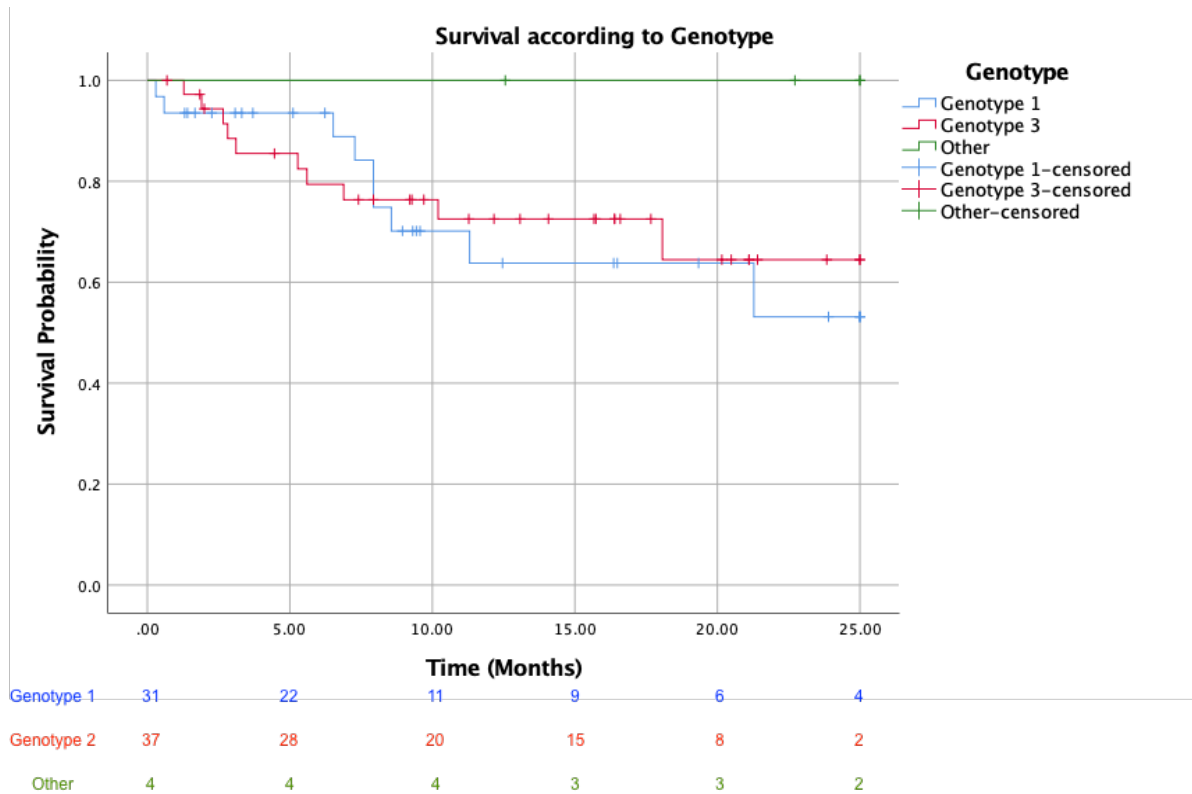
Figure 3.18: Time from HCC diagnosis to death split by genotype. Kaplan-Meier estimation depicted. Log-Rank test p=0.358.

3.1.3.15.2 <u>Child-Turcotte-Pugh</u>

We then looked at the severity of the current liver cirrhosis to ascertain whether this was a factor and found no differences between the Childs-Turcotte-Pugh grades for either progression (p=0.196) (Figure 3.19) or survival (p=0.159) (Figure 3.20). However, there was a non-significant impact of the scores with higher scores appearing to fare less well than patients with lower scores. The failure to detect statistical significance may therefore be related to the small number of patients with very low and very high scores.
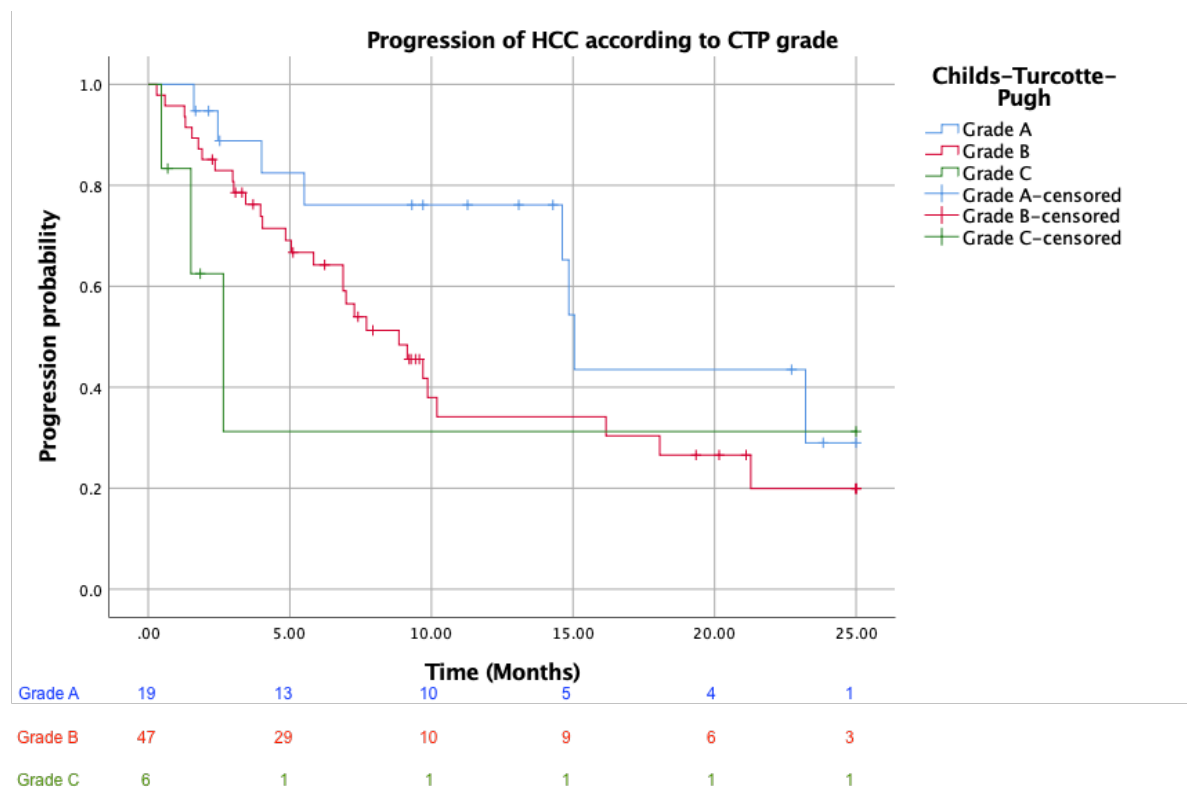
Figure 3.19: Time from HCC diagnosis to the first progression split by Childs-Turcotte-Pugh grade. Kaplan-Meier estimation depicted. Log-Rank test p=0.196.
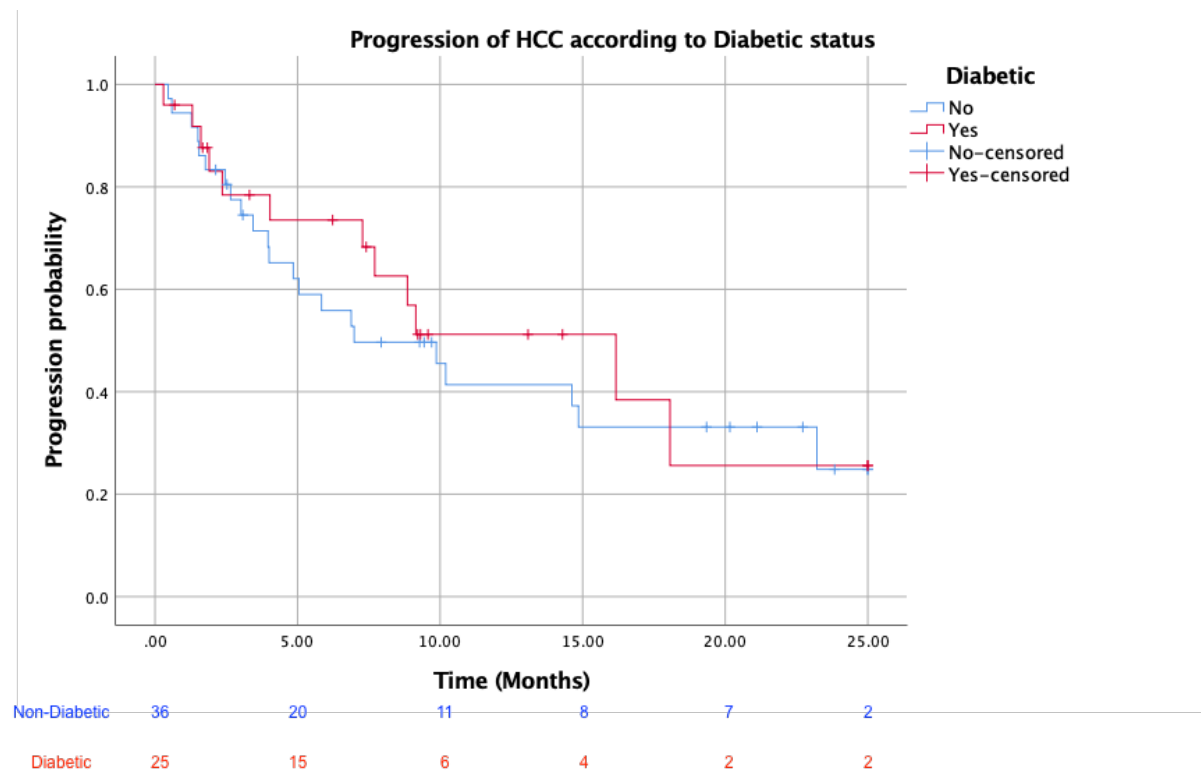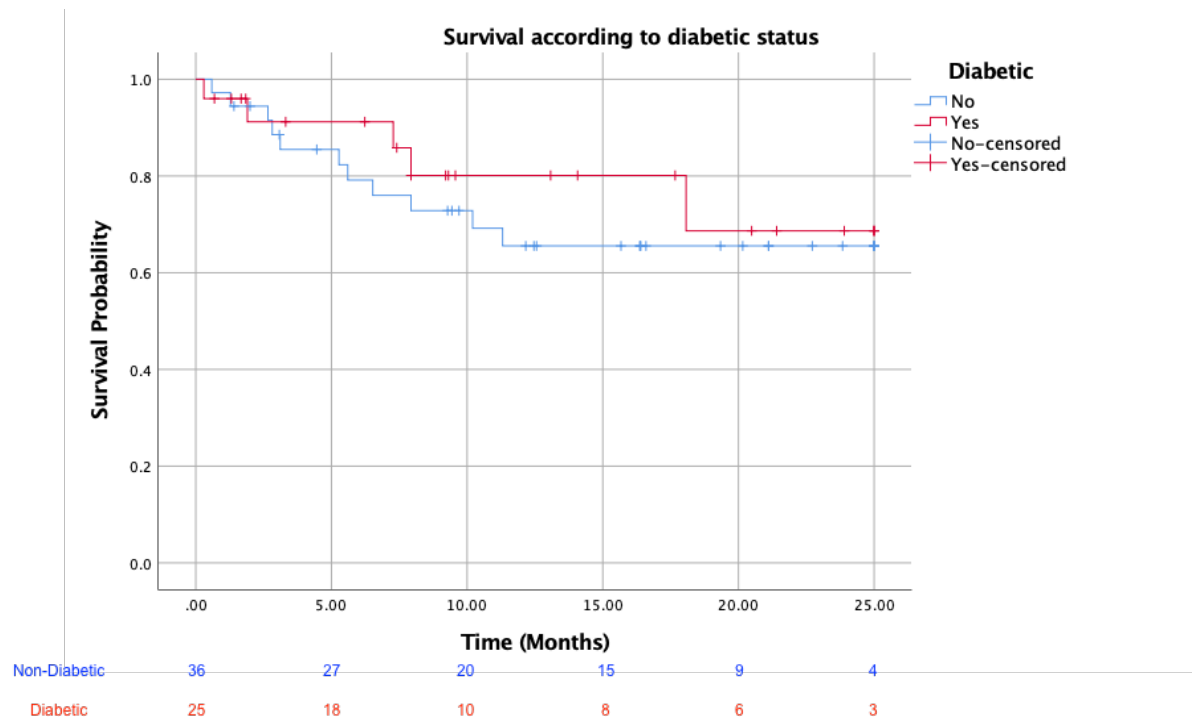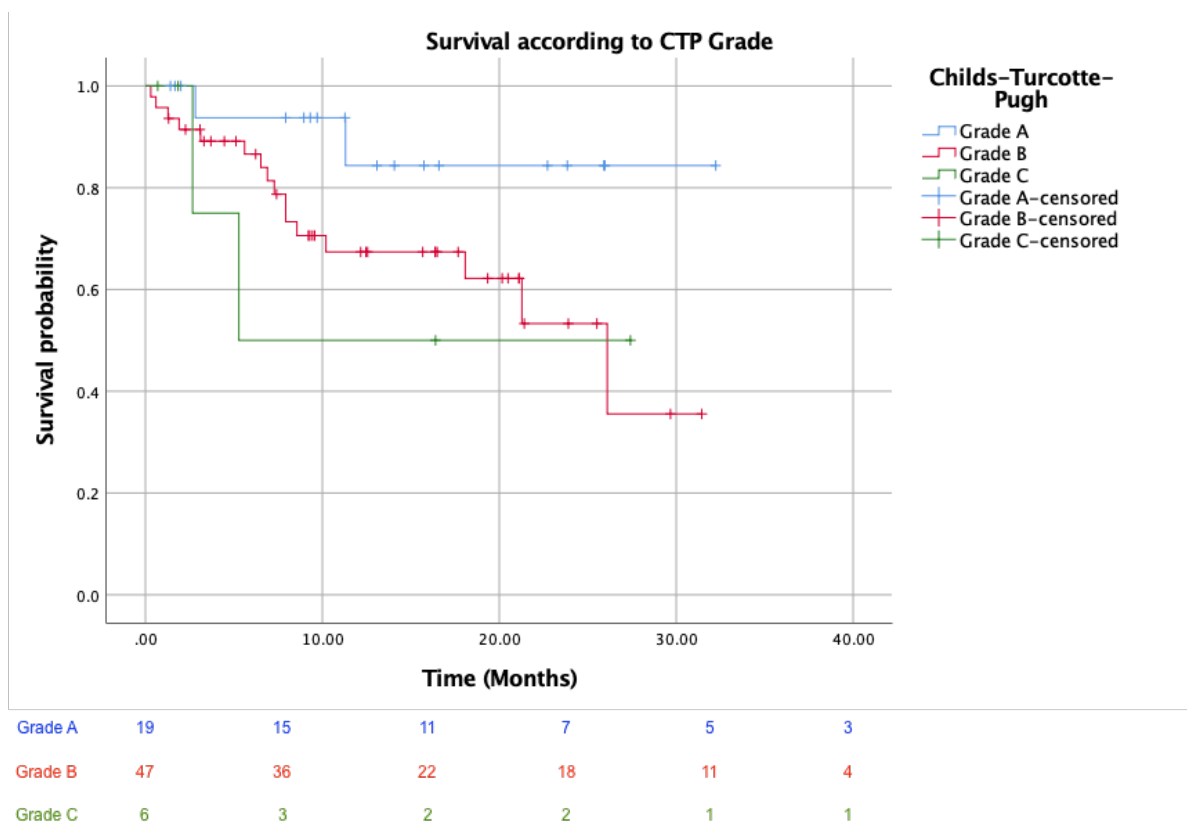

Figure 3.20: Time from HCC diagnosis to death split by Childs-Turcotte-Pugh grade. Kaplan-Meier estimation depicted. Log-Rank test p=0.159.

167

3.1.3.15.3 <u>GALAD and ALBI Grade</u>

An initial collaboration with Prof Philip Johnson and Prof William Irving was set up to investigate whether tumours were present within pre-treatment blood tests by using the GALAD scoring system which is an amalgam of gender, age, and three specialised laboratory blood tests (AFP, AFP-L3 and DCP). This was performed on 30 HCC patients and 30 controls with the hypothesis that patients diagnosed with HCC post-onset of DAA therapy were in any case destined to present with HCC whether or not they were treated for their HCV infection. These initial samples were sent to a third-party lab and returned to find no difference in the results for those developing HCC vs the controls. This highlighted the requirement for a more in-depth investigation of these patients and the following study performed.

At the inception of the study, we also discussed the suitability of applying ALBI (Albumin-Bilirubin) Grade for hepatocellular carcinoma (HCC) which was created to predict median survival for HCC patients but has since been utilised for other diseases such as hepatitis B and hepatitis B cirrhosis and primary biliary cirrhosis [342-344]. We thus performed a chi-square analysis for the whole cohort and found no difference (χ^2=2.333, p=0.311) between the controls and those developing HCC. We then looked at its prognostic capacity within HCV inducted, decompensated cirrhosis. There was only a single case with ALBI grade 1, outside of this there was no difference for survival (p=0.856; HR: 0.850, p=0.718, 95%CI: 0.351-2.056) (Figure 3.21).
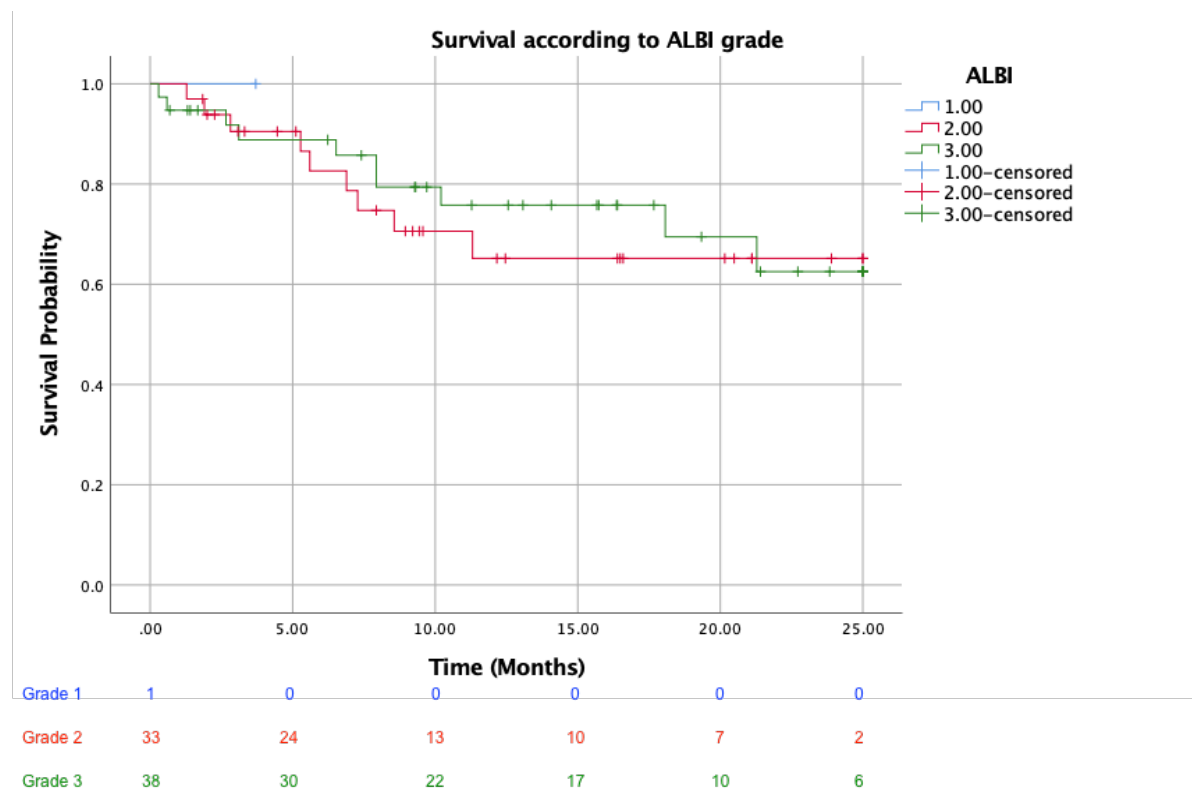
Figure 3.21: Time from HCC diagnosis to death split by Albumin-Bilirubin grade. Kaplan-Meier estimation depicted. Log-Rank test p=0.856.

3.1.4   Discussion

The evolution of DAA treatment has changed clinical practice. Here, we show data from the NHS England early access programme cohort to address issues in the management of patients at risk of cancer: (a) are there any baseline features predictive of HCC development, (b) are patients who are diagnosed with HCC during treatment less likely to achieve SVR, (c) are HCCs developing in an uninfected liver (i.e. post-SVR) more aggressive than cancers that develop in an HCV-infected liver.

To rule out non-engagement with screening as a causative factor we investigated scan frequency within the year prior to treatment and found an equal engagement (21% in each) We studied liver cancers with known outcomes and investigated them against baseline features that might be associated with malignancy and found that the presence of a 'lesion' on previous scans, diabetes and thrombocytopaenia was associated with subsequent development of malignancy. These findings are consistent with previous studies [233, 234, 236, 345-350] but require formal confirmation in a larger cohort.

The significance of pre-treatment non-malignant lesions presents a challenge for hepatologists. The LI-RADS criteria were developed to try and overcome this, but diagnostic uncertainty remains [246, 247]. We have shown that patients with apparently non-malignant lesions on scans within 12 months of DAA therapy are more likely to develop HCC. Nahon et al. found that 5/15 patients had a non-malignant nodule observed within six months before starting DAA treatment and subsequently developing HCC with this shown as a statistically significant risk factor for HCC development [346]. Alternatively, Toyoda et al. found no effect of previously identified non-hypervascular hypointense nodules (NHHNs) on HCC incidence; however, these were all compensated cirrhotic patients with all nodules found on

contrast-enhanced MRI scans as opposed to the less sensitive ultrasound scanning [351]. Our study is in agreement with a recently published Spanish study with both studies suggesting an increased rate of de novo HCC in those with non-characterised nodules or other lesions; however, as our follow-up period is a year longer, this suggests that the progression of these nodules occurs early following DAA initiation [352]. This view was supported by Sangiovanni et al. following review of 1,161 patients to find patients with non-characterised nodules to have a HR of 3.11 with this being at 18 weeks following the initiation of DAA treatment and 2-year cumulative incidence of 13.6%, though it should be noted the majority of patients investigated had compensated cirrhosis with only 8% CTP B [353]. An alternative explanation for these findings is that patients with pre-existing lesions receive more attentive follow up and therefore our results are related to ascertainment bias rather than an increase in HCC incidence per se.

We found that patients diagnosed with HCC within 6 months of the onset of DAA therapy are less likely to achieve SVR12. Prenner et al. reported that in a cohort of 137 patients with pre-existing HCC treated with a variety of regimens 21% failed to achieve SVR, significantly more than those patients without HCC (p=0.009) [242]. A meta-analysis performed for 277 patients with active HCC showed a reduction of 18.8% SVR12 rate when opposed to inactive HCC and an overall decrease for those with a history of HCC (active and inactive) against non-HCC [354]. These data may be interpreted as indicating a difficulty for DAAs to penetrate a small pre-existing liver cancer effectively. Alternatively, a strain of HCV which has a higher oncogenic effect, may be present which renders DAAs less effective. However, in our study, we also detected a lower SVR12 rate (65%) in patients who were diagnosed with HCC more than six months after the onset of therapy. This suggests that either virus-infected premalignant/malignant cells that are treatment-resistant are present for a very long

time before presenting as overt malignancy or viral or host factors that predispose to malignancy are also involved.

We investigated the importance of other factors on the behaviour of HCCs including all those found to be important in the production of HCC and only found a difference for those with baseline lesions. This may be due to better engagement by these patients or that tumours forming in these livers are less aggressive.

Our study is a nationwide prospectively collected real-world study of decompensated cirrhotic patients. The standard of data collection was high throughout the study and carried out to a clinical trial standard, although not formally audited.

Although our study is one of the larger studies examining HCC in the post-DAA era, we nevertheless had only 80 HCC patients treated with DAAs. This may limit our ability to detect small yet significant differences in populations and is compounded by the relatively short period of follow-up. Another limitation of our study is the selection of controls which, although frequency matched to remove bias for age, gender, stage of disease and length of follow-up, were not otherwise matched. However, as liver function has the greatest impact on the development of hepatocellular carcinoma, we felt that these measures would be most sensitive. We excluded patients without data for SVR and this may have led to loss of ultra-aggressive cancers in the very early stages of follow-up. We chose to use the worst value for the blood tests in the year before treatment to provide an assessment of 'baseline, most severe' liver function. Other approaches are possible, but as liver function values are often modified by specific treatments (e.g. albumin infusions), we believe that it is appropriate to use the worst value to avoid artificially adjusted values. As this is a real-world observational study,

some data were unavailable due to patient engagement or ability to gain this from the records, this also meant some well-validated scoring measures could not be investigated, such as APRI; nevertheless, the clear outcomes from the majority of patients provides us with confidence that the conclusions are robust.

3.1.5 <u>Conclusion</u>

In conclusion, we have shown the presence of baseline non-malignant lesions in addition to diabetes and a lower platelet count, to be indicative of HCC development. An absence of effect of DAA treatment on HCC progression as well as an absence of effect of viraemia on patient survival was evident.

3.2 Changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy

3.2.1 Chapter summary

This chapter will investigate the changes in liver function following treatment of hepatitis C virus (HCV) in patients with cirrhosis based on measurements at baseline, four and 52 weeks post-treatment, to investigate whether changes can be predicted prior to treatment to allow more focussed follow up in high-risk patients.

3.2.2 Hypothesis

Evaluation of liver function with Indocyanine green excretion and/or TE will allow a classification of patients into those who may improve liver function after viral clearance.

3.2.3 Results

3.2.3.1 Baseline characteristics

Patients were recruited between 1/5/2016 and 31/1/2018. There were 107 eligible patients – 52 willing to be enrolled. The threshold TE of 11.5kPa was not reached by six patients and thus not enrolled. Two patients were unable to be contacted after their initial appointment and one developed a recurrence of breast cancer and withdrew. Therefore, 43 patients had two or more investigations, with one having an HCC recurrence following his second appointment, who was censored at this point. Two patients withdrew consent, with one unwilling to undergo cannulation due to difficult access. Unfortunately, one patient attended on a day when the Fibroscan® machine was at another site and therefore could only have her ICG

reading. There were no adverse events reported. Further breakdown is available in the

CONSORT diagram, Figure 3.22.
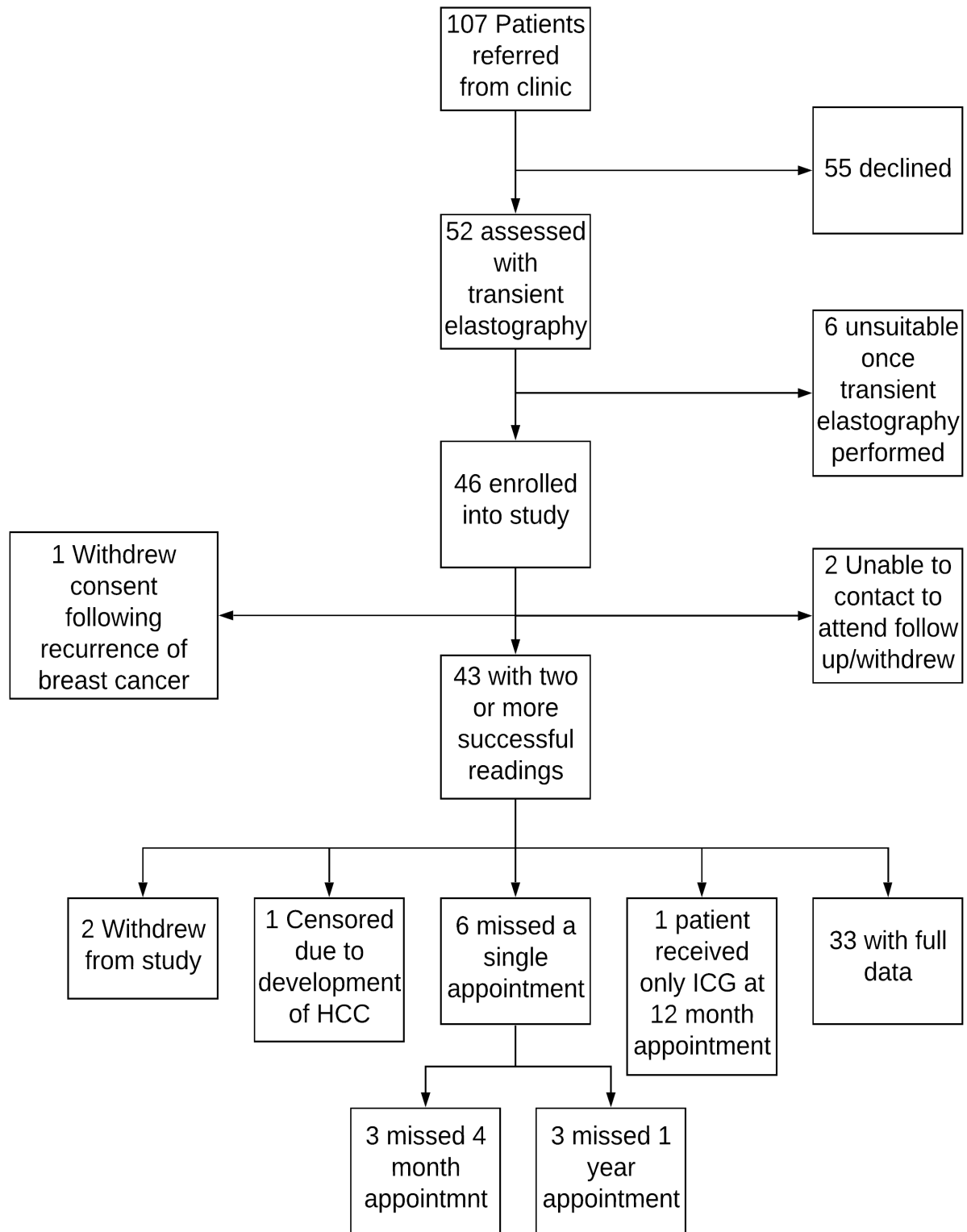
Figure 3.22: CONSORT diagram of patient recruitment

Baseline demographics for the 43 patients recruited are shown in Table 3.11. The cohort was predominantly male (58.1%), with a median age of 54 years old. Just over a quarter of patients (27.2%) had a history of heavy alcohol intake, defined as more than six units per day, with two patients continuing to drink alcohol. Most of the cohort was not diabetic (74.4%).

The median time from HCV diagnosis was six years. Eight patients failed treatment previously, one with solely interferon, one with interferon and ribavirin, five with pegylated interferon and one who was unsure of their previous treatment. No patient had been previously treated with DAAs. Three patients had previous HCC and one had liver metastases from colorectal cancer; no patient had active cancer upon commencing treatment. Most patients were Childs-Turcotte-Pugh (CTP) grade A (88.5%) and nine had a history of decompensation. Median MELD and AST to Platelet Ratio Index (APRI) scores were 7 and 1.35, respectively.

Among 31 patients who underwent an endoscopy prior to HCV treatment, 8 (18.6%) were found to have varices. A single patient had an episode of encephalitis. Of those with a history of decompensation, five were CTP grade B and overall had a median MELD of 11. Liver biopsies had been performed on 13 patients - most over five years prior to treatment; six showing cirrhosis (14%), seven (16.3%) showing fibrotic changes (Ishak score 3-4). Treatment regimens reflected the study's time course with reducing Ribavirin administration; Sofosbuvir and Velpatasvir for 12 weeks were the most commonly used treatment (44.2%). A single patient had 24 weeks Sofosbuvir and Velpatasvir (as part of another trial for initial treatment failure).

Table 3.11: Baseline characteristics

Characteristic	n=43
Median age, (IQR), yrs.	54 (49-62)
Male gender, n, (%)	25 (58.1)
Ethnicity, n, (%)	
White-British	5 (11.6)
White-Other	14 (32.6)
Asian	12 (27.9)
Other	12 (27.9)
Alcohol use, n, (%)	
Severe alcohol use (>6units/day)	12 (27.9)
Still drinking alcohol, n (%)	2 (4.7)
Diabetes, n (%)	
Yes	11 (25.6)
No	32 (74.4)
Metformin	8 (18.6)
Genotype, n, (%)	
Genotype 1	15 (34.9)
Genotype 3	25 (58.1)
Other	3 (7)
Median time from HCV diagnosis, (IQR), yrs.	6 (1-10.5)
Previous treatment failure, n, (%)	8 (18.6)
Past history of HCC, n, (%)	3 (7)
Childs-Turcotte-Pugh grade, n, (%)	
Grade A	38 (88.5)
Grade B	5 (11.6)
Previous decompensation, n, (%)	9 (20.9)
Varices, n, (%)	8 (18.6)
Past hepatitis B exposure, n, (%)	11 (25.6)
Median MELD score (IQR)	7 (6-10)
Median APRI score (IQR)	1.35 (0.67-2.43)
Median albumin, (IQR), g/L	41 (39-44)
Median alanine aminotransferase, (IQR), U/L	67 (43-107)
Median alkaline phosphatase, (IQR), U/L	96 (82-130)
Median gamma-glutamyl transpeptidase, (IQR), U/L	83 (51-187)
Median bilirubin, (IQR), μmol/L	10 (7-25)
Median platelet, (IQR), x10^9/L	154 (96-187)
Median sodium, (IQR), mmol/L	140 (137-142)
Median creatinine, (IQR), μmol/L	64 (57-77)
HCV RNA, (IQR), IU/mL	960364 (282459-2572775)
Treatment regimen, n, (%)	
Sofosbuvir/Velpatasvir	19 (44.2)
Sofosbuvir/Ledipasvir	6 (14)
Sofosbuvir/Daclatasvir	3 (7)
Sofosbuvir/Pegylated interferon	4 (9.3)
Ombitasvir/Paritaprevir/Ritonavir	1 (2.3)
Ombitasvir/Paritaprevir/Ritonavir/Dasubavir	6 (14)
Ribavirin used, n, (%)	16 (37.2)
12-week duration, n, (%)	40 (93)

n: Number, yrs: Years, %: percentage, IQR: Inter-quartile range, MELD: Model For End-Stage Liver Disease, APRI: AST to Platelet Ratio Index, HCV: Hepatitis C virus, HCC: Hepatocellular carcinoma, RNA: Ribonucleic acid, dl: Decilitre, L:

Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, μg: Micrograms, mmol: Millimole, μmol: Micromole, U: Units, IU: International units.

All qualifying patients had baseline TE and indocyanine green measurements (Table 3.12). The median TE was 18.4kPa and ICG excretion were both delayed with readings for PDR of 13.5%/min (normal ≥18%/min) and ICGR15 of 13.2% (normal <10%). Of the patients with a previous decompensation, all but one had a stiffness of above 20kPa, with an increased ICGR15.

Table 3.12: Baseline transient elastography and ICG excretion tests

Investigations	n=43
Stiffness, (Range), kPa	18.4 (11.5-69)
PDR, (Range), %/min	13.5 (0.2-26.3)
ICGR15, (Range), %	13.2 (1.9-97)

kPa: Kilopascal, PDR: Plasma disappearance rate, ICGR15: Indocyanine green retention after 15 minutes, %: Percentage, min: Minutes

3.2.3.2 Changes in liver fibrosis and function

At the end of the study, one patient had not achieved sustained virological response (SVR) and there were no relapses between four months and one year. One patient that had not achieved SVR at four months went on to have a liver resection for hepatocellular carcinoma and was therefore not re-tested as part of this study. The patient who did not attain SVR at four months subsequently had an extended course of treatment for 24 weeks and achieved SVR at one year and a single patient did not achieve SVR during the study period.

This study's primary outcome was to determine liver fibrosis and function changes in patients with cirrhosis undergoing antiviral therapy for chronic HCV.

3.2.3.2.1 Transient Elastography

We initially plotted each case (Figure 3.23), investigating these, we find that most patients improve their stiffness scores between each time point, with these usually most evident for the patients with the highest baseline stiffness.
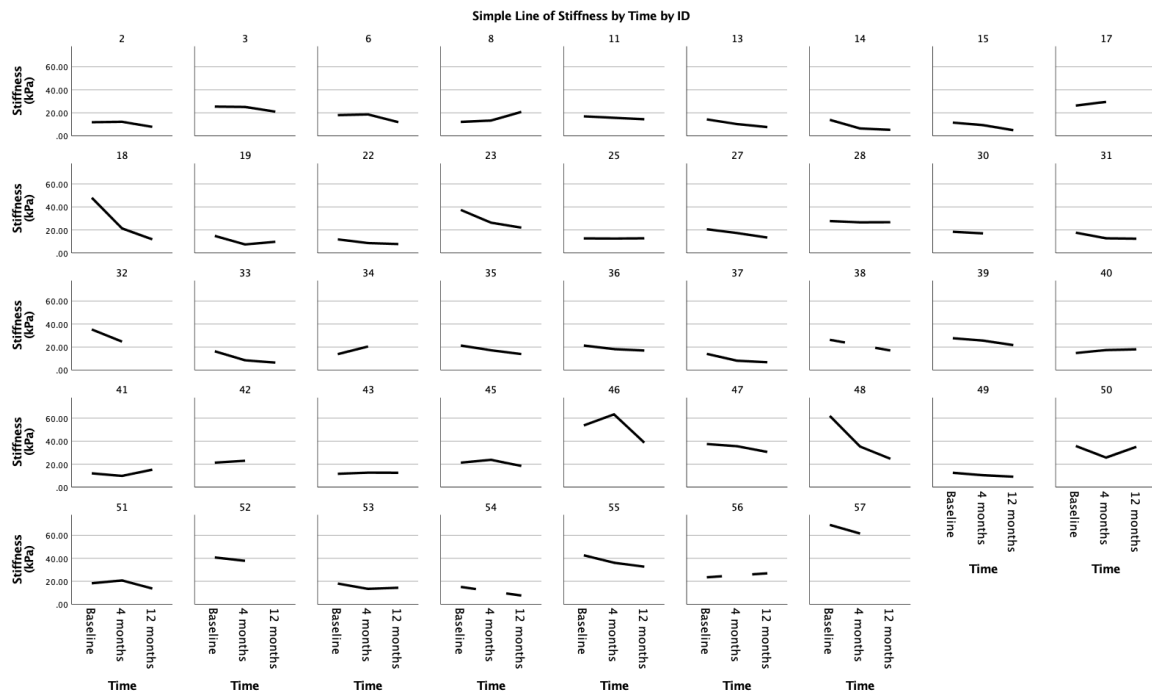
Figure 3.23: Panel of individual changes in liver stiffness over one year following treatment initiation for hepatitis C

We then amalgamated this information (Figure 3.24) and plotted the median value to confirm this trend (Figure 3.25).
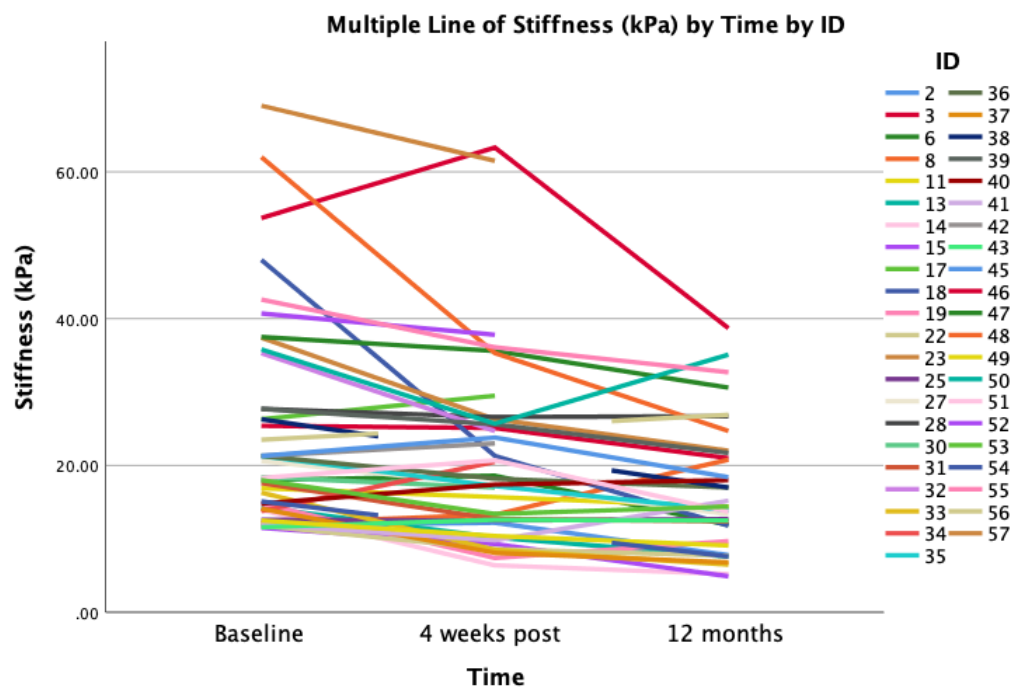
Figure 3.24: Multiple line graph showing all patients transient elastography readings over one year following treatment initiation for hepatitis C
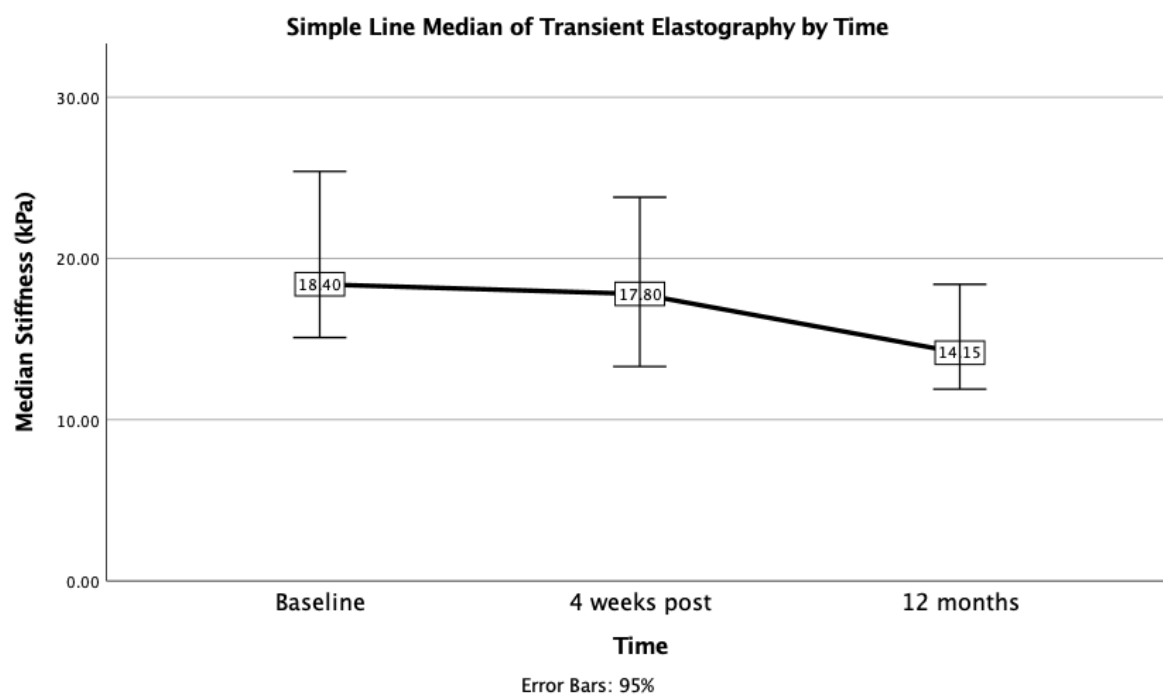
Figure 3.25: Line graph for the median stiffness changes for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

181

Following the introduction of a repeated measures analysis the adjustments that are found vary with an IQR of -8.25 to 5.58 and are shown below (Figure 3.26).

Figure 3.26: Scattergram displaying the adjustments of values following the introduction of fixed effects modelling on baseline liver stiffness.

We fitted the model for TE readings from baseline to four weeks post-treatment and found an average difference of 3.44kPa. Between this reading and one-year post-treatment, there was a further improvement of 3.09kPa. Overall, within the first year, there was an improvement of 6.53kPa. We then applied mixed effects modelling which incorporates the fixed effects, which are the quantifiable impact that certain predictors have on the model, such as time, and random effects, which accounts for factors such as the correlation between observations that come from the same subject over the observed period. The random effects are not included in the model to explain what the predictors do on the outcome, but more to optimise the fit and account for the non-independence of the observations that come from the same individuals as such, the mixed effect confirmed this overall improvement over the year (p <0.001), with the overall fitness of the model showing an R^2 of 92.8%.

3.2.3.2.2 Plasma disappearance rate (PDR)

ICG excretion evaluates liver function. We initially plotted each case (Figure 3.27) and found that, for most patients, there was a marked improvement initially following treatment, followed by a slight decrease, although this was an improvement from baseline. Some patients continue to improve when they have their final measurement at one year, namely patients 2, 3 23,43,48,51, 55 and 57.
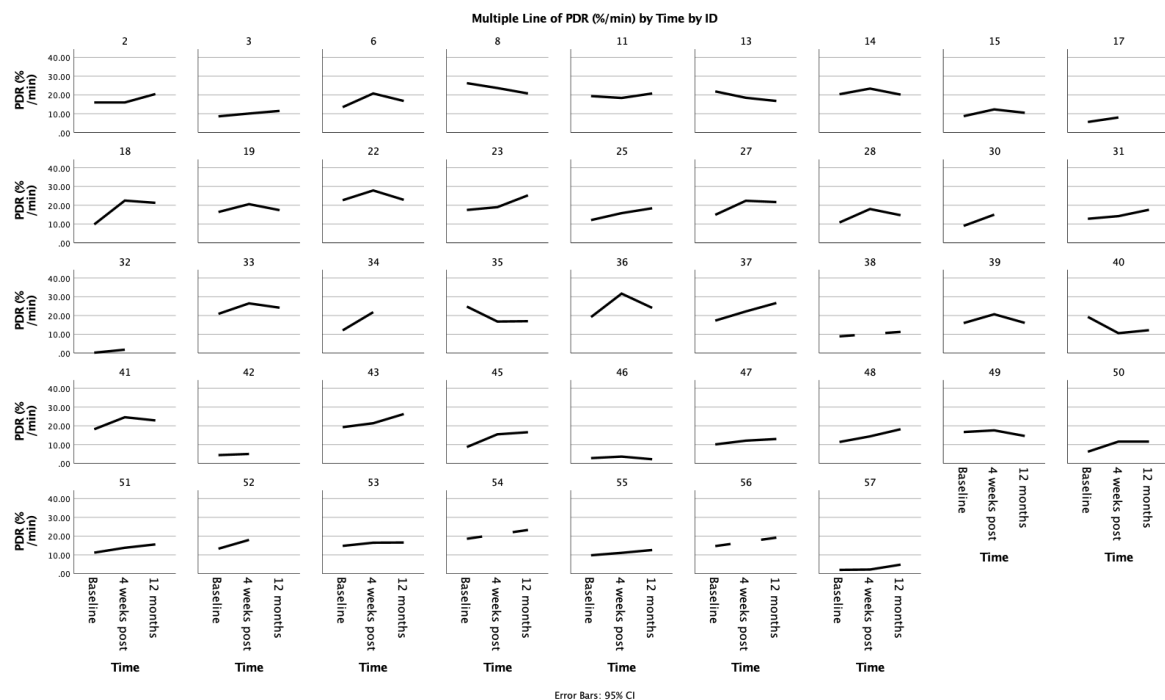
Figure 3.27: Panel of individual changes in plasma disappearance rate of ICG over one year following treatment initiation for hepatitis C

Viewing all scores does not show a clear effect for baseline score on final reading (Figure 3.28).

Figure 3.28: Multiple line graph showing all patients change for plasma disappearance rate of ICG over one year following treatment initiation for hepatitis C

The median readings support that, on the whole, patients improve drastically up to 4 weeks post therapy and this then plateaus with a slight improvement up to the one-year mark (see Figure 3.29).
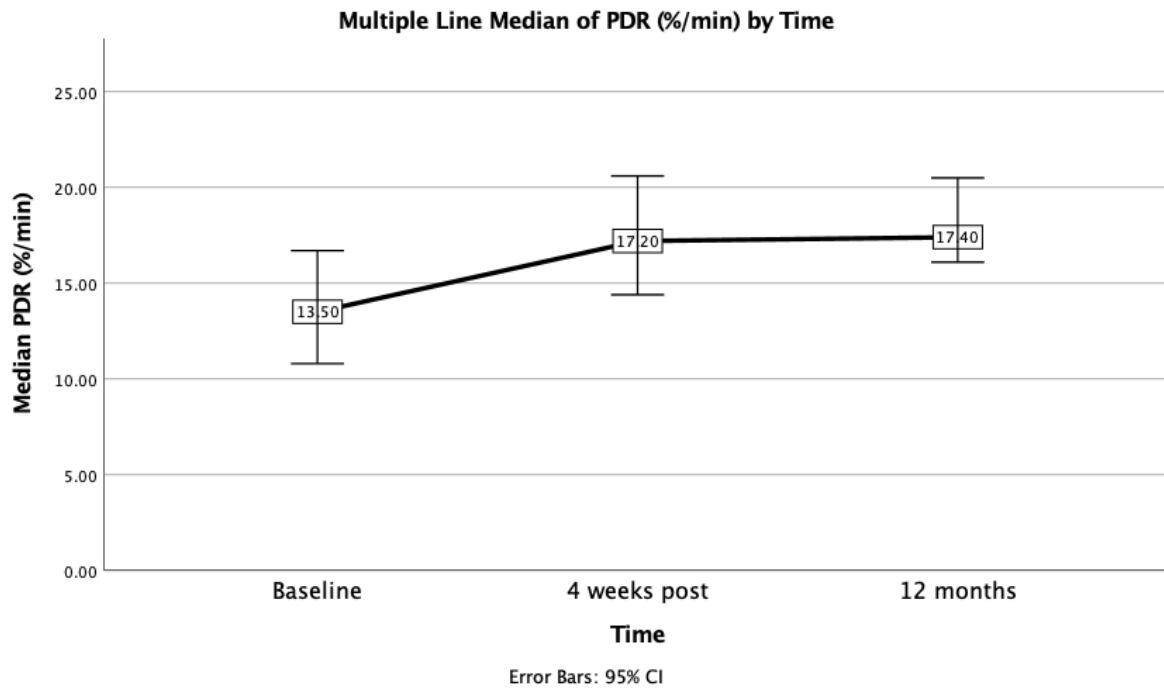
Figure 3.29: Line graph for the median plasma disappearance rate for ICG for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

As with TE, a repeated measures analysis was performed for PDR with the adjustments

displaying an IQR of -3.33 to 3.63 and are shown below (Figure 3.30).
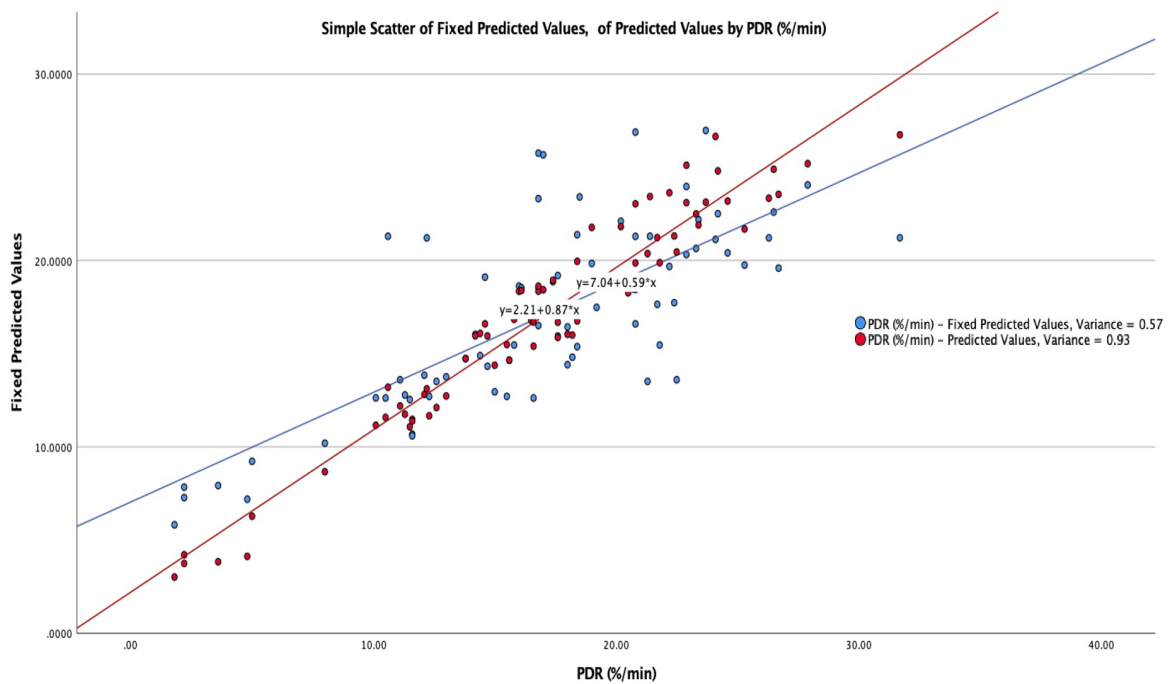
Figure 3.30: A scattergram displaying the adjustments of values following the introduction of fixed effects modelling on baseline PDR reading.

Following the fitting of the model, we found a marked improvement in the initial period post-treatment reading of 3.07%/min, with a further slight decrease to the end of the year with a loss of 0.55%/min. Overall, an improved function was found for the year of 2.52%/min (p<0.001). The mixed effect of the visit on the plasma disappearance rate readings was statistically significant (p<0.001), with the overall fitness of the model showing an R^2 of 82.09%.

3.2.3.2.3 Indocyanine Green Retention after 15 minutes (ICGR15)

The remaining percentage of ICG at 15 minutes (ICGR15) is the second reading pertinent to ICG excretion. Individual measures are displayed in Figure 3.31. Most patients demonstrated an overall improvement from baseline.
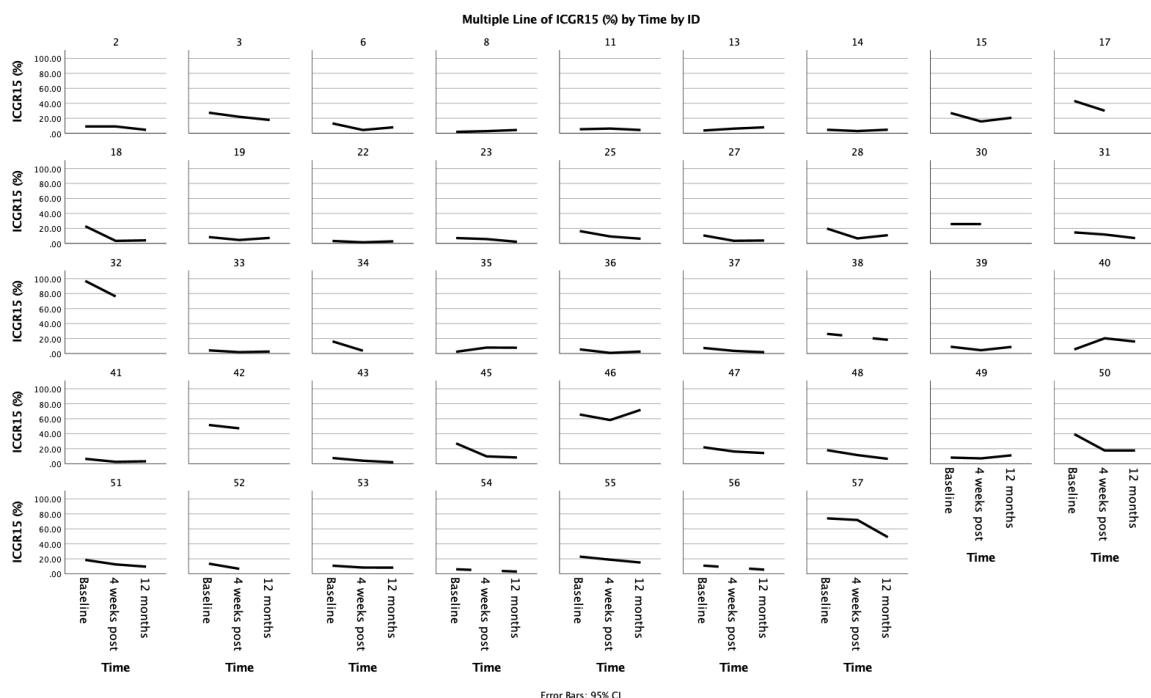
Figure 3.31: Panel of individual values for percentage of ICG remaining after 15 minutes (ICGR15) over one year following treatment initiation for hepatitis C

186

We then looked at the overall values together (Figure 3.32) and unfortunately, many of the patients with high values, though having an improvement initially, did not attend their follow-up appointments, so this valuable information is lost. However, for the majority, there is a general trend to improvement.
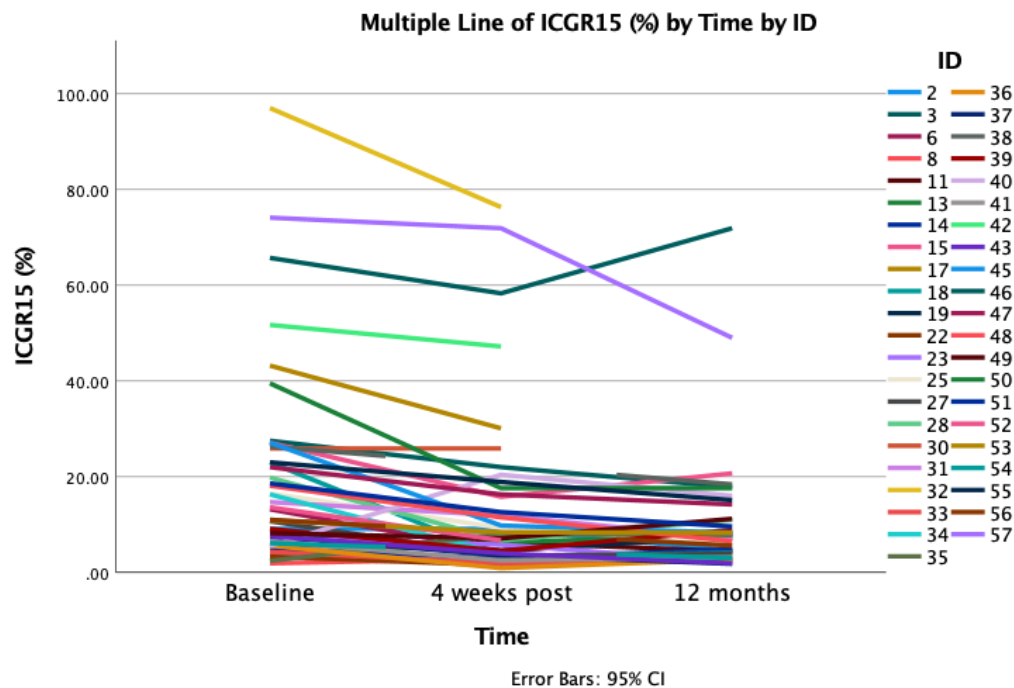
Figure 3.32: Multiple line graph showing all patients change for percentage of ICG remaining after 15 minutes (ICGR15) over one year following treatment initiation for hepatitis C

This overall result is reflected when looking at the median values with a drastic improvement at four weeks post-treatment, with this plateauing to the one-year time point (see Figure 3.33).
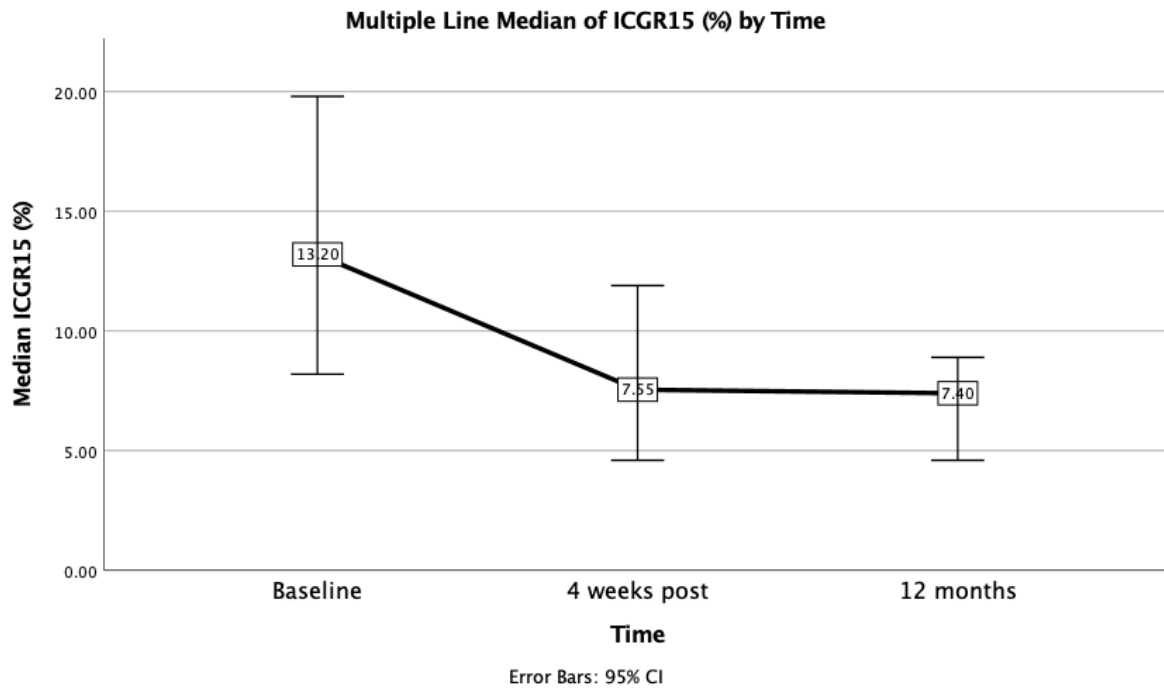
Figure 3.33: A line graph for the median percentage of ICG remaining after 15 minutes (ICGR15) for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

As with TE, a repeated measures analysis was performed for ICGR15 with the adjustments displaying an IQR of -9.88 to 2.79 and are shown below (Figure 3.34).
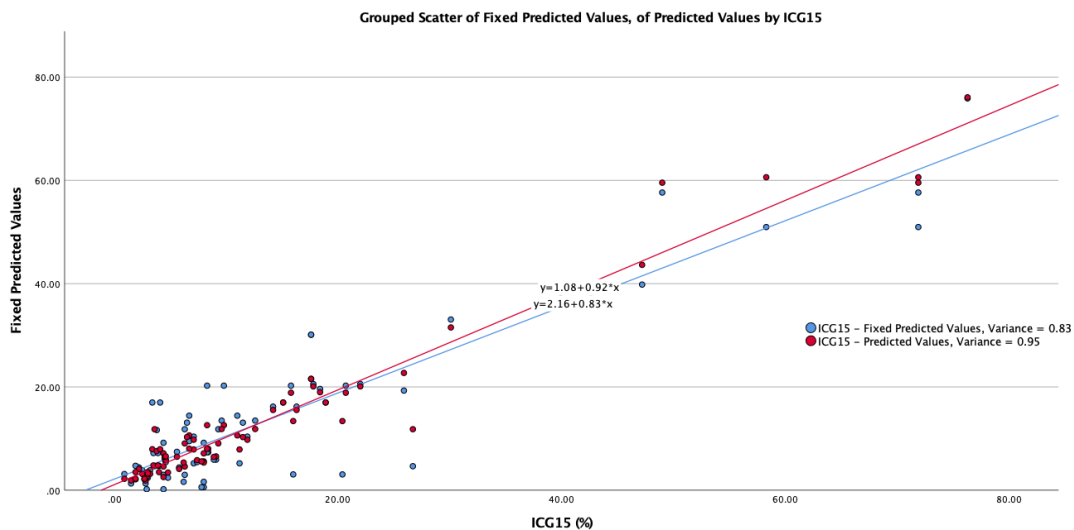
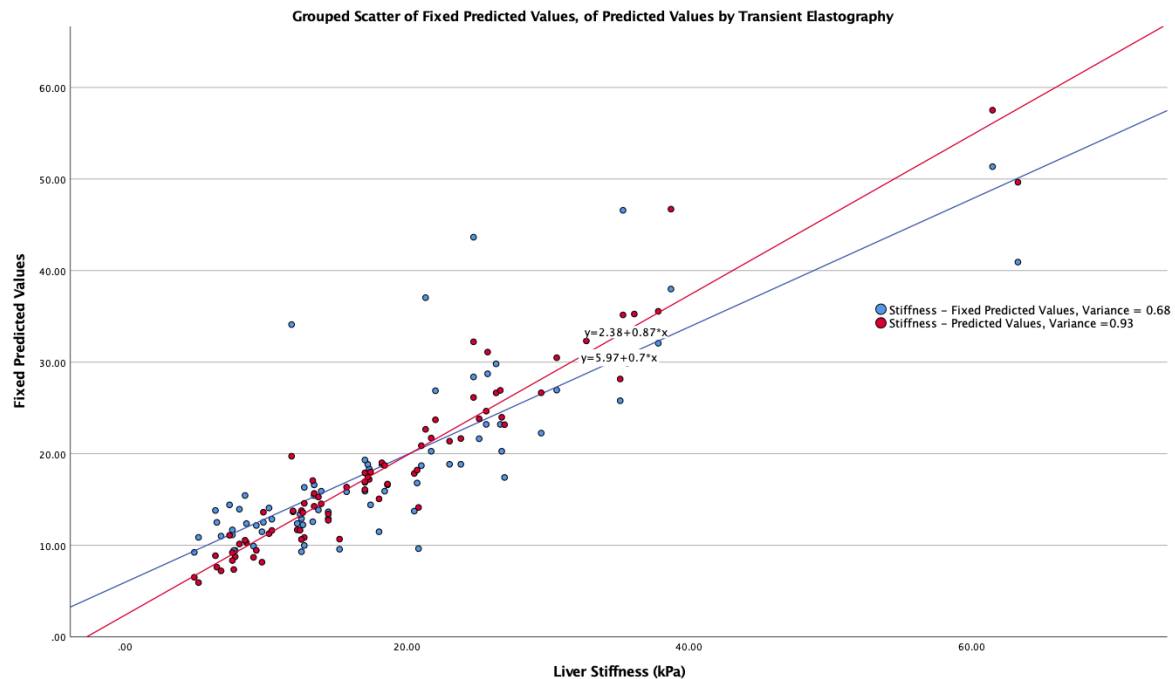**Figure 3.34:** A scattergram displaying the adjustments of values following the introduction of fixed effects modelling on baseline ICGR15 reading.

Following the fitting of the model, we found an improvement in function immediately post-treatment of 5.38% with a slight improvement of 1.58% at 1 year. This meant an overall improved excretion of 6.95% over the year (p<0.001). The mixed effect of the visit on the ICGR15 readings was statistically significant (p<0.001), with the overall fitness of the model showing an R^2 of 94.3%.

3.2.3.2.4 Correlation

We have found different responses to therapy, in some stiffness worsened despite normal function, in others we saw improving stiffness with improved function, and in some a deterioration in both. This is shown for all patients in Table 3.13 and Table 3.14 and may indicate that patients with worse scores are likely to improve stiffness and function – with a moderate association (r=-0.520, p<0.001) between stiffness and PDR. The correlation was also seen for ICGR15 (r=-0.519, p<0.001) and PDR and ICGR15 showed an almost perfect correlation as expected (r=-0.994, p<0.001).

Table 3.13: Baseline median values, grouped by overall change in stiffness and ICG excretion, at the 4 months follow up appointment.

	n	Median baseline TE	Median baseline PDR	Median baseline ICGR15
All scores improve	25	21.3	13.3	13.6
All scores decline	2	13.45	22.8	3.7
Other patterns	13	18.3	12.1	16.3
Unavailable	3	N/A	N/A	N/A

n: Number, TE: Transient elastography, N/A: Not available, PDR: Plasma disappearance rate, ICGR15: Indocyanine green retention after 15 minutes

Table 3.14: Baseline median values, grouped by overall change in stiffness and ICG excretion, at the 1 year follow up appointment.

	n	Median baseline TE	Median baseline PDR	Median baseline ICGR15
All scores improve	25	20.6	13.5	13.2
All scores decline	2	13.45	22.8	3.7
Other patterns	9	13.9	18.2	7.6
Unavailable	7	N/A	N/A	N/A

n: Number, TE: Transient elastography, N/A: Not available, PDR: Plasma disappearance rate, ICGR15: Indocyanine green retention after 15 minutes

3.2.3.3 MELD

Scoring systems have been used to understand patients' fibrotic process and overall liver function in a non-invasive manner. We found improvement in MELD scores for some patients, particularly at the first time point (patients 11, 17, 47 and 57), however certain patients show an improvement after this time (patient 13, 51 and 53), albeit a slight improvement. Interestingly, patient 47, though having a large shift in indocyanine green clearance, indicating a markedly worsening liver function, actually improved their MELD score (Figure 3.35). Overall, the median MELD score did not show any changes (see Figure 3.36 and Figure 3.37). We investigated several markers similarly and these to find significant improvements in ALT and γGT and can be seen in appendix 2.6.
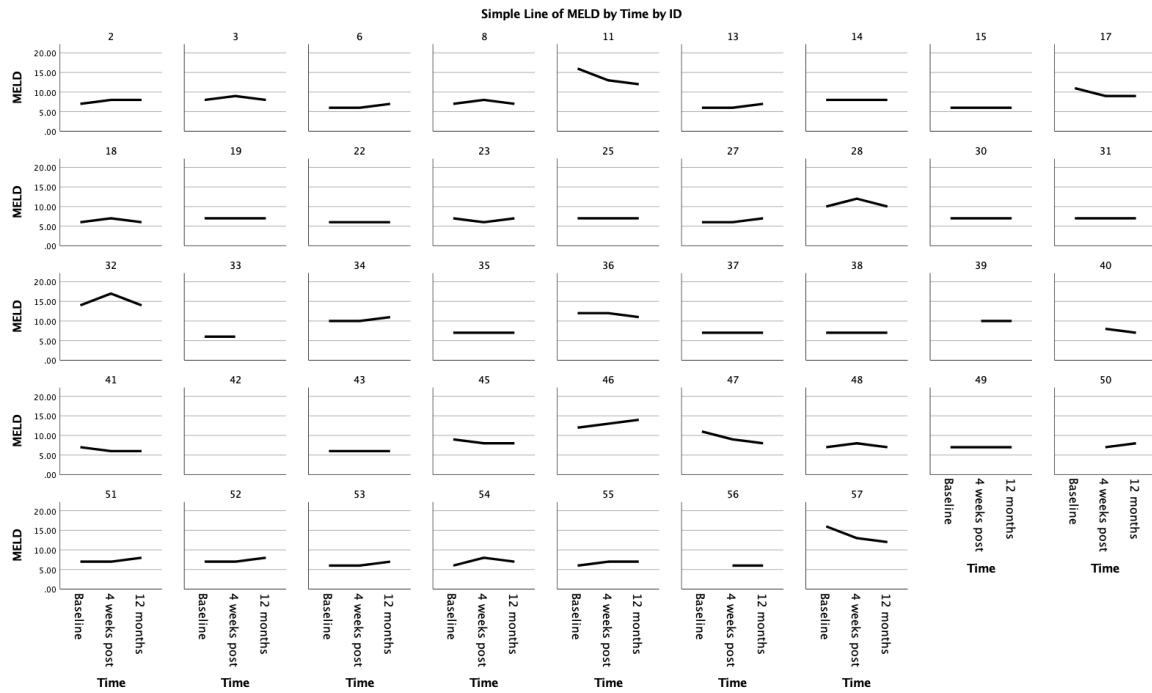
Figure 3.35: Panel of individual changes in MELD over one year following treatment initiation for hepatitis C
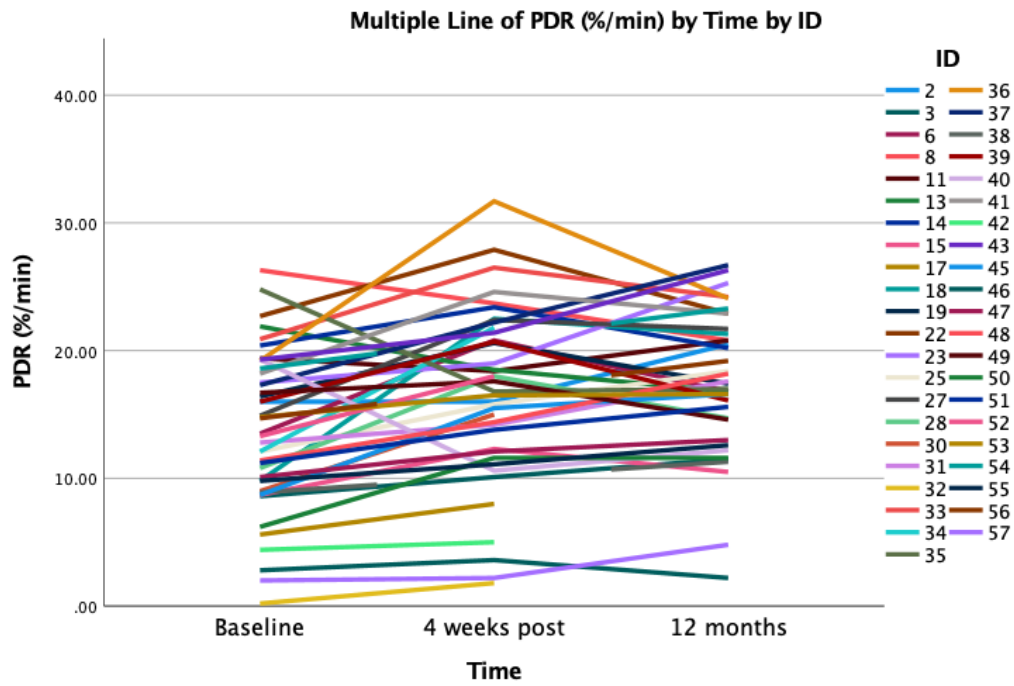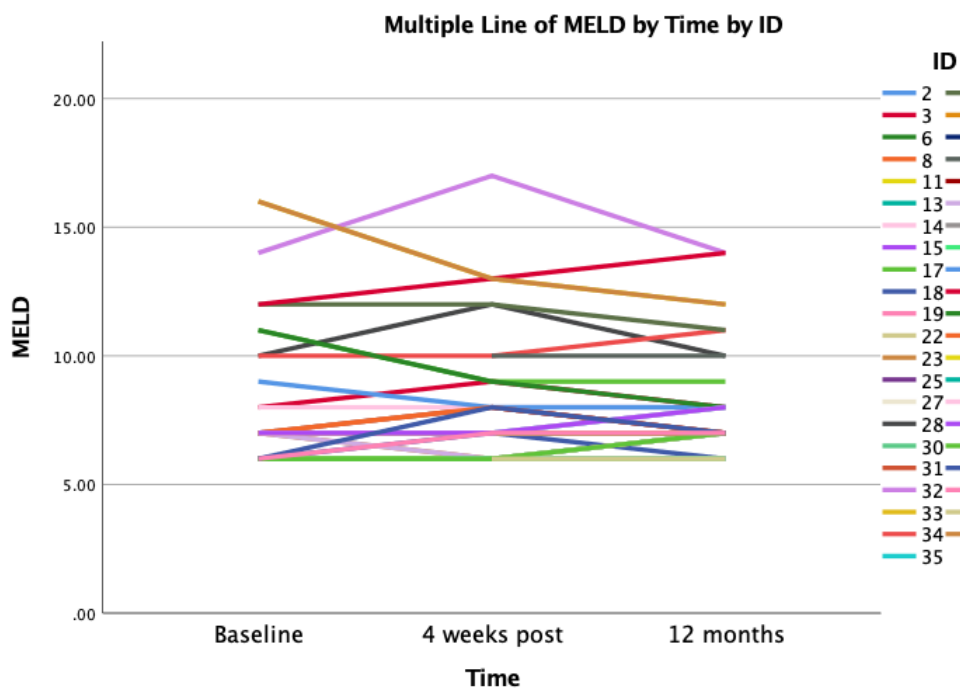

Figure 3.36: Multiple line graph showing all patients change for MELD over one year following treatment initiation for hepatitis C

191
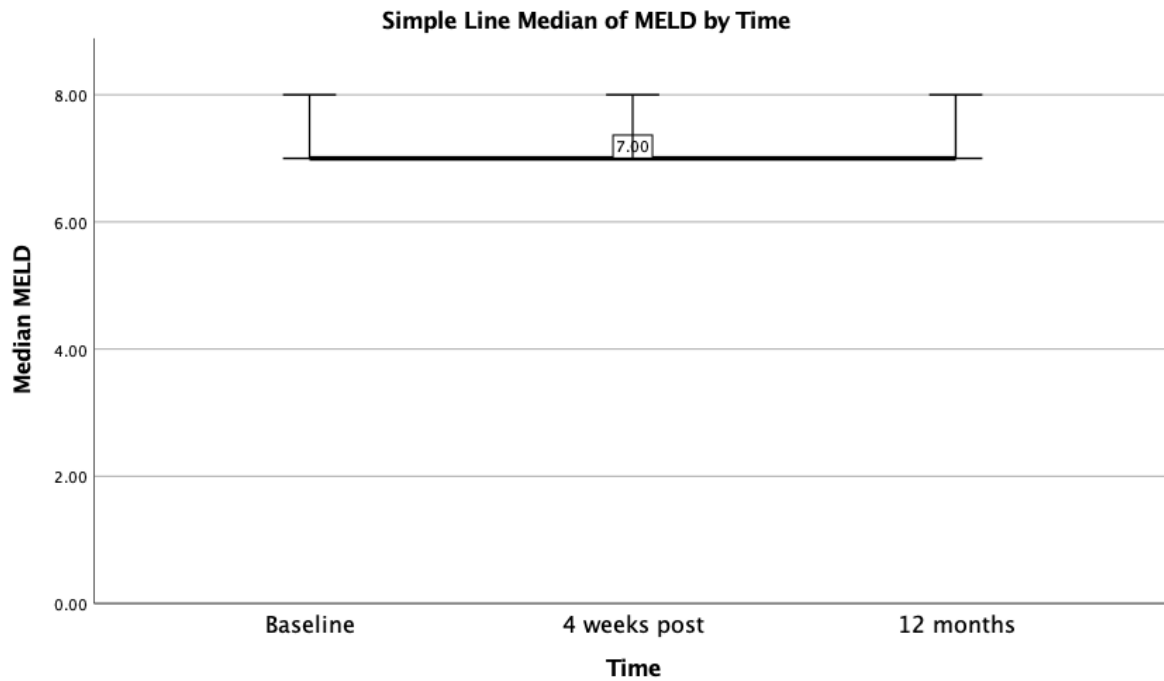
Figure 3.37: A line graph for the median MELD for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.2.3.4 **Predictive model**

With the utilisation of ICGR15, we investigated whether a value at baseline could be used to predict which patients would improve at each time point. As we did not have any patients that went on to be placed on the liver transplant list or died, we assigned reduction to a value of 14% to indicate 'meaningful improvement' as this has been shown to rule out clinically significant portal hypertension [165] and is used as the cut-off for partial hepatectomies in patients with cirrhosis, with those having values greater than 14% being regarded as unsuitable for resection.

Inspection of the data four months after treatment initiation, indicated that patients with an ICGR15 value of below 20.9% would display a meaningful improvement following treatment, with an AUROC of 0.92 (95% C.I: 0.80-1.00, p<0.001) (Figure 3.38). This gives a sensitivity of 92% and specificity of 93% with a PPV of 92.9% and an NPV of 87.5%.

192

However, these data were based on a total cohort of 40 patients of whom only seven had a pre-treatment ICGR15 of >20.9%.
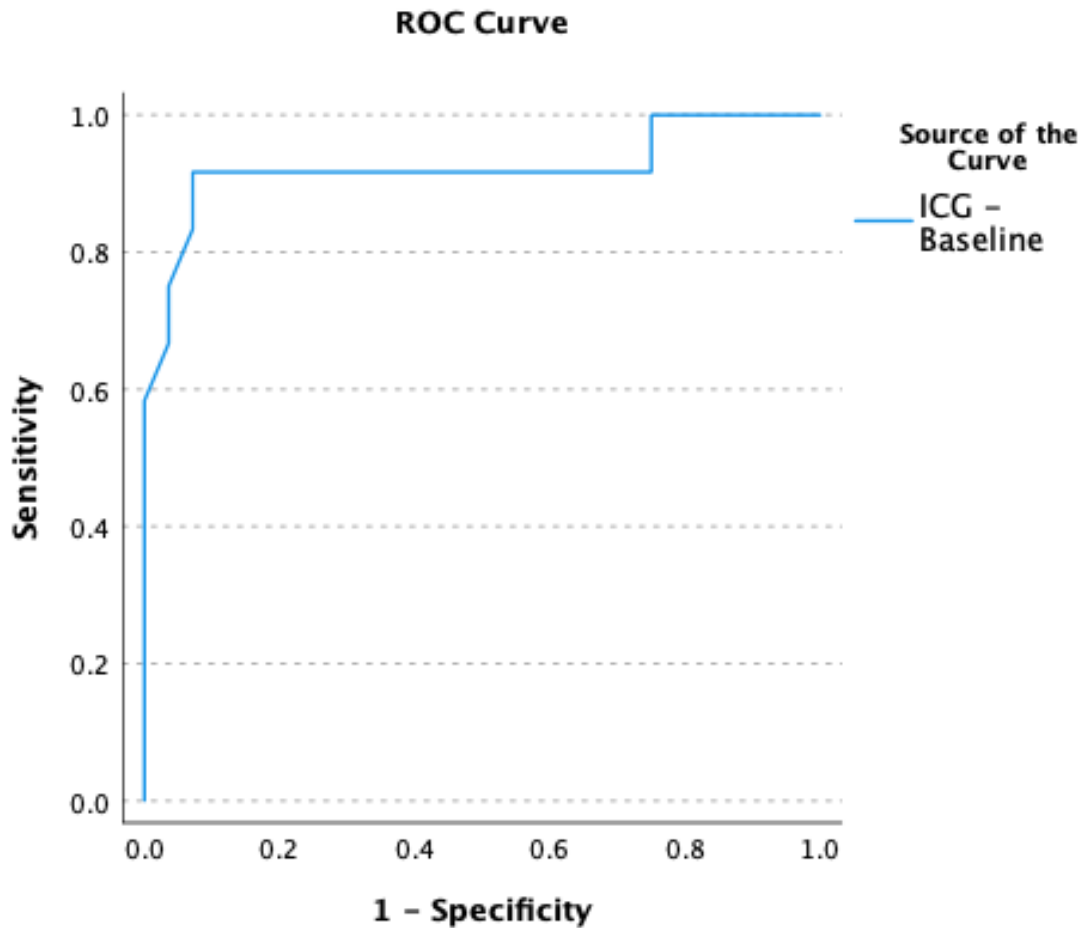
Figure 3.38: ROC curve for baseline ICGR15 for the reduction of ICGR15 to below 14% at four months post-treatment start, AUROC of 0.93 (95% C.I: 0.82-1.00, p<0.001).

This process was then repeated for one year with the same cut-off of <20.9%, (AUROC of 0.88 (95% CI: 0.683-1.08, p<0.001) (Figure 3.39), with a sensitivity of 85.7% and specificity of 87% with a PPV of 92.86% and an NPV of 88.89%. However, these data were based on a total cohort of 37 patients of whom only two had a pre-treatment ICGR15 of >20.9%.
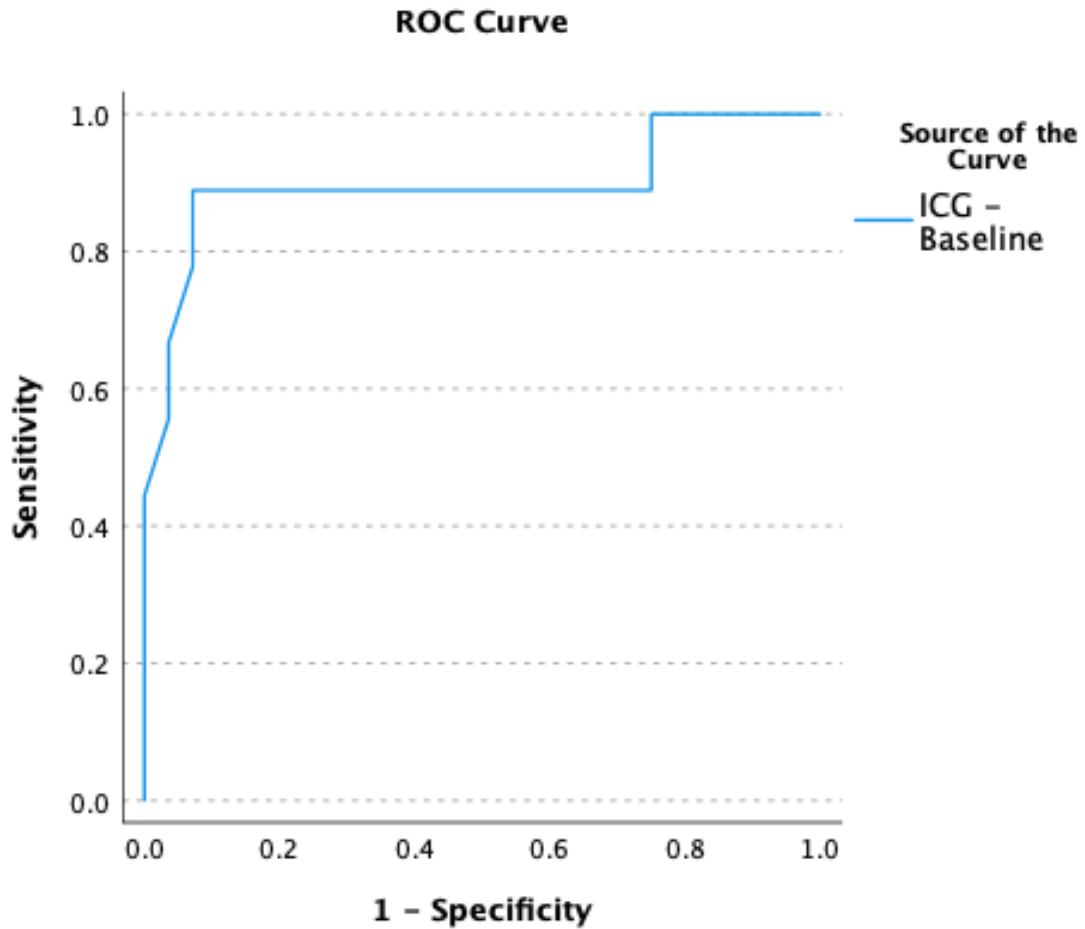
ROC Curve

Figure 3.39: ROC curve for baseline ICGR15 for the reduction of ICGR15 to below 14% at 12 months post treatment start, AUROC of 0.897 (95% C.I: 0.741 - 1.01, p<0.001).

We have then amalgamated the outcomes from the mixed effects models and ROC analysis to produce an equation, which may be used at baseline, to predict the final ICGR15 value either at the first time point of four months or one year and can be seen below.

ICG_AFTER= -0.15 + BL_ICG*0.818 – 0.043BL_ICG*time (time is either 1 or 2).

3.2.3.5 **Univariate analysis**

Utilising the cut-off value obtained from the AUROC assessments, we used this as the outcome measure to delineate any baseline factors, which may indicate which patients will, or will not, improve following treatment. We found those with advanced age (median 61 vs 53 years old), thrombocytopenic (median 102×10^9/L vs 171×10^9/L), raised INR (median 1.3 vs 1.1), an increased liver stiffness (median 30.8kPa vs 16.3kPa) or with an advanced ALBI grade were less likely to improve their ICGR15 to below 14% following DAA treatment. Bilirubin and albumin also showed statistical significance, however, as the values were within normal clinical limits, they were disregarded. (See Table 3.15)

Table 3.15: Univariate analysis of baseline variables indicating which may show an improvement in liver function post treatment according to ICGR15 of 20.9% at baseline

Category	p-value
Age	0.028
Gender	0.3
Genotype	0.41
HCC	0.11
DM	0.45
Haemoglobin	0.06
Platelet	*0.016
Bilirubin	*0.004
ALT	0.085
Albumin	*0.001
INR	*0.014
Creatinine	0.856
Sodium	0.314
Stiffness	*0.001
CTP	0.32
APRI	0.387
MELD	0.079
ALBI	*0.013

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as $p < 0.05$, *denotes significant results. HCC: Hepatocellular carcinoma, DM: Diabetes Mellitus, ALT: Alanine aminotransferase concentration, INR: International normalised ratio, CTP: Childs-Turcotte-Pugh, APRI: AST to Platelet Ratio Index, MELD: Model For End-Stage Liver Disease, ALBI: Albumin-Bilirubin grade

3.2.4 **Discussion**

Liver function has been investigated in both the pre- and current-DAA eras, via several means [190, 208, 221, 225, 355-358], with variable outcomes observed. Changes in inflammation and fibrosis have equally been investigated using liver biopsy [359, 360] and TE [361]. However, the utilisation of direct excretory function of the liver, in conjunction with morphological changes, for monitoring and predicting patient outcome at baseline, has not been explored in patients with cirrhosis treated with DAAs, until this study.

3.2.4.1 **Summary of findings**

Over the study, TE showed an overall improvement of 6.53kPa ($p<0.001$) from baseline. Liver function improved mainly within the first month following treatment, with this level of improvement being sustained at 1 year (PDR increased by 2.52%/min ($p<0.001$), ICGR15 reduced by 6.95% ($p <0.001$)). These results should be interpreted with caution as the patients with the worst liver function readings, though showing an improvement at their first follow up, were unable to attend their final appointment.

The correlation between the improvement in liver function and TE improvement was moderate but significant. Taken together, this indicates that there may be a reduction in liver damage to pre-cirrhotic levels following DAA treatment for hepatitis C.

We investigated changes to other variables that are routinely checked to find ALT and γGT improve significantly, whilst others improved within their normal ranges and, therefore, are less valuable clinically (see appendix 2.5). APRI score showed a significant improvement however, this result was marred by several missed readings for multiple patients. MELD

score remained static or slight improvement for most patients. It is important to note that the MELD score in our patients was relatively low and in addition to the small cohort, it may be, that reductions in liver function induced by DAA therapy and demonstrated in previous studies in people with more advanced liver disease [190], may not be detectable with MELD scores in those with pre-treatment low values.

Due to the almost perfect correlation between PDR and ICGR15, we concentrated on ICGR15 to produce a predictive model. We used the cut-off for a desired ICGR15 value of 14% and employing this value gave us a cut-off for baseline ICGR15 of 20.9% to predict those that will improve functionally. We produced a formula that may be used clinically to predict the likely ICGR15 result at either time point using a baseline result. Using either tool individually or in conjunction with each other should aid clinical decision-making. We found several baseline variables which may aid in the distinction of those that may achieve an acceptable improvement in liver function, chief amongst this being a raised liver stiffness, however, the limited sample size reduces the robustness of our conclusions.

3.2.4.2 **Interpretation**

The positive effects of achieving SVR and the regression of fibrosis of the liver has been investigated histologically with liver biopsies since treatment began with interferon for fibrotic patients. Shiratori et al. investigated 593 fibrotic patients who had pre and post-treatment liver biopsies and found the 183 patients that achieved SVR to have a regression of their fibrosis and inflammation as opposed to those who did not achieve SVR [359]. This improvement was sustained for up to 10 years for most patients [362, 363].

Our primary objective was to determine the changes in liver function and fibrosis in patients with cirrhosis undergoing antiviral therapy for chronic HCV. This was assessed at two time points over the first year, following treatment, to observe the early and late changes. The only inclusion criteria was the presence of cirrhosis. Following treatment, the majority of patients show improvement throughout the year, the most marked effect was seen in those with the most deranged scores at baseline. This translated into a median improvement over the year, mainly occurring between 4 weeks and 12 months following treatment. To confirm our findings, we subjected them to fixed-effects modelling, which confirmed this improvement. This is in agreement with Pons et al., who used histological samples to describe similar improvements mainly within the first 4 weeks following treatment starting, with smaller improvements following this, they concluded this was likely secondary to a reduction in inflammation[364]. A subsequent larger cohort confirmed this improvement over the year [365]. A German registry study investigating changes according to TE at the end of treatment and 24 weeks showed significant improvement throughout the study though this was mainly attributed to the period following treatment. It should be noted that they took measurements at the end of treatment as opposed to 4 weeks post-treatment [366]. A meta-analysis agrees with the overall improvements of stiffness over a year, with the largest declines for patients with higher baseline scores, though it should be noted they included interferon-based [361]. Elastography measured by ARFI have agreed with these findings [367]. Long term follow up studies for patients who achieve SVR, inclusive of those treated with interferon, have confirmed changes in liver stiffness to continue to improve over five years though at a slower rate and therefore may reflect the overall reduction in fibrotic burden [368]. Histological changes in association with TE readings, pre and post DAA treatment, were reviewed by two small studies showing an improvement in inflammation and likely collagen load, shown by morphometry [369, 370]. However, these changes did not constitute patients being

downgraded from cirrhosis as suggested by paired TE. They, therefore, argue that TE should not be used following SVR as it may overstate the level of improvement, this is in line with current guidance from EASL [371, 372]. Further to this, a Spanish study took biopsies at three years post SVR and matched these with TE readings taken pre and post treatment to confirm an improvement in fibrosis overall whilst 70% of patients continued to have F3-4 fibrosis with one third of patients with a TE between 9.6-9.9kPa continuing to show F4 fibrosis following treatment [373].

Alterations in liver architecture, though important, does not give us information on how the liver is functioning. To understand changes following SVR, we utilised ICG excretion and showed an improvement in liver function. This is most evident at four weeks following treatment, with this degree of improvement maintained following this. The obvious complexity for our data is the four patients with the worst liver function who did not attend their 1-year appointment. The outcome for these patients may have indicated a cut-off value for patients that would or would not improve following treatment or alternatively may have strengthened or weakened our conclusions; however, with the available data, we can be encouraged that patients will recover their liver function post-treatment.

ICG excretion has been shown to be affected by cirrhosis, with differentiation between CTP grades observable [159, 374, 375]. The utilisation of ICG excretion to measure the effect of treatment for HCV was trialled by Ocker et al. for patients with chronic hepatitis C infection and treated with interferon-based therapies. They did not find any changes in ICG excretion following three months of treatment, however, these patients showed no derangement at baseline for ICG, though it should be noted that they did find an improvement in aminopyrine breath test (ABT), galactose elimination capacity (GEC) in those achieving SVR[357]. Our

data is the first to report functional changes via ICG excretion changes following DAA treatment for cirrhotic patients. A recent Danish study has reported on the use of Galactose elimination capacity (GEC), a dynamic measure of cytosolic metabolic capacity of the liver [376]. They investigated 71 patients with a slightly higher threshold for TE of 15kPa to find a significant improvement over the year but mainly explained by an improvement when checked at 12 weeks following treatment ($p<0.001$) and was accompanied by a decrease in liver stiffness with a similar trend over the year. They also measured markers for liver fibrosis and found these mainly decreased during treatment, thus explaining these improvements. These results are similar to ours and considering this improvement in function, which occurs early following treatment initiation, the bulk of this improvement may be put down to a decrease in inflammation initially, leading to better perfusion and, therefore, the metabolic activity of hepatocytes. Regression of fibrotic tissue is a much longer and slower process and may explain the ongoing, though much slower, improvement in ICG and GEC excretion, as well as decreasing stiffness. This would be supported by histological studies investigated pre and post DAA treatment with biopsies taken within seven months following completion of treatment for 38 patients, which indicate inflammation improvement of two or more grades in 82% of patients with fibrosis improvement shown in 37% [377, 378].

HVPG is much more widespread in diagnosing the sequelae of advancing cirrhotic disease. ICG excretion has been shown to correlate with HVPG and to be increased for patients with clinically significant portal hypertension (CSPH) (ICGR15>14.1%), severe portal hypertension (SPH) (ICGR15>19.1%) as well as the likelihood of early oesophageal varices (ICGR15>22.9%) in patients with compensated cirrhosis [164-166, 375]. We can utilise the correlation of HVPG with ICG excretion to gain insights into the long-term effects of SVR

and how excretory liver function is affected. For those patients who are at risk of oesophageal varices, the Baveno VI guidelines are patients with a baseline TE <20kPa with a normal platelet count (>150 x10^o/L) are at low risk of varices and do not require endoscopy while those with a TE≥ 20kPa should have endoscopic investigation [379]. However, HVPG has been shown to decrease following SVR and may affect these recommendations long term. Reduction in HVPG has been shown both at the end of treatment SVR24 weeks and SVR48 weeks with the largest decreases for those with the most advanced disease, however, 66-78% of patients still have an HVPG displaying clinically significant portal hypertension[208, 225]. Afdhal et al. investigated HVPG measurements in patients following DAA treatment and found these to improve mildly at the end of treatment. The observed improvement accelerated at 48 weeks post-treatment with this recovery also true for the nine patients with HVPG≥12mmHg, with eight of these showing a ≥20% improvement in HVPG and three of these achieving HVPG of below 12mmHg, thereby reducing their risk of ascites, varices and encephalopathy[208]. Lens et al. extended their study to 96 weeks post-SVR and found continued reductions in HVPG, with 47% of patients now demonstrably clear of CSPH, with fewer patients at risk of variceal bleeds (78% to 36%) or mortality (41% to 15%)[380]. Patients with the highest HVPG and a history of decompensation at baseline were likely to have persistent CSPH. Although there was an overall reduction in TE baseline, this did not correlate with changes in HVPG or to be predictive for resolution of CSPH, however, a TE≥21 kPa was shown to have a high positive predictive value to rule-in CSPH following SVR. Mandorfer et al. agreed with a reduction in HVPG and that absolute values for TE did not correspond to changes in HVPG though they contrarily found a reduction of HVPG of ≥10% was protective against decompensation and the use of follow up TE sequentially can be used to rule in (25.3 kPa; specificity, 94.4%) or rule out (12.4 kPa; sensitivity, 97.9%) CSPH with these values being validated in a further 276 patients [381, 382].

Regarding alterations in serum markers, MELD is a measure of 90-day survival and has been shown to correlate with ICG excretion in differentiating grades of liver disease and dysfunction, particularly in advanced disease, though MELD is superior in predicting patient survival [148, 161, 375]. Though we showed an improvement in liver function, we did not find a change to MELD scores. This is slightly different from others, including Laursen et al., who found a half point improvement in MELD, which was significant [376]. Verna et al. report a decrease in MELD for 56% of patients in the short term, however, this was only clinically significant in 24% of patients and mainly attributable to those with a MELD ≥16 and did not change when re-evaluated at 4 years [383]. Our cohort has a median MELD of 7 and therefore, the minimal changes in this small cohort is not surprising though broadly in line with most other studies.

We have shown a statistically significant improvement in APRI score mainly explained directly following treatment, with this improvement also being shown by a study investigating 388 patients with a decrease from 1.19 to 0.52 twelve weeks after treatment completion[384]. They also found similar improvements to ALT, which several others reported as an early improving marker [376, 385, 386]. Interestingly, the improvement we observed with albumin has been agreed upon by others, with the ranges stated usually being within the normal ranges [365, 376, 387]. Another marker of liver function is the ALBI score which has recently been used to show improved liver function for patients achieving SVR regardless of whether this was via DAAs or interferon [388].

Our secondary objective was to determine whether pre-treatment TE and ICG testing allowed stratification of patients into those likely to recover liver function following treatment for chronic HCV and those that will have a worsening or static liver function and would require

202

assessment for liver transplantation. ICG excretion has been incorporated with MELD by Zipprich et al., using the venepuncture method, and showed an improved prognostic discernibility for those with a MELD score between 10-30 points, and the AUC improved from 0.65 to 0.69 [163]. They did note, a non-invasive measurement for ICG excretion would improve the applicability of their findings today. Similar approaches to improve MELD scoring or CTP score with functional liver tests have also been described for other functional liver tests such as galactose elimination and MEGX [389, 390]. Within our cohort, we did not have any patients that died or went on to require liver transplantation, therefore, we used a surrogate marker for ICGR15 improvement to below 14%, which would rule out clinically significant portal hypertension and thus selected the cut-off value of 20.9%[165]. As this is above the cut-off for SPH, the hope is that these patients would significantly improve their clinical condition; this is supported by the long-term trials as described above. The value of the equation to clinical staff is invaluable, in not only being able to predict patient improvement and thus plan services around this, but also in giving patients a tangible value, which is realistic, and hopefully, would aid in motivating them to complete the treatment course. We had hypothesised that the use of TE at baseline would also allow us to refine this model further, but with few patients with severe readings and no patients progressing to require transplantation, we felt that the data should not be overextended.

Using our cut-off value, we see several variables which, at baseline, may be able to aid prediction for patients who are at risk of deteriorating, several of which have been highlighted by others, including albumin and bilirubin. The use of albumin has recently gained increased interest for decompensated cirrhosis and utilising its further breakdown components, which goes beyond the scope of our population [391, 392]. Of the scoring systems, neither APRI nor MELD indicated improvement though ALBI grade was.

Interestingly, baseline stiffness was significant in this univariate analysis and given a larger sample size, it may indicate that it may be used with ICG at baseline to indicate prognosis.

3.2.4.3   <u>Limitations</u>

We performed a prospective observational cohort study to investigate changes in liver function and liver stiffness. We did not include a control group as it was unethical to withhold treatment for patients in the context of this study and as ICG is not measured routinely, there was no historical data available. Observational studies have inherent limitations within their design, which we tried to mitigate. Firstly, we tried to be as inclusive as possible with our inclusion criteria to allow as many patients as possible to be assessed within this real-world study. However, this does lead to the possibility of confounding as although baseline assessments occurred for alcohol excess, diabetes mellitus, metformin use, HIV co-infection and previous hepatic disease were noted these were not re-assessed during follow-up and therefore, may confound recovery of liver function. However, as this is a real-world study, it was felt that excluding these patients would not allow the maximum transferability of the results and it was thought unlikely that significant lifestyle changes would occur following therapy.

The multidisciplinary team specified treatment type and all patients undergoing treatment were considered; this inherently may have led to some differences in the outcome as changes in liver function were not considered in the administration of prescriptions and therefore may add to confounding. Despite our best efforts fewer than 50% of the eligible patients enrolled in the study raising questions about the characteristics of the cohort. However, those patients who withdrew did so because of practical concerns rather than because of any particular liver

disease and we therefore believe that our results are likely to apply to the general population although further confirmatory studies are required. Additionally, following the commencement of the study, patients who did not achieve SVR were given treatment extensions to between 16 and 24 weeks, which required an amendment to the original ethics application. Any factors that may have caused the single patient not to achieve SVR may not have been adequately mitigated here.

Certain selection biases may remain, though specific measures were put into place to mitigate these. However, biases such as non-participation bias undoubtedly remain. Patients with compensated liver cirrhosis were included at study entry, with previous decompensating events noted but not a barrier to entry. Those with ongoing decompensation were not included as the study aimed to identify factors associated with future liver deterioration and individuals with ongoing liver failure were likely to undergo either transplantation or serious sequelae of infection. The criteria used to determine cirrhosis matched those used in the NHS England HCV early access scheme. It should be noted that the cut-off used for TE score by NHSE was 11.5kPa and is slightly lower than other guidelines, which use 12.5-13kPa to define cirrhosis.

The study was carried out prospectively, logged in real-time, and designed to keep data collection as objective as possible throughout to avoid reporting and recall bias. Patients were seen throughout the day and on different days to avoid systematic bias.

Treatment for patients with compensated cirrhosis due to HCV was fast-moving and meant the most unwell patients were treated early, possibly with some overlap with decompensated patients. This was a trial of adjunctive measurements, thereby getting patients treated took

priority and delays in attaining ethical and site approval may have meant that those patients with the most severe disease were missed and the potential pool of patients who would have been eligible to be studied reduced. The potential effects of this were that we may have missed any patients who had a decline in liver function and possibly required transplantation or died; however, 107 patients were approached overall. Several patients did not complete their follow up due to various reasons, including worsening health, recurrence of malignancy and several who were not contactable. It is a regular occurrence for our local population to take extended holidays during the summer months, which added to missed appointments. Unfortunately, the patients with the worst ICG 15, though showing an improvement at their first follow-up, were unable to attend their final appointment and we were thus unable to verify whether this continued. This means that important information is missing and we are unable to state whether patients with poor baselines have not reached a level of overall improvement. These factors in accumulation, mean a small dataset was available with no patients having a hard outcome (transplantation/death) with which to be stratified against. We thus, utilised various statistical methods, including mixed modelling, to compensate for this where possible, however, a robust predictive model could not be produced due to this lack of power and so was not attempted to avoid misleading results.

Finally, regarding the generalisability of this cohort, we had a split of mostly genotype 1 and 3 patients with only three patients with other genotypes. This lack of representation may mean further similar investigations should occur in areas where these genotypes are more prevalent. Also, since the completion of this study, Glibenclavir/paritaprevir has become one of the standard pan-genotypic treatment options, but we did not have any patients on this treatment regimen.

3.2.4.4 Generalisability

We have performed a prospective cohort study aimed at identifying changes in liver function and stiffness following treatment for hepatitis C virus, with direct-acting antiviral therapy, for patients with compensated cirrhosis. We have utilised dynamic liver tests in the form of green, which is excreted solely by the liver, to observe an increase in the rate of expulsion from the body following treatment. We have also shown a decrease in liver stiffness over this period with associated improvements in blood tests. Taken together with prior knowledge and understanding, this is likely due to an initial decrease in liver inflammation following SVR, which then allows an improvement in perfusion and hepatic activity followed by a slower, but important regression of fibrosis which allows for a continued improvement in these markers. It is likely that utilising post-treatment TE alone to declare patients as not cirrhotic is unwise until further information is gathered, however, we can say there is an improvement in liver function following DAA treatment, which is encouraging for patients and clinicians alike. Though these improvements had been indicated previously, this is the first study to show an improvement in actual liver function using ICG following treatment with DAAs.

We have found a value at which patients are likely to improve their liver function to an acceptable degree. This will allow clinicians to translate the findings of this study into their daily practice by predicting which patients will improve their liver function prior to treatment and would allow more individualised patient care and engagement as shown following the HepCATT study [393]. This can also be used to forecast service requirements for patients following treatment, including those which will require extra support regarding liver failure and the possibility for liver transplantation. Several reports show liver transplant waiting lists being reduced following patients achieving SVR and thus liver function following DAA treatment [394-396], which our findings support. The improvement in the liver function we

have shown has already been shown by fewer patients with compensated cirrhosis having new decompensatory events [397, 398], thereby decreasing medication requirements and hospital admissions into the future [399-401] as well as a reduction in mortality [398, 402, 403]. The final question will be regarding ongoing surveillance for HCC following SVR and whether patients with cirrhosis may need lifelong surveillance as it has been shown that epigenetic changes associated with HCC persist post-SVR [404], this is under intense scrutiny currently. It would be interesting to review whether those patients who improve their ICGR15 would have a lower risk for HCC and thereby not require ongoing HCC surveillance and whether the cut-off value we have suggested holds true in this context.

3.2.4.5 Conclusion

We have shown that patients with compensated cirrhosis due to the hepatitis C virus will improve their liver function and stiffness following direct-acting antivirals treatment. We have also shown an improvement for those with an ICGR15 of below 20.9% to improve to below the level for clinically significant portal hypertension.

3.3 What is the distribution of transient elastography results in patients deemed at risk of Sickle cell liver disease

3.3.1 Chapter summary

This chapter will present the results of an audit, following the introduction of transient elastography (TE) measurements for patients suspected of having adult-onset sickle cell liver disease (SCLD), within a tertiary referral centre. We then present our work to ascertain if there are any lab investigations which may indicate which patients should have further assessment for liver disease, such as liver biopsy or more active follow up.

3.3.2 Aim

To determine the prevalence of sickle cell liver disease in terms of both severe fibrosis and cirrhosis in a single tertiary centre and to identify factors indicating a deterioration in liver health. An important secondary aim was to determine the need for a Fibroscan®/liver service embedded within the haematology department.

3.3.3   Results

3.3.3.1   Baseline Demographics

A total of 324 patients attended the haematology clinic and blood tests were reviewed for abnormalities indicating a need for liver review; 135 patients were highlighted and 76 patients had completed their hepatology assessment (including TE) between 1/7/17 and 1/8/18 and data on this cohort will be presented here.

Table 3.16 summarises the baseline characteristics. The median age of this cohort was 29.5 years. Of these, 47% were male and 97% had HbSS genotype. Most patients had transfusions either as exchange (53%) or simple transfusions (10%) and 13% with hydroxyurea. Median ferritin of 1019μg/L and a median liver iron concentration (LIC) of 7.7 mg/g for the 38 patients where reading was available both of which are elevated, with the recommended level for starting chelation therapy being 3.3mg/g.

In keeping with SCD, the median haemoglobin concentration was low at 75 g/dl despite treatment. Median liver function tests were at the upper end of the normal range for alkaline phosphatase (130IU/L), alanine transaminase (40.5units/L) and albumin (41g/L).

Table 3.16: Baseline characteristics for all patients

Characteristic	n=76
Median age, yrs., (IQR)	29.5 (21.25-42.75)
Male, n, (%)	36 (47)
Genotype	
HbSS, n, (%)	74 (97)
HbSb-thalassaemia, n, (%)	2 (3)
Previous cerebrovascular event, n, (%)	14 (18.4)
Treatment	
Exchange Transfusion, n, (%)	40 (53)
Simple transfusion, n, (%)	8 (10)
Hydroxyurea, n, (%)	10 (13)
No treatment, n, (%)	18 (24)
Chelation therapy, n, (%)	17 (22)
Cholecystectomy, n, (%)	33 (43)
Median Hb, g/dl, (IQR)	75 (67.25-87)
Median Platelets, x10^9/L, (IQR)	238.5 (197-300.8)
Median MCV, fL, (IQR)	83.6 (77.8-86.6)
Median Ferritin, µg/L, (IQR)	1019 (216-2597)
Median HbF, %, (IQR)	2.6 (1.6-5.7)
Median HbS, %, (IQR)	55 (34.6-72.9)
Median Sodium, mmol/L, (IQR)	137 (134-138)
Median Potassium, mmol/L, (IQR)	4.2 (3.9-4.6)
Median Chloride, mmol/L, (IQR)	99 (97-101)
Median Urea, mmol/L, (IQR)	2.3 (1.9-3.6)
Median Creatinine, µmol/L, (IQR)	51 (39-68)
Median Bilirubin, µmol/L, (IQR)	70 (45-124)
Median ALP IU/L, (IQR)	130 (85.5-189.5)
Median ALT, units/L, (IQR)	40.5 (24.3-62.3)
Median albumin, g/L, (IQR)	41 (36-44)
Ferriscans® performed, n, (%)	38 (50)
Median Liver iron concentration, mg/g, (IQR)	7.7 (3.8-17.9)

HbSS: Homozygous for the sickle mutation, Hb: Haemoglobin, MCV: Mean corpuscular volume, HbF: Foetal haemoglobin, HbS: Sickle Haemoglobin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase concentration, n: Number, yrs: Years, %: Percentage, IQR: Inter-quartile range, dl: Decilitre, L: Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, µg: Micrograms, mmol: Millimole, µmol: Micromole, U: Units, IU: International units.

The distribution of transient elastographies overall shows 36 patients (47%) to have no fibrosis (<5.6kpa), 12 (16%) having mild fibrosis (5.6-7.1kPa), 21 (28%) with severe fibrosis (7.2-11.9kPa) and 7 (9%) having cirrhosis (see Figure 3.40).

As all 135 patients were offered a hepatology assessment and only 76 patients attended, this is a subset of the overall cohort. We consider this a random sampling and therefore, we assume the prevalence of SCLD found within those who attended for a TE reading to be the same within the total population 135 which is 37% (95% C.I: 26%-49%). However, it is possible that the subset of patients who attended are a selected cohort at higher risk of liver disease and if we assume that only those with liver disease attended for assessment the overall prevalence falls to 15.4% (95% C.I: 10.8%-18.8%). We speculate that the true prevalence lies between these extremes but is more likely to be the higher prevalence estimate.
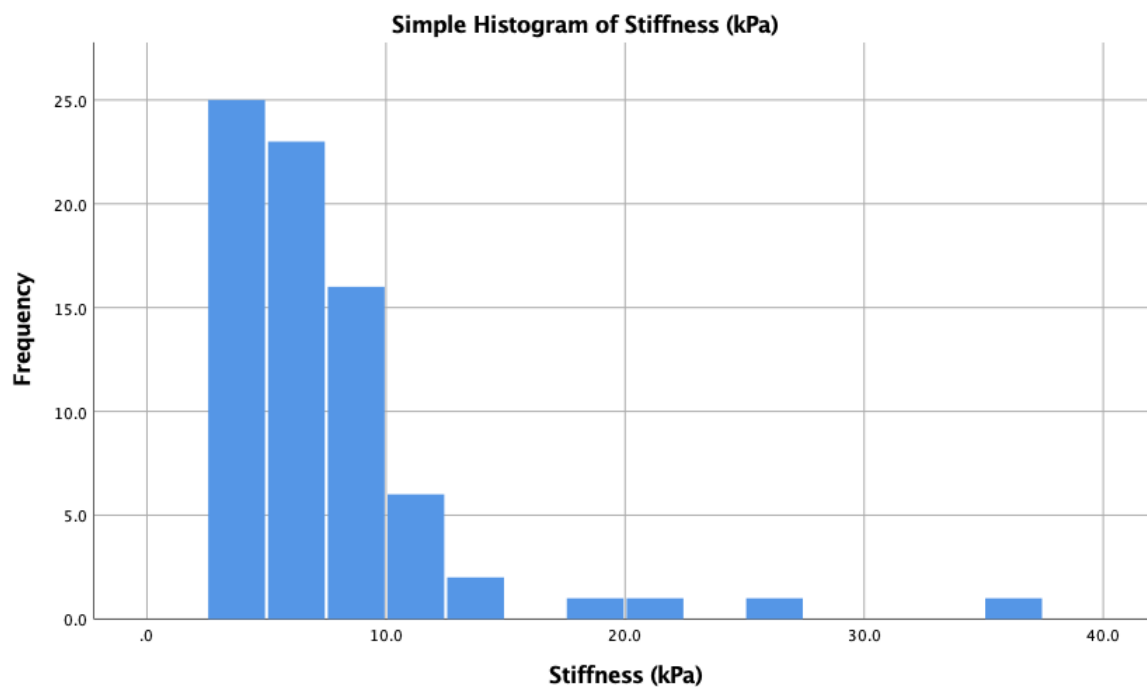
Figure 3.40: Distribution of transient elastography scores for all patients

3.3.3.2 Non-severe fibrosis vs Severe fibrosis

We investigated the various factors and whether they indicated a high TE reading. We assumed a TE of below 7.2kPa excludes severe fibrosis and thus this was used as a cut-off. Below is the distribution of stiffness scores as well as a breakdown of baseline characteristics according to this division (Figure 3.41 & Table 3.17).
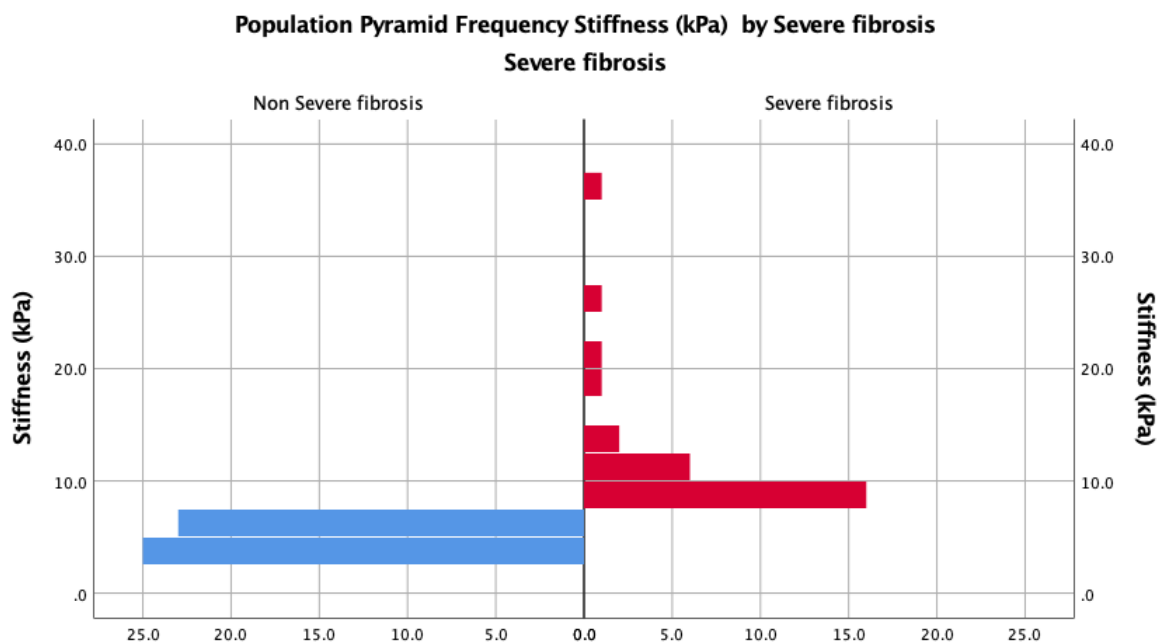
Figure 3.41: Histogram showing the distribution of transient elastography when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

Table 3.17: Patient characteristics when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa inclusive of patients with cirrhosis)

	Non-severe n=48	Severe n=28	p-value
Median Stiffness, kPa, (IQR)	4.9 (4.4-5.6)	9.55 (8.5-12.1)	
Median age, yrs., (IQR)	27.5 (21-40.3)	32.5 (22-43.8)	0.321
Male, n, (%)	21 (44)	15 (54)	0.408
Genotype			
HbSS, n, (%)	46 (96)	28 (100)	0.53
Treatment			
Exchange Transfusion, n, (%)	24 (50)	16 (57)	
Simple transfusion, n, (%)	5 (10)	3 (11)	
Hydroxyurea, n, (%)	7 (15)	3 (11)	
No treatment, n, (%)	12 (25)	6 (21)	
Average transfusions, n/year	7	7	0.982
Chelation therapy, n, (%)	13 (37)	4 (17)	0.259
Cholecystectomy, n, (%)	21 (44)	12 (43)	0.940
Cerebrovascular event, n, (%)	9 (18.8)	5 (17.9)	0.923
Median Hb, g/dl, (IQR)	81.5 (70.3-88.8)	72 (61-83.3)	*0.027
Median Platelets, x109/L, (IQR)	252.5 (194.8-308.5)	223.5 (198-277)	0.352
Median MCV, fL, (IQR)	82.15 (77.4-85.9)	84.25 (80.1-87)	0.173
Median Ferritin, µg/L, (IQR)	712 (216-2249)	1246 (197.8-3784)	0.399
Median HbF, %, (IQR)	2.6 (1.6-5.2)	2.65 (1.6-6.1)	0.881
Median HbS, %, (IQR)	51.7 (39.9-73.2)	58.25 (28.6-73.2)	0.982
Median Sodium, mmol/L, (IQR)	137 (134-138)	136.5 (134.3-138)	0.890
Median Potassium, mmol/L, (IQR)	4.2 (3.8-4.6)	4.25 (4.1-4.7)	0.254
Median Chloride, mmol/L, (IQR)	99 (97-101)	99 (96.3-101)	0.847
Median Urea, mmol/L, (IQR)	2.3 (1.8-4.2)	2.5 (2-3.4)	0.865
Median Creatinine, µmol/L ,(IQR)	51 (37-70)	50.5 (40.3-66.8)	0.869
Median Bilirubin, µmol/L ,(IQR)	68 (44-115)	87 (45.3-146)	0.335
Median ALP, IU/L, (IQR)	101.5 (80-154)	171.5 (111.5-210)	*0.009
Median ALT, units/L, (IQR)	38.5 (21.5-59.8)	42.5 (29.3-71.8)	0.349
Median albumin, g/L, (IQR)	42 (36.3-45)	40 (34-42.8)	0.194
Ferriscans® performed, n, (%)	24 (50)	14 (50)	1
Median Liver iron concentration, mg/g, (IQR)	7.15 (3.9-17.4)	7.7 (2.9-27.6)	0.846
Median Liver iron concentration, mmol/kg, (IQR)	128.5 (70.3-312)	137 (52.5-493)	0.870

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as p<0.05, *denotes significant results. HbSS: Homozygous for the sickle mutation, Hb: Haemoglobin, MCV: Mean corpuscular volume, HbF: Foetal haemoglobin, HbS: Sickle Haemoglobin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase concentration, n: Number, yrs: Years, %: Percentage, IQR: Inter-quartile

range, dl: Decilitre, L: Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, µg: Micrograms, mmol: Millimole, µmol: Micromole, U: Units, IU: International units.

Four patients were diagnosed clinically as SCLD. There were no patients identified as SCLD clinically that were not identified by TE.

To examine the associations which may contribute to fibrosis, in more detail, we examined individual factors in relation to TE.

3.3.3.2.1 <u>Haemoglobin concentration</u>

To determine whether severity of anaemia was associated with increased fibrosis we compared haemoglobin with elastography.
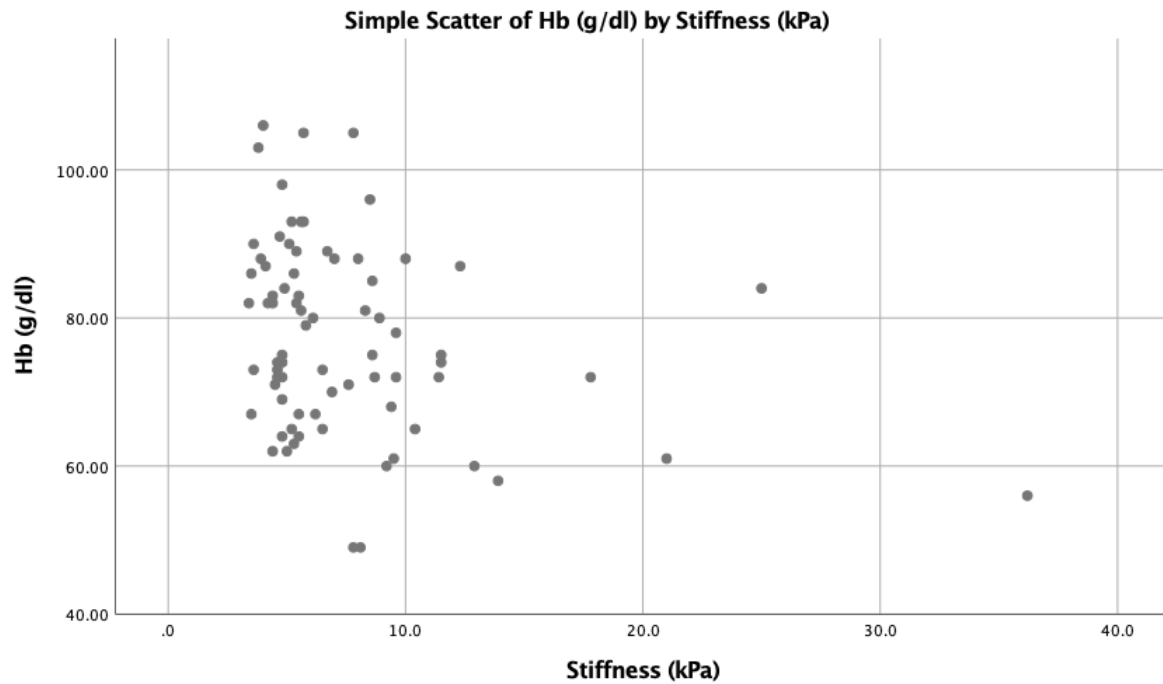
Figure 3.42: Scattergram showing haemoglobin concentration against liver stiffness. Spearman's rho test, r=-0.277, p=0.015
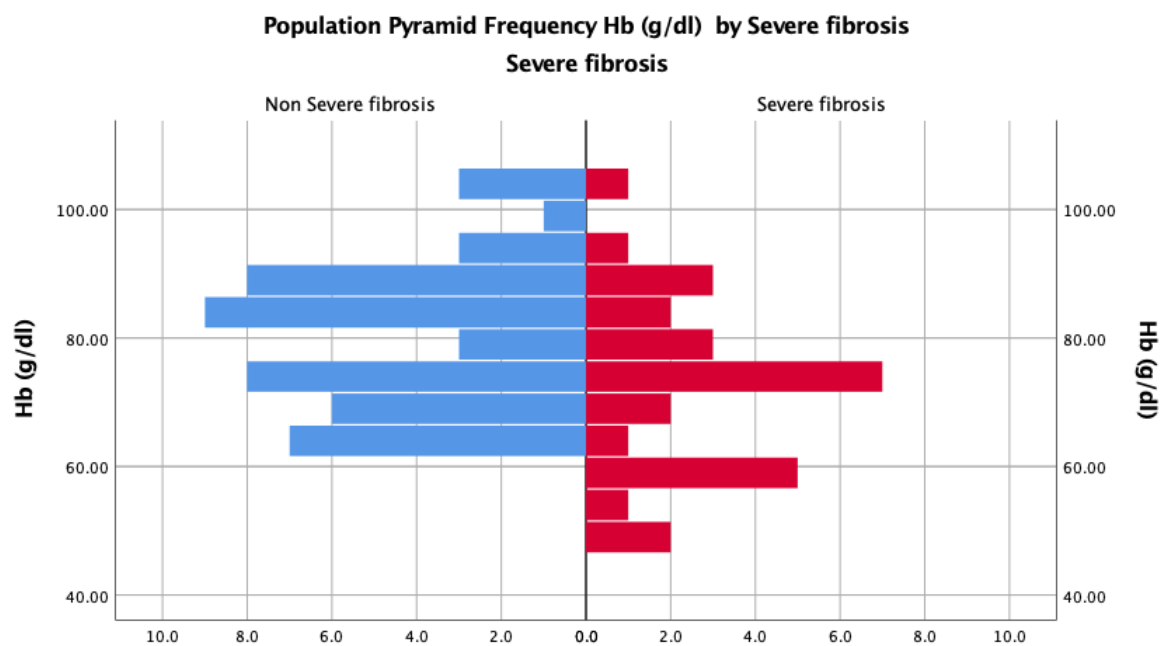
Figure 3.43: Histogram showing distribution of haemoglobin concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was a statistically significant, weak negative correlation between an increasing liver stiffness and haemoglobin (Figure 3.42). Haemoglobin concentration for patients without

216

fibrosis (mean rank = 42.77) were significantly higher than for those with severe fibrosis

(mean rank =31.18), p=0.027 (Figure 3.43).

We then investigated whether haemoglobin type was associated with an increased liver

stiffness and found this not to be the case (appendix 4.2)

3.3.3.2.2 ALP

Attenuated excretion of bile through the biliary system secondary either to mechanical causes

(e.g. gallstones) or inflammation and/or fibrosis can cause the stasis of bile acids and thus

damage to cholangiocytes. This can be measured using serum alkaline phosphatase.
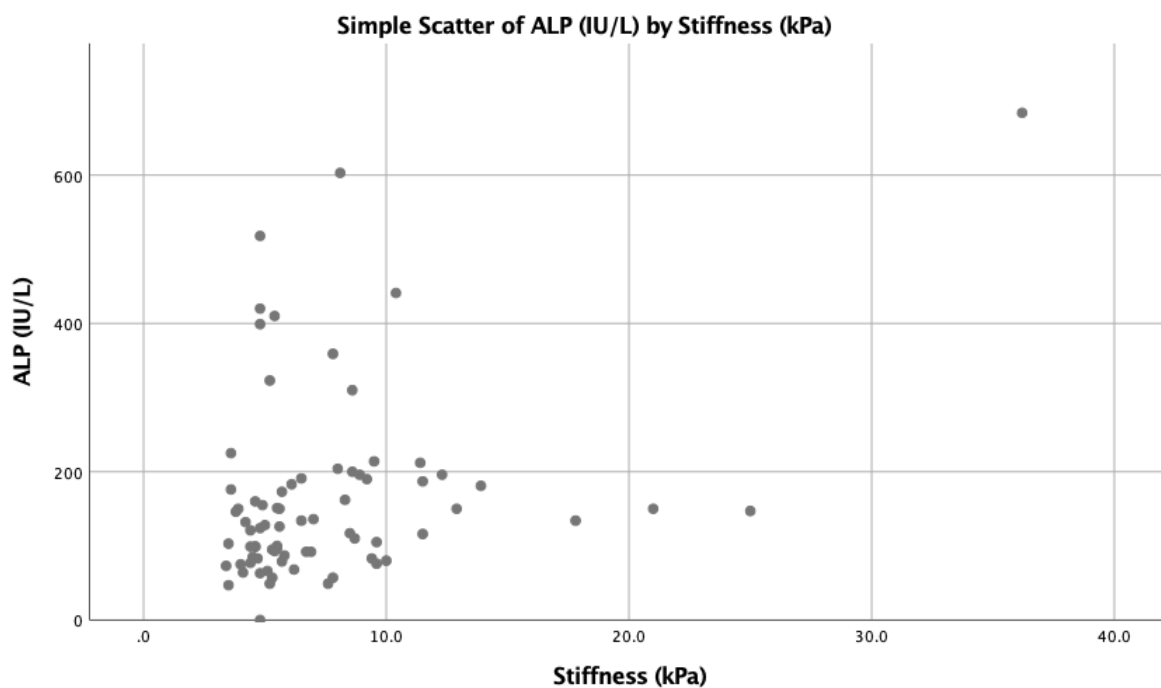
Figure 3.44: Scattergram showing serum alkaline phosphatase concentration against liver stiffness. Spearman's rho test, r=0.302, p=0.008
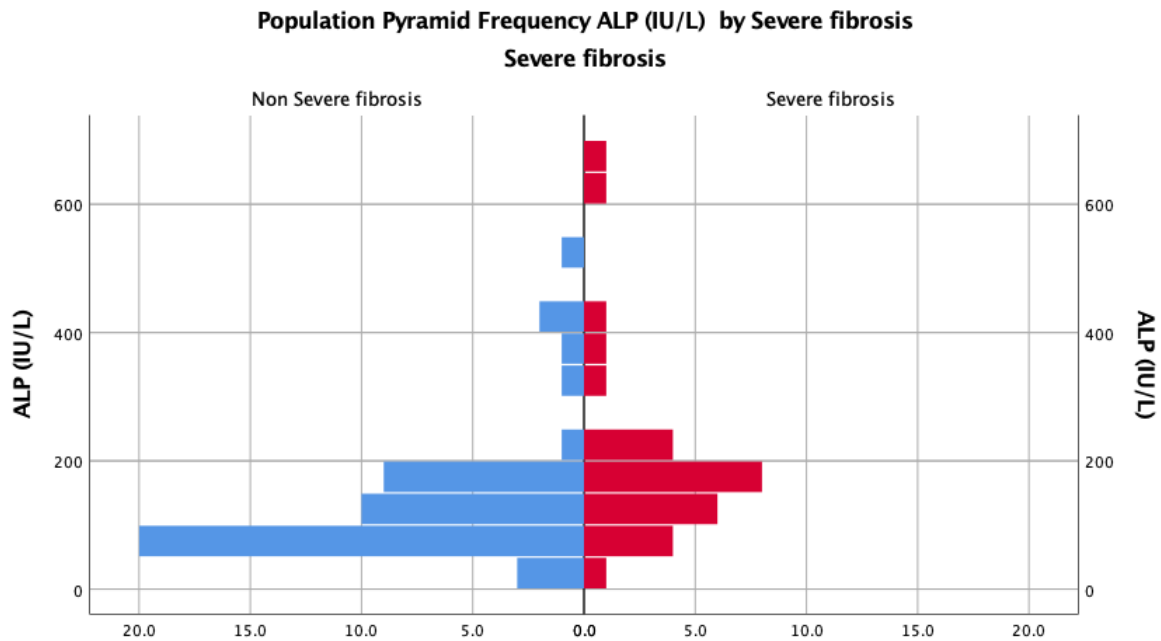
Figure 3.45: Histogram showing the distribution of serum alkaline phosphatase concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was a statistically significant, weak positive correlation between an increasing liver stiffness and serum alkaline phosphatase concentration (Figure 3.44) and median alkaline phosphatase was significantly lower in non-severe fibrosis (101.5 IU/L) than in severe fibrosis (171.5 IU/L), p=0.009 (Figure 3.45). Analysis was also performed with exclusion of the outlier, with the results remaining significant.

We investigated whether other static liver function tests (ALT and bilirubin) indicated patients at risk of severe fibrosis and did not find any relationship (appendix 4.3)

3.3.3.2.3 Albumin

Synthesis of several proteins occurs within hepatocytes, serum albumin may be used to monitor this function, as, with increasing damage, serum albumin may fall, though this may also be affected by poor nutrition.
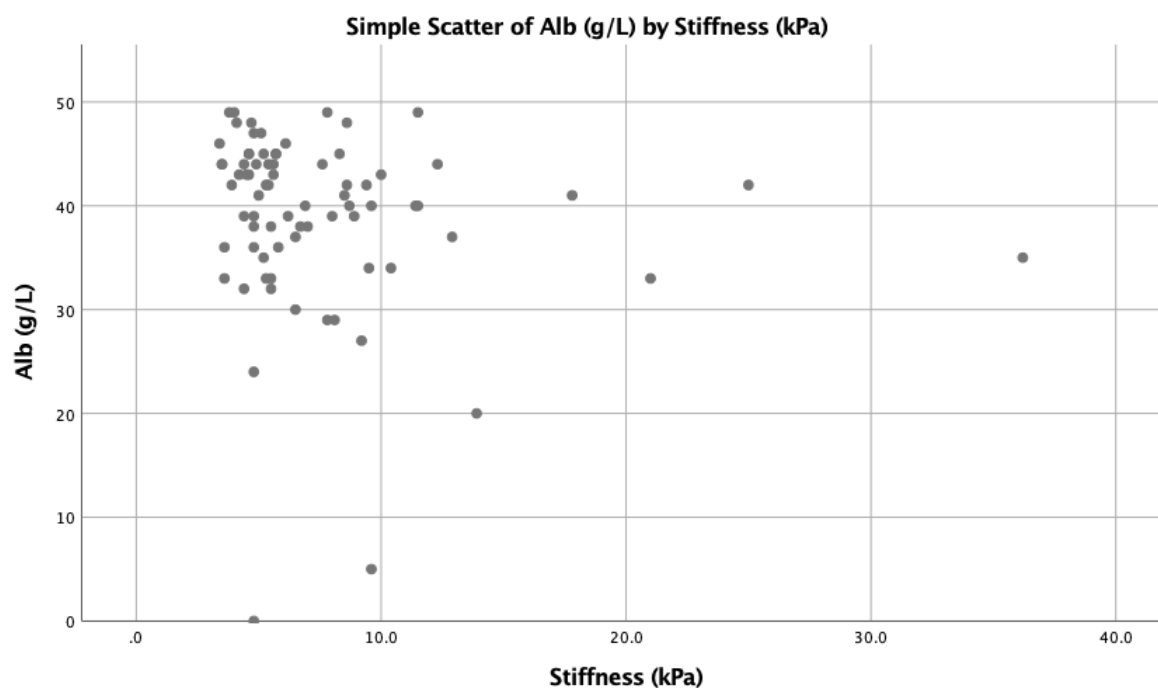
Figure 3.46: Scattergram showing serum albumin concentration against liver stiffness. Spearman's rho test, r =-0.258, p=0.024
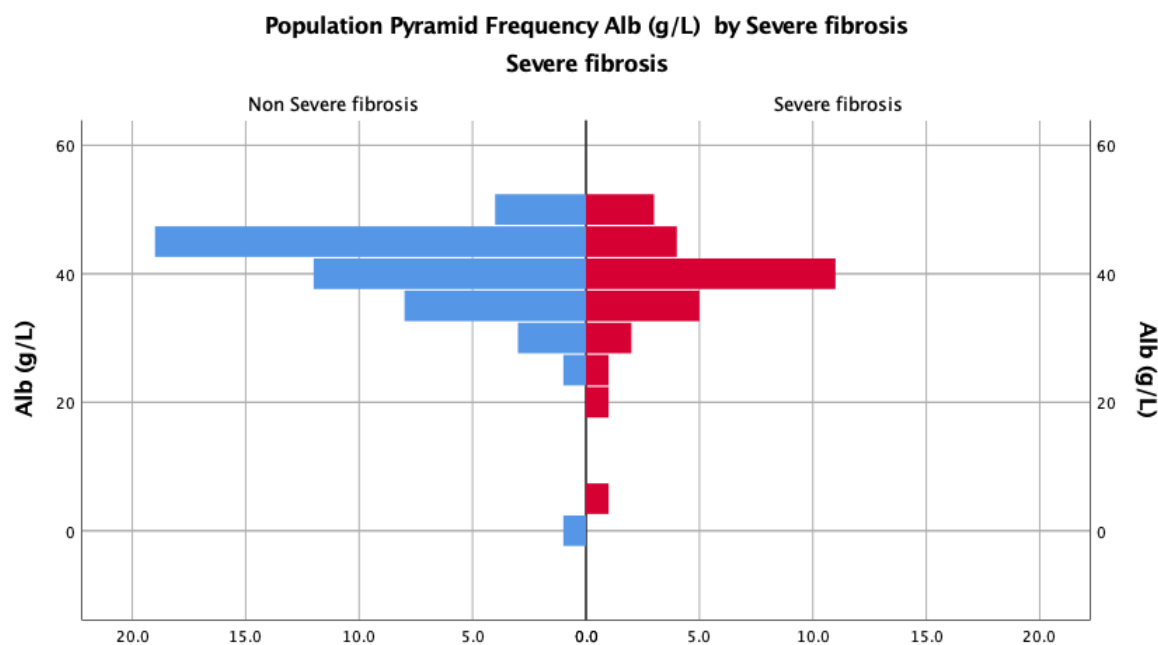
Figure 3.47: Histogram showing the distribution of serum albumin concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was a statistically significant, weak negative correlation between an increasing liver stiffness and serum albumin concentration (Figure 3.46) but median albumin concentration was not statistically significantly different between non-severe (42g/L) and severe fibrosis (40g/L), p=0.194 (Figure 3.47).

3.3.3.2.4 Liver iron concentration

Patients receiving blood transfusions have an increased concentration of iron. This is stored within the liver and has been intimated as a cause of hepatocyte damage and may compromise estimation of liver damage by imaging studies such as TE. The median LIC for this cohort was 7.7 mg/g for the 38 patients where a reading was available, with the recommended level for starting chelation therapy being 3.3mg/g.
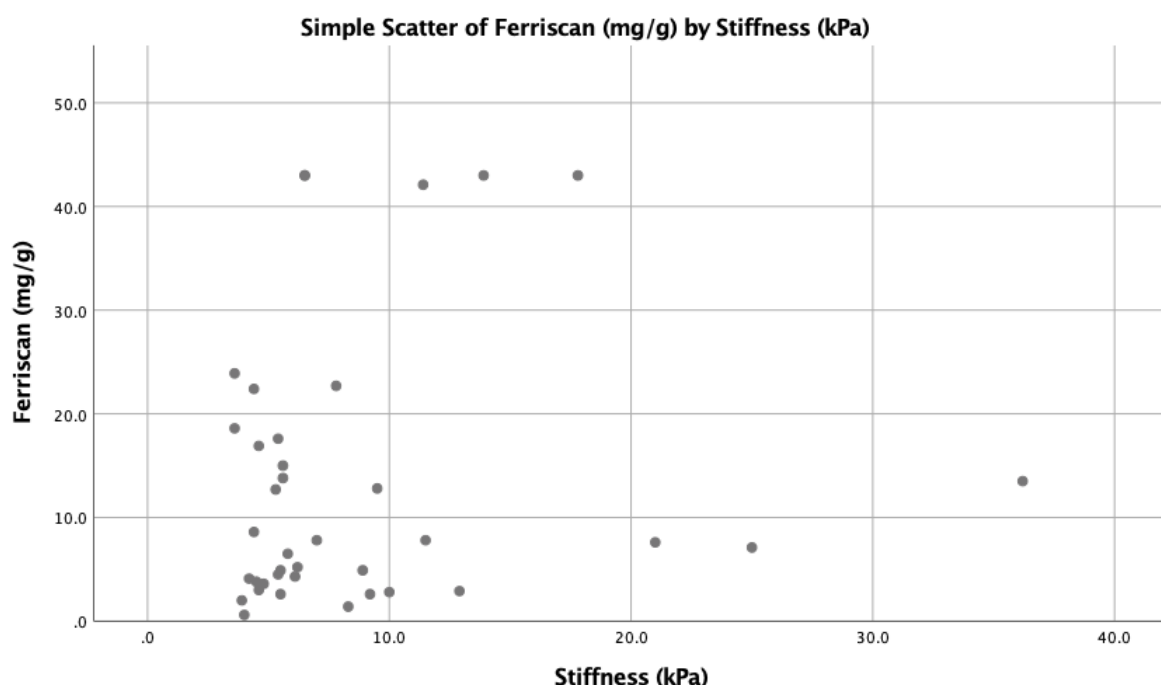
Figure 3.48: Scattergram showing liver iron concentration against liver stiffness. Spearman's rho test, r=0.149, p=0.371

Population Pyramid Frequency Ferriscan (mg/g) by Severe fibrosis
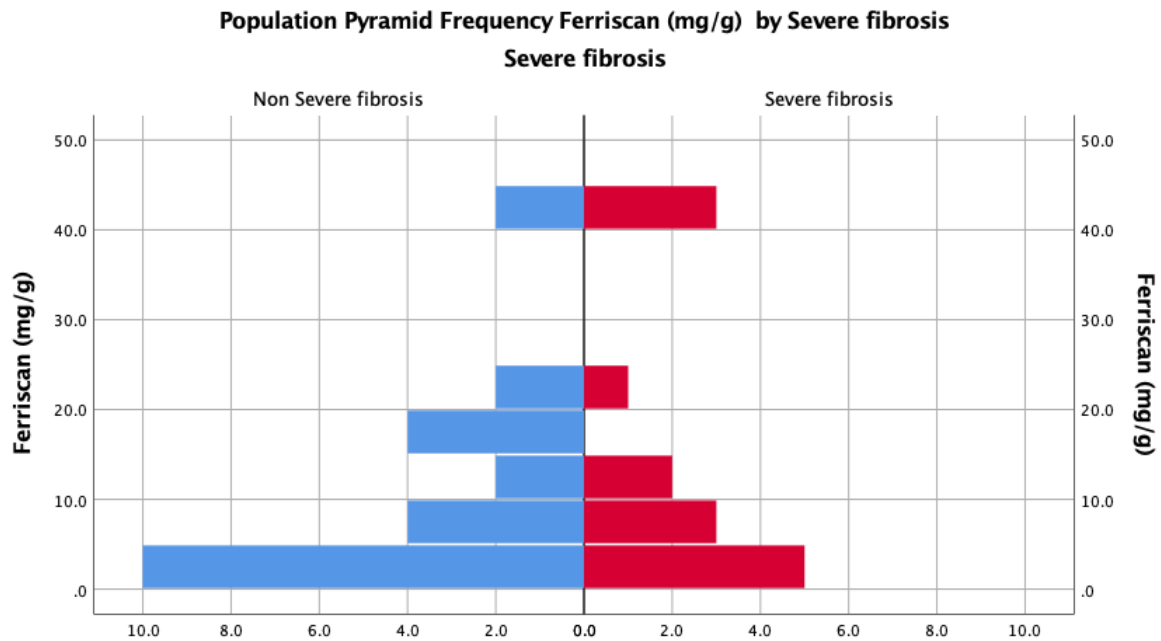
Figure 3.49: Histogram showing the distribution of liver iron concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was no correlation between an increasing liver stiffness and liver iron concentration (Figure 3.48) and median liver iron concentration was not statistically significantly different between people with non-severe (7.15mg/g) and severe fibrosis (7.7mg/g), p=0.844 (Figure 3.49). In addition, other surrogated of total body iron, namely serum ferritin, transfusion frequency and chelation therapy did not signify an elevated liver stiffness (appendix 4.4).

3.3.3.2.5 US changes

Ultrasound scanning is the routine test used to screen patients for the presence of fibrosis. Findings of coarsened echotexture, irregular margins, nodularity, hepatomegaly or cirrhosis were deemed indicative of fibrosis on the ultrasound report. We investigated whether the level of fibrosis found by TE correlated with US scans and found no association (p=0.777, OR: 1.18, 95% CI: 0.371-3.76) (see Figure 3.50). One patient showed nodularity on US scanning with a normal TE (4.9kPa) but had slight derangement of serum liver function tests,

otherwise there were no patients with ultrasound abnormalities that were not associated with a raised TE score. Splenomegaly was noted for four patients, with one having a raised TE (5.6kPa) and hepatomegaly, otherwise normal TE was noted.
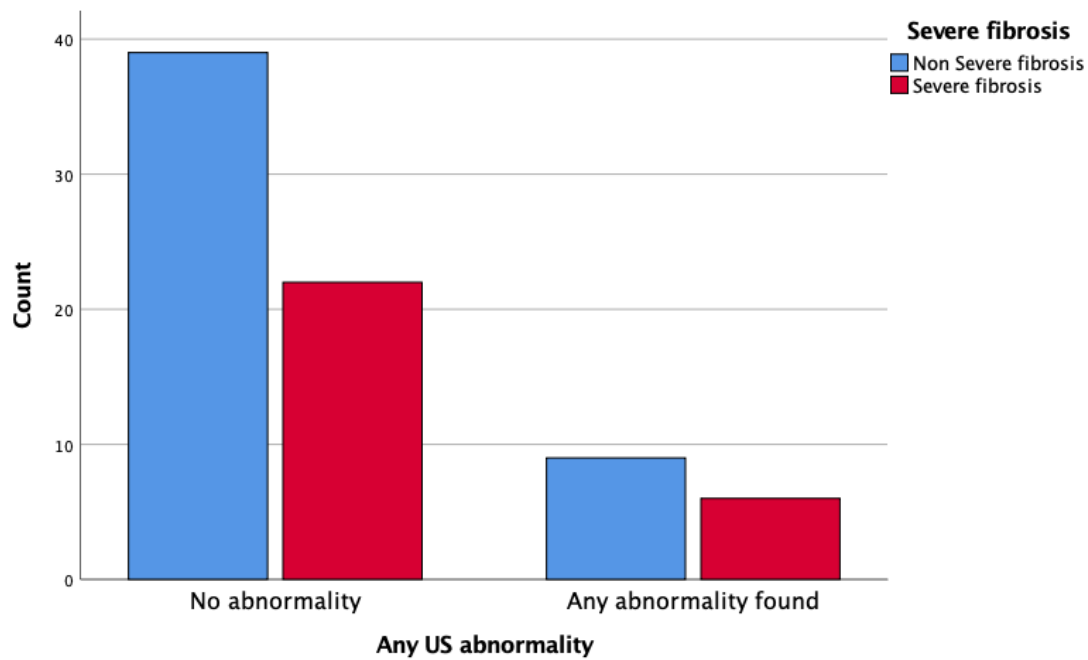
Figure 3.50: A clustered bar chart displaying the number of patients with any abnormality found on ultrasound scan according to degree of fibrosis. Chi square test, p=0.777, OR: 1.18, 95% CI: 0.371-3.76

3.3.3.3 Cirrhosis sub group

Recognition of severe fibrosis allows early intervention that may arrest progression to cirrhosis. However, identifying those patients that have already crossed into cirrhosis (kPa>12) is imperative to clinical management.

Table 3.18 presents the baseline characteristics. In brief, seven patients were found to have cirrhosis (12.3–36.2kPa). The overall prevalence of patients with cirrhosis within all the patients with sickle cell disease is 3.8% (95% C.I = 1.57%-7.52%). They had a median age of 24 years old. Most patients were receiving exchange transfusions (57.1%). Higher median ferritin, bilirubin and ALP was demonstrated for people with cirrhosis, however, other liver function tests were similar between the groups. All patients with cirrhosis had Ferriscans® but only 46.4% of those without cirrhosis underwent this procedure. Two patients were diagnosed clinically as having cirrhosis, there were no patients with cirrhosis that were not detected by TE.

Table 3.18: Patient characteristics when divided by absence (TE <11.9kPa) or presence of cirrhosis (TE>12.0kPa)

	Non-Cirrhotic (n=69)	Cirrhotic (n=7)
Stiffness, kPa, (IQR)	5.5 (4.7-8)	17.8 (12.9-25)
Median age, yrs., (IQR)	30 (21.5-43)	24 (21-38)
Male, n, (%)	31 (44.9)	5 (71.4)
Genotype		
HbSS, n, (%)	67 (97)	7 (100)
Treatment		
Exchange Transfusion, n, (%)	36 (52.2)	4 (57.1)
Simple transfusion, n, (%)	6 (8.7)	2 (28.6)
Hydroxyurea, n, (%)	9 (13)	1 (14.3)
No treatment, n, (%)	18 (26.1)	0
Average transfusions, n/year	6 (0-22)	10 (2-32)
Chelation therapy, n, (%)	15 (22)	2 (29)
Cholecystectomy, n, (%)	30 (43.5)	3 (42.9)
Median Hb, g/dl, (IQR)	78 (69.5-88)	61 (58-84)
Median Platelets, $x10^9$/L, (IQR)	243 (196-303)	234 (201-274)
Median MCV, fL, (IQR)	82.6 (77-86)	86.9 (85.8-92.6)
Median Ferritin, μg/L, (IQR)	711 (147-2495)	1685 (685-6151)
Median HbF, %, (IQR)	2.45 (1.6-5.3)	3.3 (2.5-13.4)
Median HbS, %, (IQR)	54.6 (34.7-74.7)	55 (29.7-71)
Median Sodium, mmol/L, (IQR)	137 (135-138)	136 (136-138)
Median Potassium, mmol/L, (IQR)	4.2 (3.9-4.6)	4.1 (3.3-4.2)
Median Chloride, mmol/L, (IQR)	99 (97-101)	98 (95-101)
Median Urea, mmol/L, (IQR)	2.3 (1.9-3.5)	2 (1-5.6)
Median Creatinine, μmol/L, (IQR)	51.5 (39-67.8)	40 (32-69)
Median Bilirubin, μmol/L, (IQR)	69 (44.3-120.2)	118 (47-242)
Median ALP, IU/L, (IQR)	121 (83-189)	150 (147-196)
Median ALT, units/L, (IQR)	40 (24-60)	48 (44-125)
Median albumin, g/L, (IQR)	41 (36-45)	37 (33-42)
Ferriscans® performed, n, (%)	32 (46.4)	7 (100)
Median Liver iron concentration, mg/g, (IQR)	7.15 (3.7-17.4)	10.55 (6.05-43)
Median Liver iron concentration, mmol/kg, (IQR)	128 (68-312)	188 (107.75-770)

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as $p<0.05$, *denotes significant results. HbSS: Homozygous for the sickle mutation, Hb: Haemoglobin, MCV: Mean corpuscular volume, HbF: Foetal haemoglobin, HbS: Sickle Haemoglobin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase concentration, n: Number, yrs: Years, %: Percentage, IQR: Inter-quartile

range, dl: Decilitre, L: Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, µg: Micrograms, mmol: Millimole, µmol: Micromole, U: Units, IU: International units.

3.3.3.4 Univariate analysis

The factors shown to be important for severe fibrosis were haemoglobin concentration, ALP concentration with a correlation only for lower albumins. Age, alanine aminotransferase concentration, bilirubin, serum ferritin concentration and liver iron concentrations may be important and we have further investigated them and found only anaemia identified patients with cirrhosis (p=0.044) (Table 3.19).

Table 3.19: Univariate analysis for patients with sickle cell disease when divided by absence (TE <11.9kPa) or presence of cirrhosis (TE>12.0kPa)

Characteristic	p-value
Age	0.646
Haemoglobin concentration	*0.044
Alkaline phosphatase concentration	0.074
Alanine aminotransferase concentration	0.148
Bilirubin	0.126
Serum ferritin concentration	0.074
Liver iron concentration	0.336

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as p<0.05, *denotes significant results.

3.3.3.5 <u>Multivariate analysis</u>

Factors from both univariate assessments were inserted into an ordinal scale for degree of

fibrosis to increase sensitivity and power. We find haemoglobin concentration (p=0.042) and

ALP (p=0.025) to be significant (Table 3.20 and Figure 3.51).

Table 3.20: Multivariate analysis for patients with sickle cell disease in an ordinal manner for patients with absence, severe fibrosis and cirrhosis.

Characteristic	p-value
Age	0.386
Haemoglobin concentration	*0.042
Alkaline phosphatase concentration	*0.025
Alanine aminotransferase concentration	0.328
Bilirubin	0.291
Serum ferritin concentration	0.202
Liver iron concentration	0.547

Kruskal-Wallis test applied. Significant taken as p<0.05, *denotes significant results.

a)
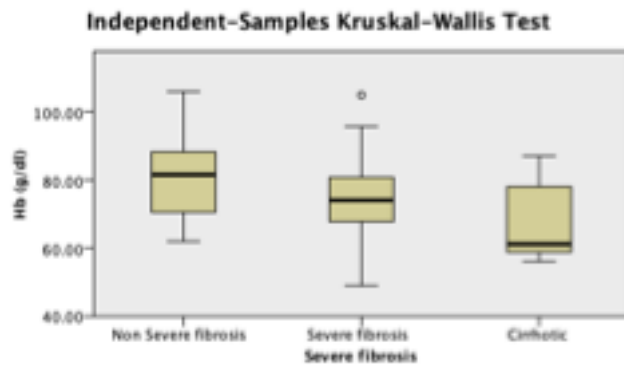
b)
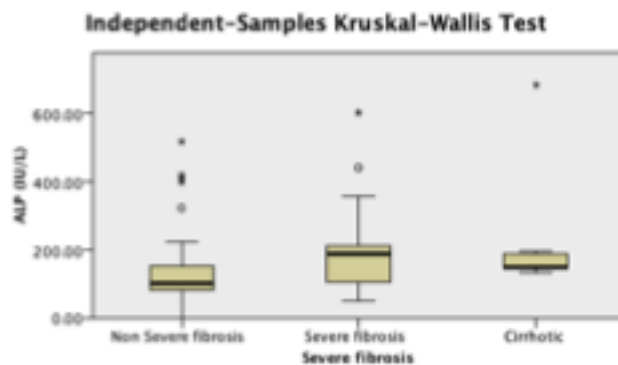
Figure 3.51: The trends for the significant variables, a) haemoglobin concentration and b) ALP, from a Kruskal-Wallis test, are displayed as box plots with medians and 95% intervals

In a multivariate model, only ALP remained significant (p=0.006) and correlated with severity of fibrosis (r=0.313, p=0.006). A ROC curve was produced (AUROC=0.706, 95% C.I: 0.57-0.84) and a clinically relevant cut-off of 148IU/L had a sensitivity of 71.4% and specificity of 68.1% (OR: 3.89 (95% C.I: 0.70-21.5) p=0.12; PPV: 15.6% (95% C.I: 9.6-24.4%), NPV: 95.5% (95% C.I: 86.5-98.6%)).

3.3.3.6 <u>Fibrosis vs cirrhosis</u>

The differentiation of when patients transition from fibrosis to cirrhosis and therefore require follow up is important and we examined this.

The baseline features are detailed in Table 3.21. There were 21 patients with severe fibrosis (TE 7.6 – 11.9kPa) and seven patients with cirrhosis (TE>12 kPa). Median bilirubin was again found to be higher in cirrhosis whilst ALP was lower, though a tighter IQR in cirrhosis.

Table 3.21: Patient characteristics when divided by absence (TE <11.9kPa) or presence of cirrhosis (TE>12.0kPa)

	Severe fibrosis (n=21)	Cirrhosis (n=7)
Stiffness, kPa, (IQR)	8.9 (8.2-9.8)	17.8 (12.9-25)
Median age, yrs., (IQR)	33 (22-44.5)	24 (21-38)
Male, n, (%)	10 (47.6)	5 (71.4)
Genotype		
HbSS, n, (%)	21 (100)	7 (100)
Treatment		
Exchange Transfusion, n, (%)	12 (57.1)	4 (57.1)
Simple transfusion, n, (%)	1 (4.7)	2 (28.6)
Hydroxyurea, n, (%)	2 (9.6)	1 (14.3)
No treatment, n, (%)	6 (28.6)	0
Average transfusions, n/year	11 (7-25.5)	10 (2-32)
Chelation therapy, n, (%)	2 (10)	2 (29)
Cholecystectomy, n, (%)	9 (42.9)	3 (42.9)
Median Hb, g/dl, (IQR)	74 (66.5-83)	61 (58-84)
Median Platelets, x10^9/L, (IQR)	216 (176-280)	234 (201-274)
Median MCV, fL, (IQR)	82.8 (77-86)	86.9 (85.8-92.6)
Median Ferritin, µg/L, (IQR)	634 (122-3198)	1685 (685-6151)
Median HbF, %, (IQR)	2.3 (1.6-5.5)	3.3 (2.5-13.4)
Median HbS, %, (IQR)	59.1 (27.9-81.6)	55 (29.7-71)
Median Sodium, mmol/L, (IQR)	137 (136-139)	136 (133-138)
Median Creatinine, µmol/L, (IQR)	53 (45-66)	40 (32-69)
Median Bilirubin, µmol/L, (IQR)	80 (43-128)	118 (47-242)
Median ALP, IU/L, (IQR)	187 (94-213)	150 (147-196)
Median ALT, units/L, (IQR)	40 (27-66)	48 (44-125)
Median albumin, g/L, (IQR)	40 (34-44)	37 (33-42)
Ferriscans$^®$ performed, n (%)	8 (38.1)	7 (100)
Median Liver iron concentration, mg/g, (IQR)	6.35 (2.65-20.23)	10.55 (6.05-43)
Median Liver iron concentration, mmol/kg, (IQR)	113 (47-362)	188 (107.75-770)

HbSS: Homozygous for the sickle mutation, Hb: Haemoglobin, MCV: Mean corpuscular volume, HbF: Foetal haemoglobin, HbS: Sickle Haemoglobin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase concentration, n: Number, yrs: Years, %: Percentage, IQR: Inter-quartile range, dl: Decilitre, L: Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, µg: Micrograms, mmol: Millimole, µmol: Micromole, U: Units, IU: International units.

There are no indicators which indicate which patients are cirrhotic as opposed to severe

fibrosis (Table 3.22)

Table 3.22: Univariate analysis for patients with sickle cell disease when divided by absence (TE <11.9kPa) or presence of cirrhosis (TE>12.0kPa)

Characteristic	p-value
Age	0.348
Haemoglobin concentration	0.249
Alkaline phosphatase concentration	0.756
Alanine aminotransferase concentration	0.155
Bilirubin	0.208
Serum ferritin concentration	0.140
Liver iron concentration	0.282

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as p<0.05, *denotes significant results.

3.3.4 <u>Discussion</u>

Liver disease in adult patients with sickle cell disease (SCD) is an under recognised condition. Early diagnosis may allow an arresting of progression and thereby an improvement in prognosis. We thus performed an audit following the introduction of TE within a tertiary centre. We aimed to determine the prevalence of sickle cell liver disease (SCLD) in terms of both severe fibrosis and cirrhosis and to identify factors indicating a deterioration in liver health. An important secondary aim was to determine the need for a Fibroscan®/liver service embedded within the haematology department.

3.3.4.1 <u>Summary of findings</u>

We found the prevalence of SCLD to be 15.4 % with 3.8% showing cirrhosis. TE did not miss any patients diagnosed as SCLD clinically and identified 24 further cases. This may be due to a higher threshold to diagnose patients with SCLD by the haematology team.

Patients with severe anaemia were at highest risk of fibrosis but interestingly, haemoglobin type or treatment did not indicate severity of fibrosis. ALP was the only serum test indicative of raised TE. It has been postulated the deposition of iron within the liver may cause an interference and thereby derangement of TE readings, we found no evidence of this. Together these findings display the inherent difficulty in diagnosis of SCLD.

3.3.4.2 <u>Interpretation</u>

Prevalence was investigated by Draser et al. in 134 consecutive patients and showed severe fibrosis for 27% with 5% classified as cirrhosis on TE [296]. Another study of 37 patients

found 19% to have severe fibrosis and 8% had cirrhosis. Post-mortem data has shown 10% of patients to have cirrhosis with no other cause attributable, although this was before the discovery of hepatitis C [278]. Our findings are in line with these.

Factors which may indicate or contribute to liver fibrosis are important. We found severe anaemia, hypoalbuminaemia and raised ALP to indicate severe fibrosis. Severe anaemia is known to indicate severe fibrosis [296, 298]. This may be a result of incomplete treatment of patient's anaemia or indicate the severity SCD. However, there is the possibility that the screening blood tests were taken prior to transfusion or increasing of Hydroxyurea dosages which may give an artificially low result. Also, older patients with SCD have a more significant anaemia than younger patients possibly due to impaired erythropoiesis secondary to kidney injury [405]. Patients with SCD who are not in crisis generally tolerate a significant anaemia and guidelines advise treatment according to symptoms rather than aiming for normal values[263]. With the current understanding of pathophysiology to include areas of ischaemic necrosis within the liver, we recommend those patients that have shown signs of liver injury should be managed at a higher haemoglobin concentration.

Patients requiring aggressive treatment modalities, as well as those not completely responsive to their current treatment, are likely to have increased fibrosis. A study found those on exchange blood transfusion regimes showed an increased liver stiffness as opposed to transfusion, which was followed by Hydroxyurea. They did not find any differences in fibrosis for patients who were not initially responsive to Hydroxyurea and therefore progressed to transfusion [297]. Another study showed that patients who received regular transfusions had an increased TE reading in contrast to those not being transfused [298]. We found no difference between treatment type or haemoglobin type, indicating that patients

were on the correct treatment regimens for the severity of their disease, however we have few patients who had Hydroxyurea which may skew our results.

3.3.4.2.1 Liver functions tests

We found a raised ALP to be indicative of severe fibrosis in SCD in agreement with several studies, though the explanation of why it is increased is disputed, as it is produced by injury to bones as well as the biliary system. In the acute setting ALP is raised and in further testing has been shown to be secondary to bone damage [284, 406-408]. However, for patients with chronic disease, investigations of ALP isoenzymes have shown this increase was more likely from a hepatic cause [406, 409]. Multiple studies indicate ALP to be correlated with TE [281, 296, 297]. It would be interesting to study the association between more specific markers of cholangiocyte injury, such as γGT or alkaline phosphatase isoenzymes, but these data were not available to us.

We saw a correlation for TE with hypoalbuminaemia, however, the median values were normal and in accordance with other studies [296, 297].

ALT is a marker for hepatocyte damage, in accordance with our results there are no studies which show a correlation with liver damage. Unfortunately, our cohort did not have enough values for AST and GGT to allow a meaningful analysis, though these have shown to be significant elsewhere. We found no association between abnormalities found on ultrasound scanning and liver stiffness, however, this association has been shown elsewhere [296]. The inherent difficulty with ultrasound scans are the variations in reporting, the subtlety of changes and the interpretation of those results. We tried to mitigate for these by narrowing

our criteria to coarsened echotexture, irregular margins, nodularity, hepatomegaly or cirrhosis nevertheless, there may always be the risk of under or over-reporting and therefore, the use of an objective measure such as TE allows for the standardisation practice and decreased variability.

The interaction between serum ferritin, liver iron concentration, fibrosis and in turn TE is controversial. Serial serum ferritin measurements have been used as an indirect measure of liver iron concentration, with this being quantified by liver biopsy if required. Wolfe et al. found a moderate correlation between ferritin and fibrosis on liver biopsies [295], however, the cut-off they have suggested for fibrosis is 5000 ng/mL. This is markedly higher than the 1000 ng/mL, which indicates decreased survival from SCD [259, 281] raising the possibility they were actually seeing damage caused by iron deposition rather than SCD. Serum ferritin concentration and TE has been shown elsewhere, to correlate to varying degrees [296, 298, 307]. We did not find a significant relationship possibly due to the high number of patients on exchange transfusions, thus keeping the ferritin levels lower for the majority of patients.

The correlation between serum ferritin and LIC, is poorly to moderately correlated [291, 297, 410] and the damage caused by increased LIC variable. The Stroke Prevention Trial in Sickle Cell Disease (STOP) indicated all patients have iron deposition within the liver, though this only led to a low grade of fibrosis for most and no correlation, though this was mainly in children and young adults [291]. We therefore hypothesised that, LIC would not affect the TE readings and this was borne out within our data in agreement with several studies in both adults and children [291, 297, 411], though it stands in contrast to others [296, 298, 307], however, ours is the largest study in HbSS patients with a Ferriscan performed.

3.3.4.2.2 Use in assessing severity of disease

We used the cut-off values for severe fibrosis as 7.5kPa and cirrhosis as equal to or above 12kPa. These values were adopted arbitrarily and based on the available data for hepatitis C as this was the most validated disease process. We found ALP to be the best indicator for severity of fibrosis. The use of ALP has also been associated with increased mortality [259] and would thus support its use as indicated increasing severity of fibrosis.

3.3.4.3 Limitations

This is an audit of clinical practice within a tertiary centre and is therefore performed in a highly selected cohort, though care is provided to all patients within the covered region. Satellite clinics and local expertise may impact on the aggressiveness of the, such as the recognition and possibly varying management of sickle cell crises. Other potential confounders may include the local management of associated diseases such as gallstone disease and immediacy of cholecystectomies. To avoid selection bias, inclusion and exclusion criteria were as broad as possible, however as was an observational study, data may not have been collected during clinical practice. Also, baseline blood test results for patients with frequent attendances can be difficult to ascertain, which is particularly important as these are likely to have aggressive SCD, which may be affecting the liver. A way to overcome this would be to take a single shot blood test on all patients, however this is not practice and does not fit with the observational design of this study.

This was particularly prominent with AST, which is required for several scoring systems for fibrosis, such as APRI, and therefore restricted our ability to incorporate these within our patient selection or analysis. Hyperbilirubinaemia may be produced from an increased

breakdown of haemoglobin and is frequently found in SCD, though this may occur due to decreased excretion from the liver following conjugation. Bilirubin conjugation may be measured; however, this is not performed routinely and so was not available. To mitigate this, we accepted a higher bilirubin level of 100μmol/L within our inclusion criteria. However, as a baseline result was utilised to highlight eligible patients, when a paired result with TE measurement was obtained, several results were higher than this cut-off, highlighting the dynamic nature of the disease and a pitfall of an observational study.

To avoid recall and reporting bias the study was carried out in a prospective manner, logged in real time and designed to keep data collection as objective as possible. To avoid systematic bias, patients were seen on different days and throughout the day.

Non-participation bias is a real possibility within this cohort. To mitigate this appointment times were kept to a minimum and wherever possible, co-ordinated with the medical team to reduce the disruption to patients. It is recommended to have a period of fasting of 3-6 hours prior to TE reading, however, patients who required transfusions are required to eat regularly, therefore this was waived to improve attendance and not affect their other treatments. TE measurements were taken prior to transfusion commencement, as there is evidence that TE may change before and after blood transfusion, likely due to the increased circulating blood volume. We are unsure whether this was the case for exchange transfusions as the circulating volume of blood would not change, though our study was not aimed at addressing this.

Though this cohort represents one of the largest studied for SCLD in patients without the HbSC genotype, the relative paucity of cases, whilst being re-assuring, significantly affects

the conclusions which may be drawn from the data. To overcome this, a multi-centre or international study will allow for a better understanding.

A significant limitation of this study is the low number of patients being treated with hydroxyurea. These patients attend hospital less frequently with many check-ups telephone based, thus limiting our ability to investigate this group and may explain the lack of correlation between TE and HbF percentage.

We have investigated several markers for patients and considered whether a Bonferroni correction was needed, however, as simple tests were performed and overall testing was performed by regression analysis, significance correction is not required.

3.3.4.4 Implementation and further work

We have performed an audit aimed at investigating the prevalence of sickle cell liver disease, potential causative factors and the usefulness of introducing a Fibroscan® service to our haematology department. We have selected patients with HbSS and HbSβ+ Thalassaemia genotypes, as these are the most likely to develop SCLD [296] and allowed us to investigate and commence treatment for those most at risk within our population in a timely manner.

We have extrapolated the incidence of liver disease from those clinically and biochemically most at risk of liver disease. The evident next step, is to screen all patients with a TE reading, particularly as the investigation is quick overall and well tolerated, as demonstrated by no patient stopping mid test. The inherent difficulties are equipment and operator availability. It should be noted that a deranged bilirubin threshold was adjusted as part of the inclusion criteria and the inclusion of these patients would be interesting. We have shown the use of

237

ALP as a marker of fibrosis and as such, can be used to highlight which patients should have closer monitoring, initially with a full liver screen including TE and those that continue to require further work up, to also have a transjugular liver biopsy. This would allow strategic use of resources and alleviate patient concern while allowing early and aggressive treatment for a complication of SCLD, thereby reducing morbidity and in turn mortality. The lack of regular AST measurements also hampered our use of fibrosis scoring scales and the use of these and the correlation within our cohort would be beneficial.

The main limitation of this study is the lack of correlated histology. A recent American paper has begun this line of inquiry, comparing 50 patients with suspected SCLD to confirm the correlation of increased stiffness with fibrosis, though only five patients within their cohort had significant fibrosis (defined as Ishak fibrosis score >3) [412]. Further validation is required with a cut-off TE for cirrhosis especially important. Due to the low numbers within this extreme scale there is scope for collaborative work. Patients being treated with hydroxyurea and the contrast in the degree of liver disease is another area to be investigated.

The overall outlook for treatment of SCD is being revolutionised by disease-modifying drugs, gene addition and gene editing strategies which may make the disease treatable, potentially from birth. The outcome of these trials and their ongoing safety is under investigation currently and may markedly reduce the burden of SCD, though their effect on peripheral diseases from SCD such as SCLD will need ongoing monitoring; TE measurements would be well placed to evaluate this in the future.

3.3.4.5 Conclusion

We have shown the use of transient elastography within a cohort of patients suspected to have liver disease can have an important role in quick and early diagnosis and may be used alongside or potentially replace liver biopsies. We have also shown the prevalence of sickle cell liver disease within our population to be 15.4% with 3.8% of these being cirrhotic. Overall, the best marker for fibrosis is ALP though there are no markers to differentiate between fibrosis and cirrhosis.

3.4 What is the distribution of transient elastography results of adult patients with cystic fibrosis

3.4.1 Chapter summary

This chapter will present an audit following the introduction of transient elastography (TE) measurements for patients suspected of having adult-onset cystic fibrosis liver disease (CFLD) within a tertiary referral centre. We will then ascertain if any lab investigations indicate which patients should have a further assessment for liver disease.

3.4.2 Aims

To determine the prevalence of cystic fibrosis liver disease in terms of severe fibrosis and cirrhosis in a single tertiary centre and to identify factors indicating a deterioration in liver health. An important secondary aim was to determine the need for a Fibroscan®/liver service embedded within the respiratory department.

3.4.3 Results

3.4.3.1 Baseline characteristics

Overall, 144 patients were identified as having cystic fibrosis and of these, 79 patients attended for TE readings. They had a median age of 29 years old and were mainly homozygous for F508. The majority of patients were pancreatic insufficient and 35 patients had a diagnosis of diabetes mellitus.

Regarding liver disease, 30 patients were treated clinically as having CFLD, no patients drank alcohol excessively. Hepatomegaly was documented for three patients. Ultrasound scan found 22 patients with changes that may be indicative of CFLD, with nine described as cirrhosis and five patients showing splenomegaly. A liver biopsy was performed on six patients and two of these showed cirrhosis. Out of the 19 that had an OGD, three had varices and no patients had shown signs of encephalopathy. Further baseline demographics can be found in Table 3.23.

Table 3.23: Baseline characteristics for all patients

Characteristic	n=79
Median age, yrs., (IQR)	29 (22-36)
Male, n, (%)	44 (55.7)
Genotype, n, (%)	
Homozygous F508	39 (49.4)
Heterozygous F508	22 (27.8)
Other	18 (22.8)
Current BMI, kg/m², (IQR)	22.2 (20.5-24.9)
Lowest BMI, kg/m², (IQR)	20.7 (18.8-23.4)
Meconium ileus, n, (%)	5 (6.3)
Pancreatic insufficiency, n, (%)	63 (79.7)
UDCA, n, (%)	30 (38)
Diabetes, n, (%)	35 (44.3)
Previous Decompensation, n, (%)	3 (3.8)
Hepatomegaly on examination, n (%)	3 (3.8)
Organism, n, (%)	
Pseudomonas aeruginosa, n, (%)	27 (34.6)
NPA, n, (%)	40 (50.6)
Multidrug-resistant Pseudomonas aeruginosa, n, (%)	7 (8.9)
Other, n, (%)	5 (6.3)
Median FEV1, L, (IQR)	2555 (1800- 3492)
Median FVC, L, (IQR)	3665 (2787-4670)
Median FEV1/FVC, %, (IQR)	69 (60-79)
Median Predicted FEV1, %, (IQR)	74 (56-94)
Median Hb, g/dl, (IQR)	139 (126-148)
Median Platelets, x109/L, (IQR)	282 (224-352)
Median Sodium, mmol/L, (IQR)	140 (139-142)
Median Creatinine, μmol/L, (IQR)	64 (56-80)
Median Bilirubin, μmol/L, (IQR)	7 (4-9)
Median ALP, IU/L, (IQR)	100 (76-134)
Median γGT, U/L, (IQR)	19.5 (13.3-32.8)
Median ALT, units/L, (IQR)	20 (14-29)
Median albumin, g/L, (IQR)	45 (43-48)

BMI: Body mass index, UDCA: Ursodeoxycholic acid, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, Hb: Haemoglobin, NPA: Non-Pseudomonas Aeruginosa, ALP: Alkaline phosphatase, γGT: Gamma-glutamyl transferase, ALT: Alanine aminotransferase concentration, n: Number, yrs: Years, %: Percentage, IQR: Inter-quartile range, m: Metre, dl: Decilitre, L: Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, μg: Micrograms, mmol: Millimole, μmol: Micromole, U: Units, IU: International units.

The distribution of TE shows 60 patients to have no fibrosis (≤5.9kpa), 13 with fibrosis (6-8.9kPa) and 6 having severe fibrosis or cirrhosis (≥9kPa) (see Figure 3.52). TE did not identify two patients with known cirrhosis, one with portal hypertension proven on US, they had a stiffness of 5.7kPa. The second had a liver biopsy, proving cirrhosis with the latest US showing persisting morphological changes of irregular margins and nodularity, with deranged blood tests and a liver stiffness of 5.9kPa. In addition, 15 patients were being treated on clinical grounds as CFLD who were not diagnosed by TE, whilst 6 patients were identified solely by TE and they were in agreement for 13 patients. Taken together this would mean 36 patients were treated as CFLD diagnosed by either clinical means and/or TE. Based solely on TE, there is a prevalence of fibrosis of 14% (95% C.I. 15.4%-34.98%) with this extrapolated to include all patients diagnosed by either TE or clinically this is 45.6% (95% C.I. 34.3%-57.2%).
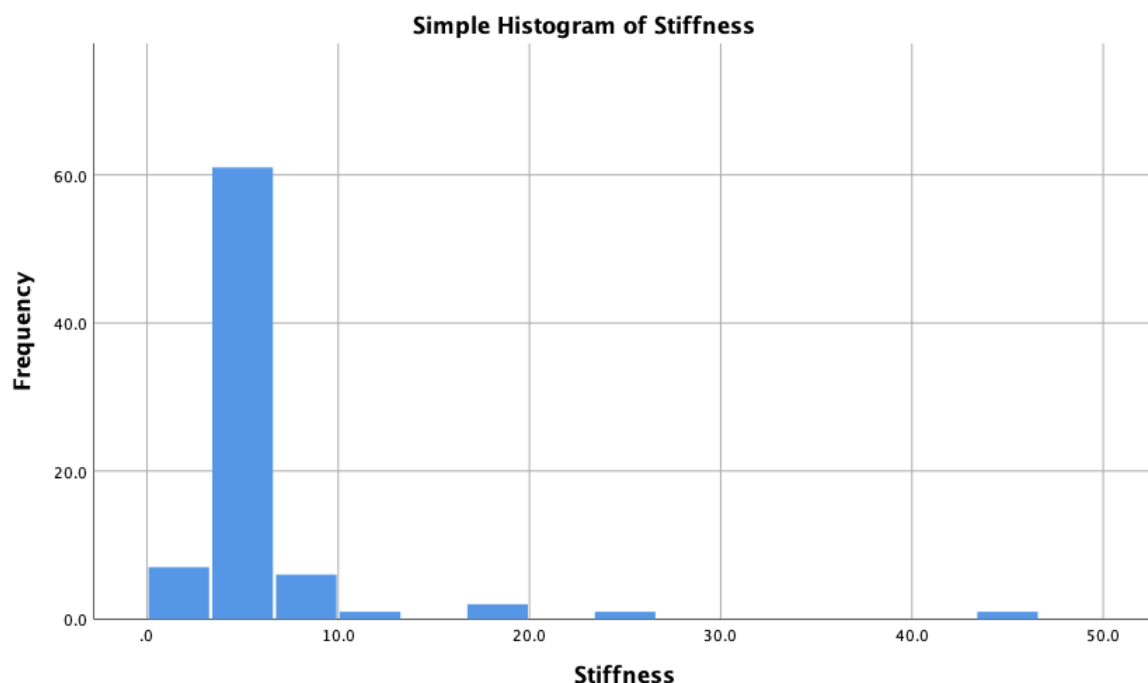
Figure 3.52: Distribution of transient elastography scores for all patients

3.4.3.2 Fibrosis vs no fibrosis

Table 3.24 displays the baseline characteristics according to no fibrosis (≤5.9kPa) or fibrosis present (≥6kPa). Pancreatic insufficiency, diabetes and ursodeoxycholic acid therapy (UDCA) was more prevalent for patients with fibrosis. All patients with a history of liver decompensation had increased stiffness. Predicted FEV1 is used to ascertain the degree of reduction in respiratory ability and we found no difference between the groups. Blood test results proved to be similar for both groups, except for platelets and albumin. The distribution of liver stiffness is shown in Figure 3.53.

Table 3.24: Patient characteristics when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa inclusive of patients with cirrhosis)

Characteristic	No fibrosis, n=60	Fibrosis n=19	p-value
Median age, yrs., (IQR)	29 (22.25-35.75)	28 (20-42)	0.735
Male, n, (%)	33 (55)	11 (57.9)	0.825
White - British, n, (%)	43 (71.7)	13 (68.4)	0.692
Genotype, n, (%)			
Homozygous F508	29 (48.3)	10 (52.6)	0.744
Heterozygous F508	18 (30)	4 (21.1)	
Other	13 (21.7)	5 (26.3)	
Current BMI, kg/m², (IQR)	22.20 (20.54-25.05)	22.20 (19.9-24.8)	0.859
Lowest BMI, kg/m², (IQR)	20.83 (18.95-23.42)	19.59 (18.47-23.36)	0.415
Meconium ileus, n, (%)	5 (8.3)	0	0.579
Pancreatic Insufficiency, n, (%)	46 (76.7)	17 (89.5)	0.332
UDCA, n, (%)	19 (31.7)	11 (57.9)	*0.040
Diabetes, n, (%)	22 (36.7)	13 (68.4)	*0.015
Previous Decompensation, n, (%)	0	3 (15.8)	*0.002
Hepatomegaly on examination, n (%)	1 (1.7)	2 (10.5)	0.088
Organism, n, (%)			
Pseudomonas aeruginosa	17 (28.3)	10 (52.6)	0.052
NPA	34 (56.7)	6 (31.6)	
Multidrug-resistant Pseudomonas aeruginosa	5 (8.3)	2 (10.5)	
Other	4 (6.7)	1 (5.3)	
Median FEV1, L, (IQR)	2760 (1960-3480)	2300 (1220-3550)	0.279
Median FVC, L, (IQR)	3650 (2930-4700)	3740 (2560-4560)	0.507
Median FEV1/FVC, %,(IQR)	71 (63-79)	65 (52-72)	*0.031
Predicted FEV1, %, (IQR)	76 (61-95)	61 (39-87)	0.086
Median Hb, g/dl, (IQR)	140.5 (127.5-150)	134 (119-140)	0.065
Median Platelets, x10⁹/L, (IQR)	287.5 (228.5-360.5)	246 (182-314)	*0.045
Median Sodium, mmol/L, (IQR)	140 (139-142)	140 (138-143)	0.772
Median Creatinine, µmol/L, (IQR)	64.5 (56.3-82.3)	64 (50-76)	0.606
Median Bilirubin, µmol/L, (IQR)	7 (4-9)	7 (4-9)	0.822
Median ALP, IU/L, (IQR)	96 (76-127.5)	115 (93-142)	0.105
Median γGT, U/L, (IQR)	18 (13-28)	22 (17.3-48)	0.079
Median ALT, units/L, (IQR)	20 (14-29)	22 (14-30)	0.877
Median albumin, g/L, (IQR)	46 (43-48)	44 (41-45)	*0.031

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as p<0.05, *denotes significant results. n: number, BMI: Body mass index, UDCA: Ursodeoxycholic acid, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, NPA: Non-Pseudomonas Aeruginosa, Hb: Haemoglobin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase concentration, γGT: Gamma-glutamyl transferase, n: Number, yrs: Years, %: Percentage, IQR: Inter-quartile range, m: Metre, dl: Decilitre, L: Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, µg: Micrograms, mmol: Millimole, µmol: Micromole, U: Units, IU: International units.
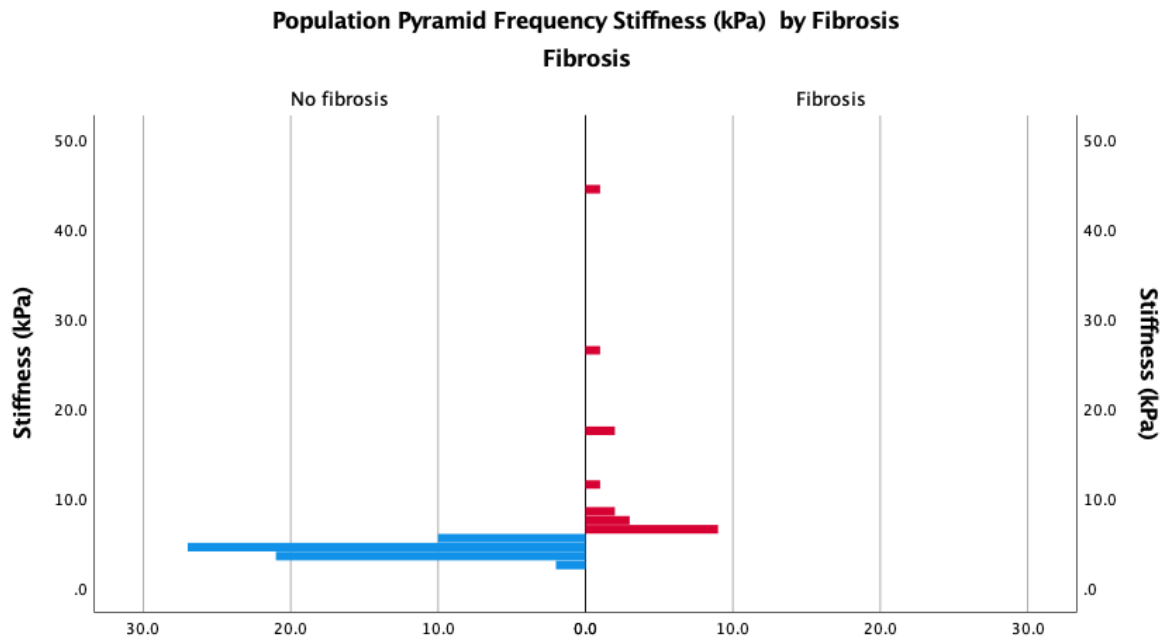
Figure 3.53: Histogram showing the distribution of transient elastography when divided by absence (TE<5.9kPa) or presence of fibrosis (TE>6kPa)

Several relationships were examined in more detail to further examine individual factors that might contribute to fibrosis development. (See appendix 5 for further details)

3.4.3.2.1 Lowest BMI

Patients with CF tend to suffer from malnutrition due to several causes, including a lower nutritional absorption and therefore, we investigated the lowest BMI recorded.
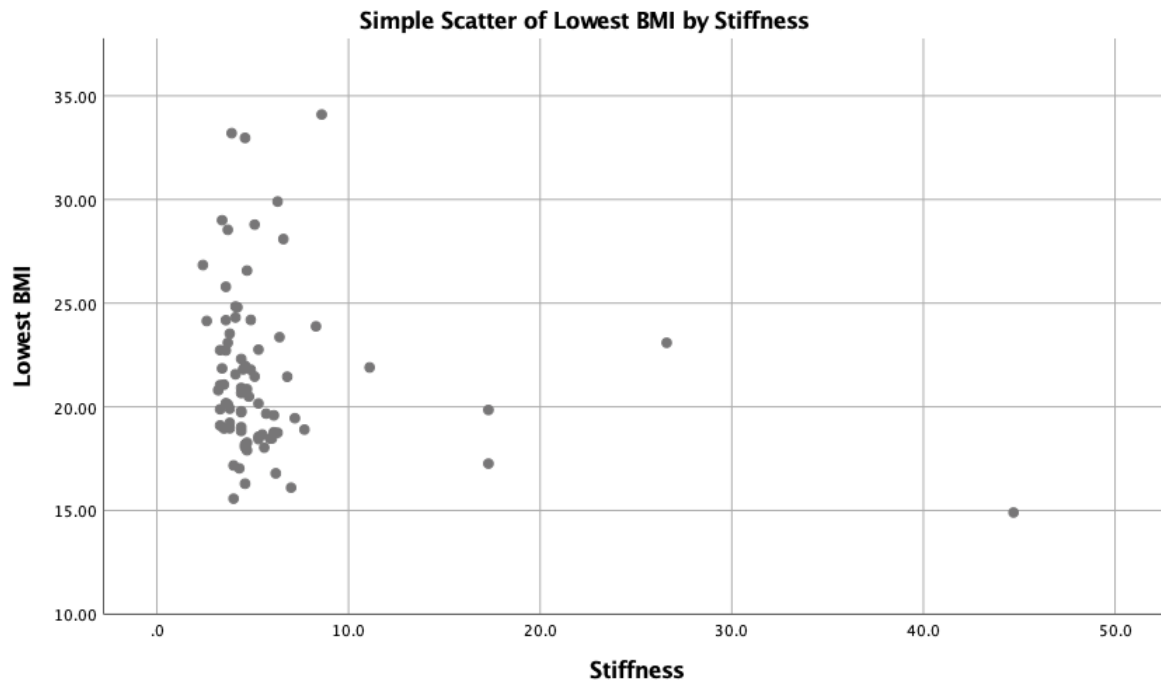
Figure 3.54: Scattergram showing lowest BMI against liver stiffness. Spearman's rho test, r=-0.227, p=0.044
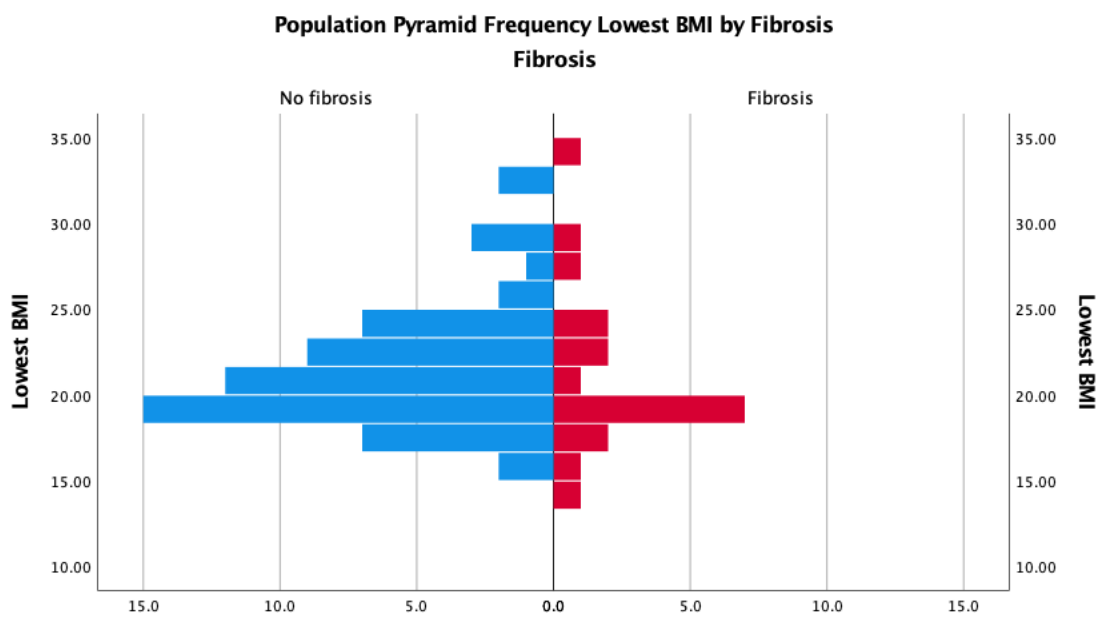
Figure 3.55: Histogram shows the lowest BMI distribution when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

A statistically significant weak negative correlation was between increasing liver stiffness and the lowest BMI (p=0.044) (Figure 3.54). The median lowest BMI for patients without

fibrosis was not different between patients with no fibrosis (20.83kg/m^2) and those with fibrosis (19.59kg/m^2), p=0.415 (Figure 3.55).

3.4.3.2.2 Diabetes Mellitus

Due to pancreatic insufficiency and reduced muscle mass, incidence of diabetes mellitus in patients with CF is increased. We found a statistically significant association with fibrosis (p=0.015, OR: 3.74, 95% CI: 1.245-11.25) (Figure 3.56). Pancreatic insufficiency did not indicate CFLD (appendix 5.3).
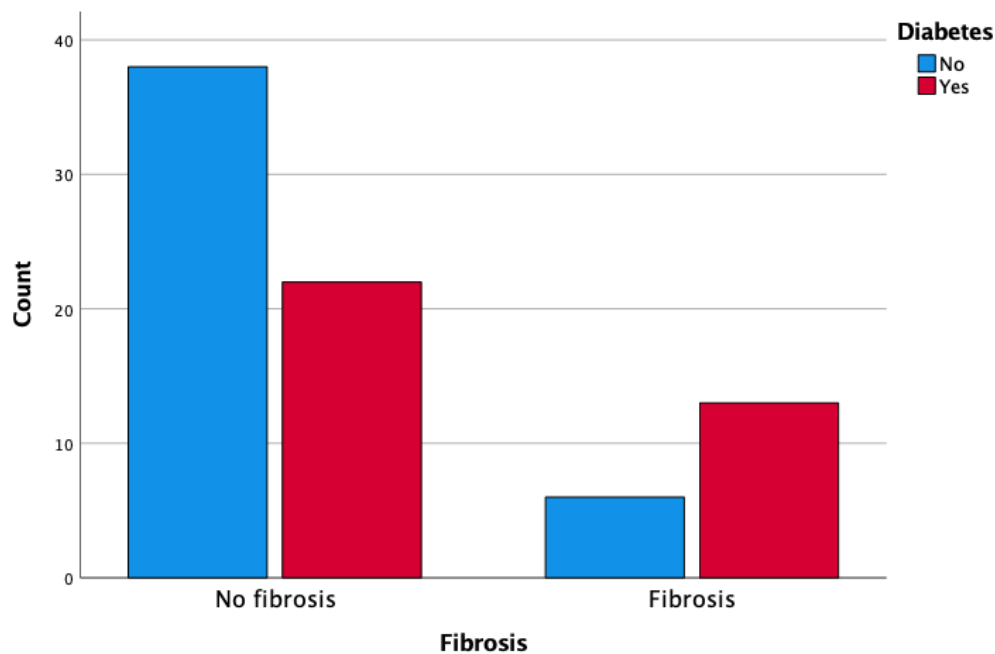
Figure 3.56: A clustered bar chart displaying the number of patients with diabetes mellitus when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa). Chi Sq test, p=0.015, OR: 3.74, 95% CI: 1.245-11.25

3.4.3.2.3 **Platelets**

Decreased platelet count is associated with severity of liver disease, therefore, we looked at platelet count against liver stiffness.
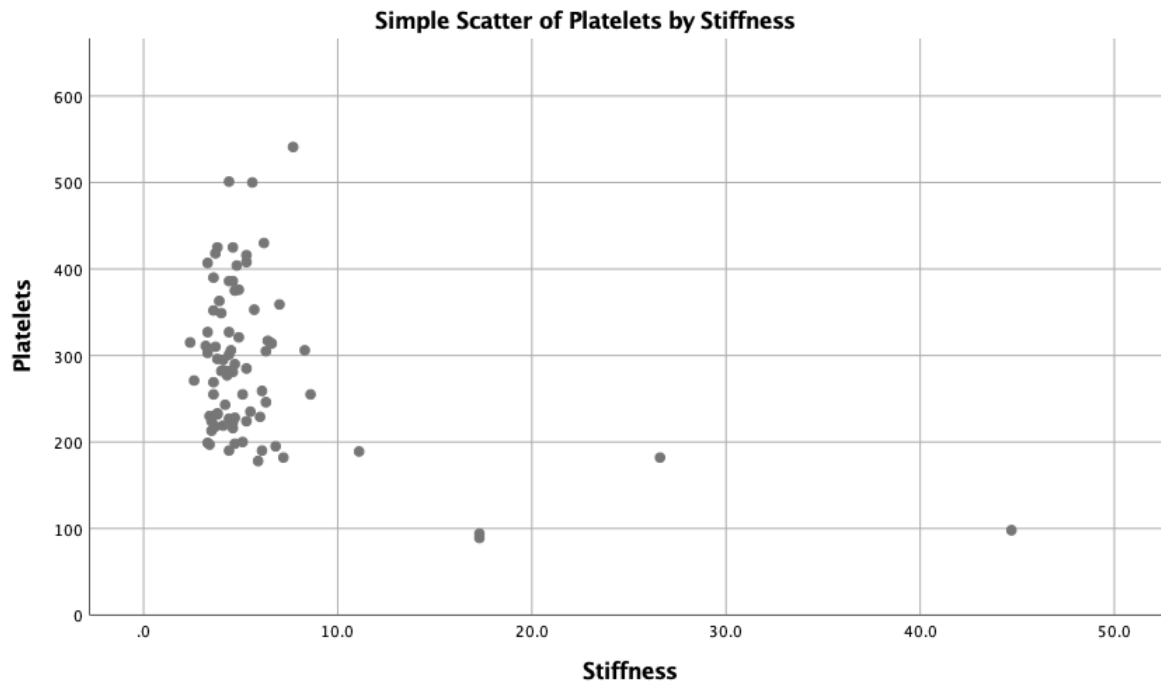
Figure 3.57: Scattergram showing platelet concentration against liver stiffness. Spearman's rho test, r=-0.162, p=0.153
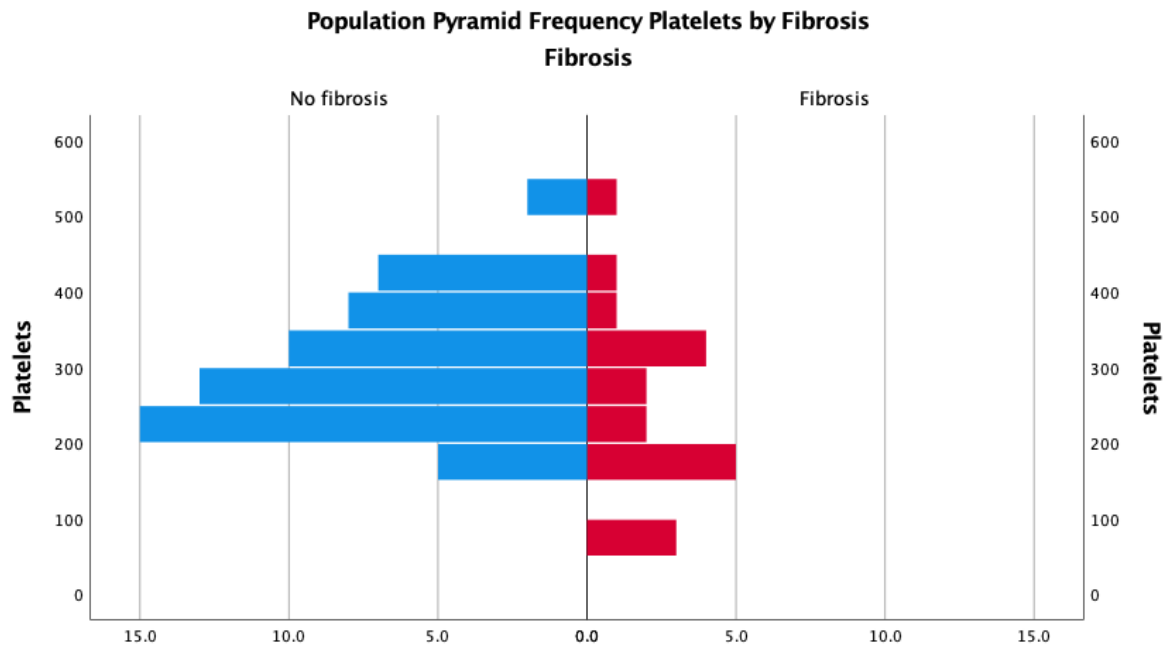
Figure 3.58: Histogram showing the distribution of platelet concentration when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

There was no correlation between increasing liver stiffness and platelet concentration (Figure 3.57) however, median platelet concentration was statistically significantly different between patients with no fibrosis (287.5x10^9/L) and those with fibrosis (246 x10^9/L), p=0.045 (Figure 3.58).

3.4.3.2.4 Static liver tests

Serum blood tests are performed routinely and we have investigated them here.

We investigated ɣ-glutamyl transpeptidase which showed a positive correlation (r=0.356, p=0.02) (Figure 3.59), however there were no significant differences in level (p=0.074) (Figure 3.60). There were no other significant liver tests (appendix 5.4)
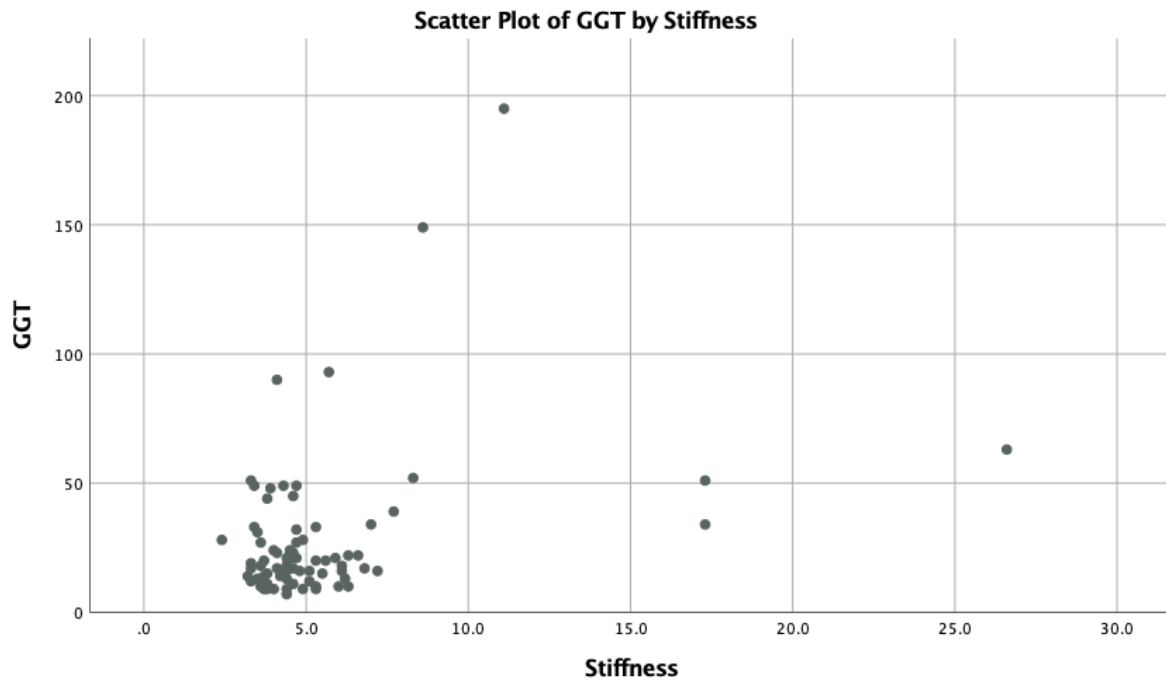
Figure 3.59: Scattergram showing serum ɣ-glutamyl transpeptidase (γGT) concentration against liver stiffness. Spearman's rho test, r=0.356, p=0.02
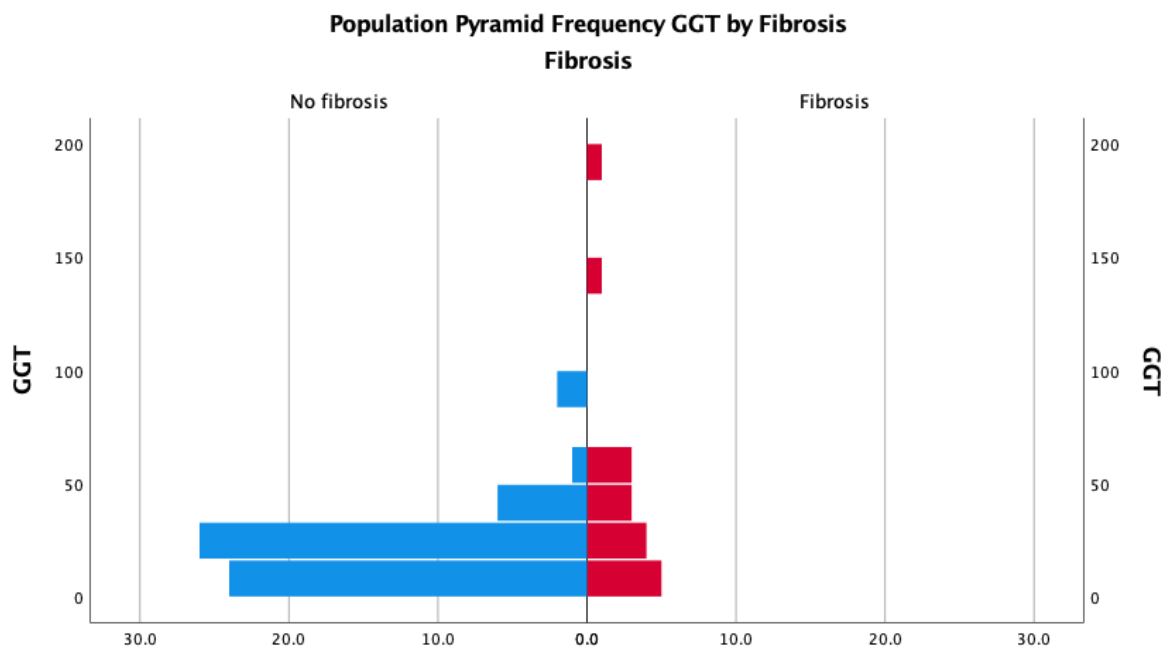
Figure 3.60: Histogram showing the distribution of serum ɣ-glutamyl transpeptidase (γGT) concentration when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

251

3.4.3.2.5 Albumin

Synthesis of several proteins occurs within hepatocytes; serum albumin may be used to monitor this function of the liver, though this may also be affected by poor nutrition or absorption, such as may be seen in severe CF.
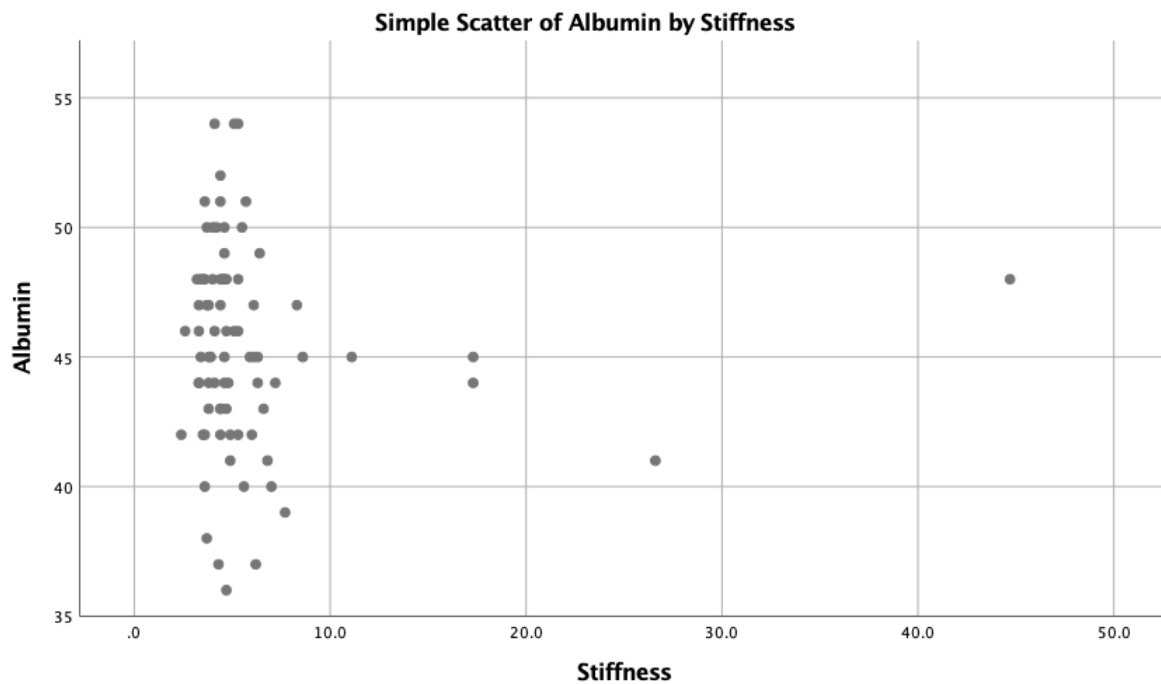
Figure 3.61: Scattergram showing serum albumin concentration against liver stiffness. Spearman's rho test, r=-0.140, p=0.220

252

Population Pyramid Frequency Albumin by Fibrosis
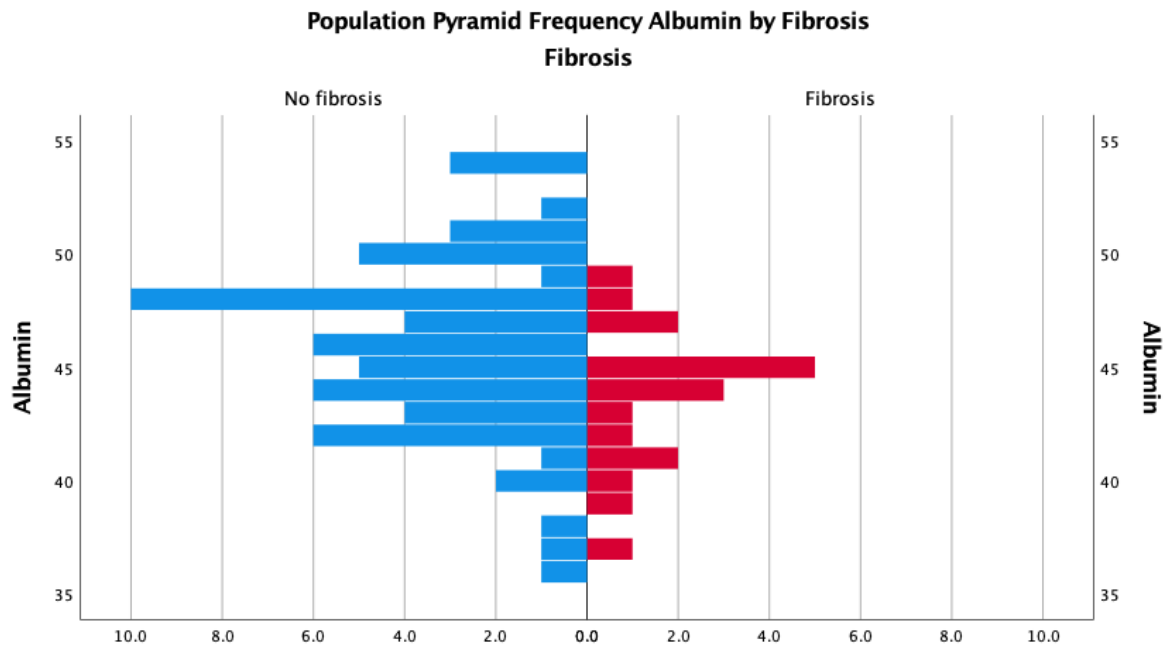
Figure 3.62: Histogram showing the distribution of serum albumin concentration when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

There was no correlation between increasing liver stiffness and serum albumin concentration (Figure 3.61), although there is a statistically significant difference between median albumin concentration for patients with no fibrosis (46g/L) and those with fibrosis present (44g/L) p=0.030 (Figure 3.62).

3.4.3.2.6 **UDCA**

Patients with suspected CFLD are often prescribed Ursodeoxycholic acid (UDCA) to improve the flow of bile. A minority (38%) were prescribed UDCA treatment, with the majority of these given for those showing fibrosis on Fibroscan® (p=0.040, OR: 2.97, 95% CI: 1.027-8.57) (Figure 3.63).
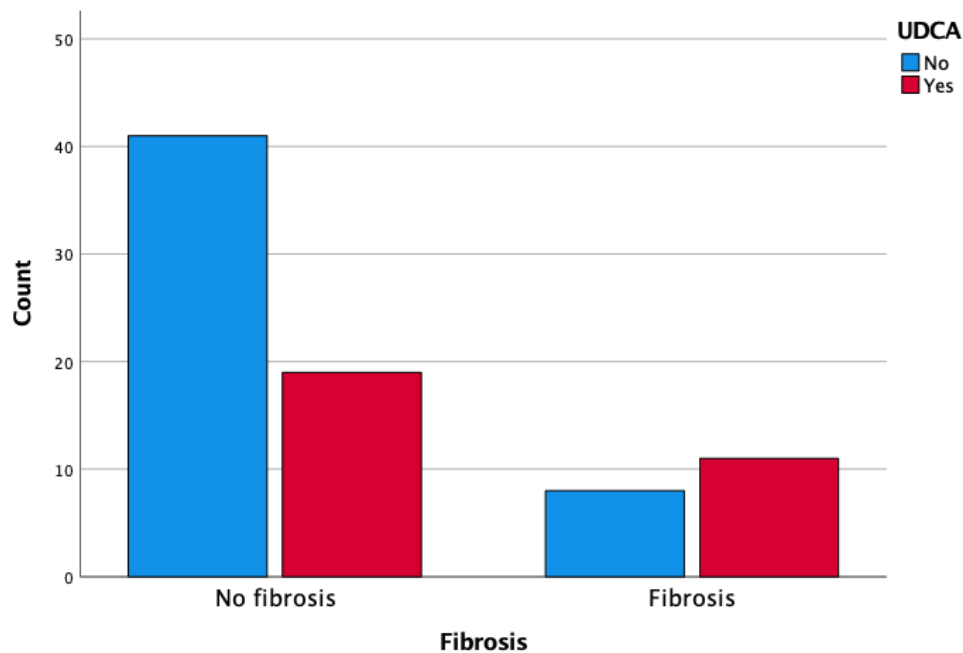
Figure 3.63: A clustered bar chart displaying the number of patients, prescribed ursodeoxycholic acid when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa). Chi Sq test, p=0.040, OR: 2.97, 95% CI: 1.027-8.57

3.4.3.2.7 US changes

CF guidelines state that patients should receive an US scan regularly for the surveillance of CFLD. All but one patient had surveillance imaging within two years of the audit. This was reported as normal in 41 cases and coarsened echotexture was the most commonly reported abnormality in 21 patients. TE indicated CFLD for patients with any signs of liver abnormalities, not secondary to fatty changes (p=0.001, OR:6.125, 95% CI: 2.00-18.79) (Figure 3.64). Splenomegaly was seen in five patients, all of whom had TE indicative of fibrosis.
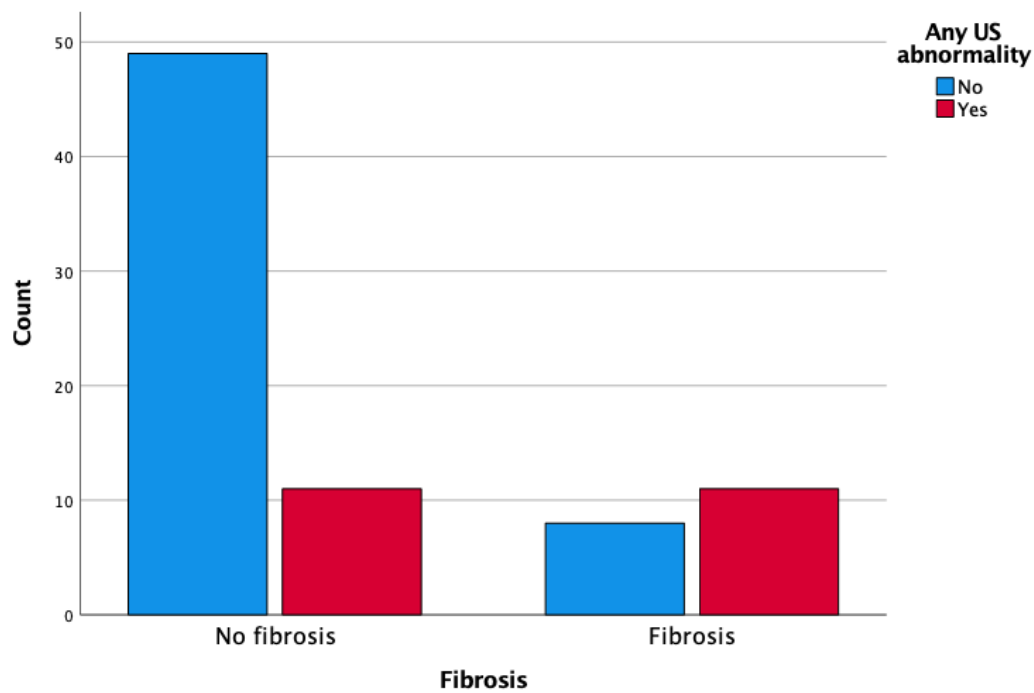
Figure 3.64: A clustered bar chart displaying the number of patients with any abnormality found on ultrasound scan when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa). Chi Sq test, p=0.001, OR:6.125, 95% CI: 2.00-18.79

3.4.3.2.8 Scoring systems

Various algorithmic scoring systems indicate the presence of liver fibrosis. We have investigated their usefulness in CFLD.
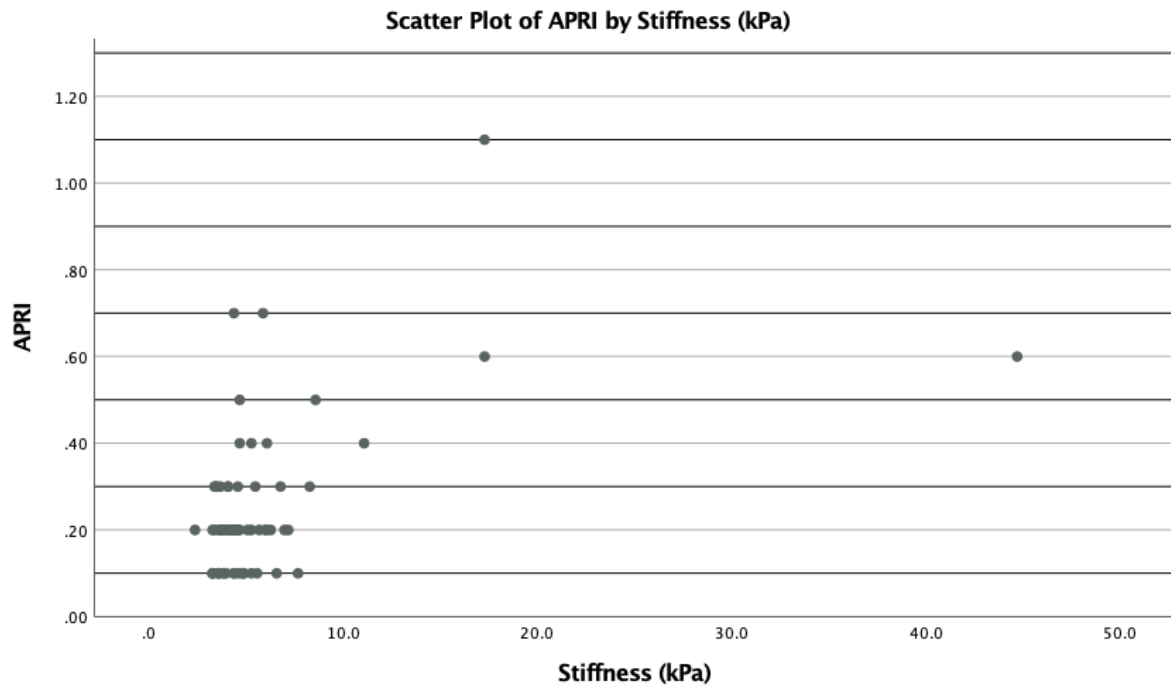
Figure 3.65: Scattergram showing AST to platelet ratio index (APRI) score against liver stiffness. Spearman's rho test, r=0.329, p=0.008
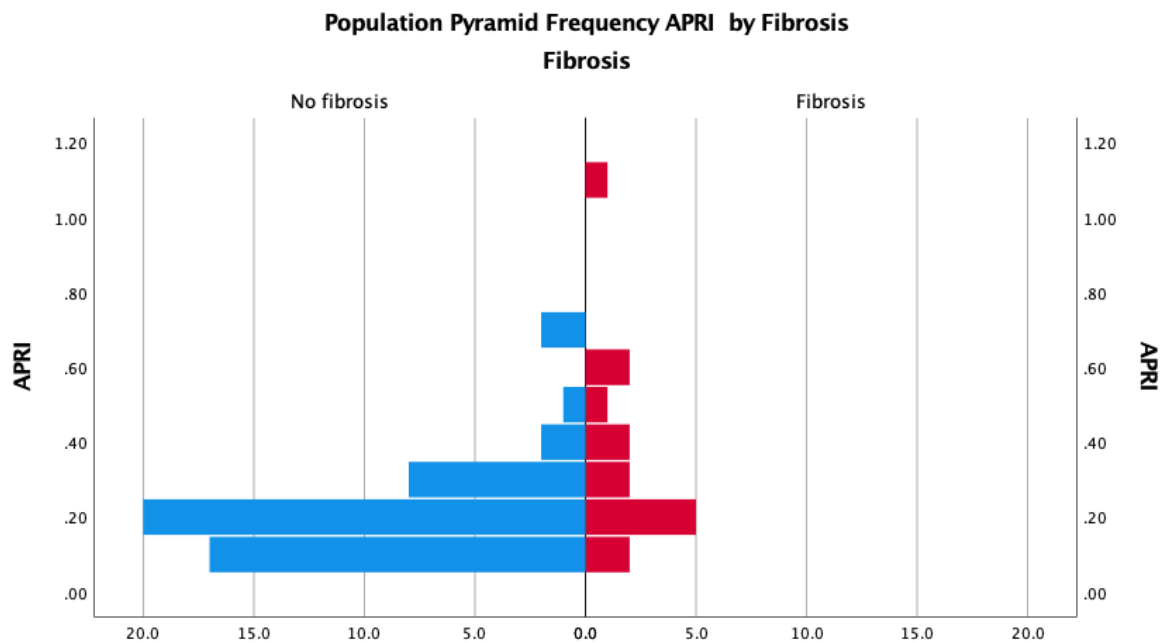
Figure 3.66: Histogram shows the distribution of AST to platelet ratio index (APRI) when divided by absence (TE<5.9kPa) or presence of fibrosis (TE>6kPa)

A statistically significant weak positive correlation exists between increasing liver stiffness

and AST to platelet ratio index (APRI) (Figure 3.65). The median APRI score was

statistically significantly different between patients with no fibrosis and those with fibrosis, p=0.019 (Figure 3.66). A lower cut-off (0.265) for fibrosis in CF has been suggested, but we did not find this to indicate a raised TE (p=0.062). However, when the APRI scores were categorised at the cut-off of 0.5, which has been shown to indicate fibrosis in other diseases, there was a significant separation (p=0.044). For Fib4 see appendix 5.8

3.4.3.2.9 <u>Multivariate analysis of predictors for fibrosis</u>

We amalgamated the factors from the univariate analysis, as can be seen in Table 3.25. following multivariate analysis UDCA (p=0.036) and albumin (p=0.030) are the strongest predictors of severe fibrosis. The model proved to be adequate with an R^2 of 10.6%. The area under the curve for this model is 0.71 (95% C.I: 0.57-0.84).

Table 3.25: Univariate analysis of factors differentiating absence (TE <5.9kPa) or presence of severe fibrosis (TE>6kPa)

Characteristic	p-value
Age	0.735
Lowest BMI	0.415
UDCA use	*0.040
Diabetes mellitus	*0.015
Pseudomonas aeruginosa infection	0.052
US abnormalities	*0.001
Haemoglobin	0.065
Platelets	*0.045
Alkaline phosphatase concentration	0.105
γ-glutamyl transpeptidase concentration	0.079
Albumin	*0.030
APRI score	*0.044

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as p<0.05, *denotes significant results. BMI: Body mass index, UDCA: Ursodeoxycholic acid, US: Ultrasound, FEV1: Forced expiratory volume in 1 second, APRI: AST to platelet ratio index

3.4.3.3   <u>Advanced fibrosis or Cirrhosis subgroup</u>

The progression to severe fibrosis or cirrhosis is important to recognise early to avoid the associated sequelae. We have extracted patients with a TE of >9kPa and compared them against normal and non-severe fibrosis patients.

Table 3.26 presents the baseline characteristics of these groups. We found five patients to have advanced fibrosis or cirrhosis (11.1–44.7kPa) this indicates a prevalence of 6.3%. TE missed four patients with known cirrhosis, two of these with portal hypertension. Two, as described above, had normal TE readings. Of those which had shown fibrosis, one had liver biopsy-proven cirrhosis with splenomegaly on the US and a stiffness of 7.2kPa, with the other showing cirrhosis on US, deranged serum tests and a liver stiffness of 8.6kPa. Had these been accounted for prevalence increases to 12.2%. All the patients with TE>9kPa had pancreatic insufficiency with associated diabetes and 80% were prescribed UDCA. US showed splenomegaly for four patients with advanced disease. Predicted FEV1 was lower for patients with advanced disease (55%).

Table 3.26: Baseline patient characteristics when divided by normal and non-severe fibrosis (TE ≤8.9kPa) or the presence of advanced fibrosis or cirrhosis (TE≥9.0kPa)

Characteristic	Normal and non-severe fibrosis n=74	Advanced fibrosis or cirrhosis, n=5
Median age, yrs., (IQR)	29 (22-36.3)	28 (22-37.5)
Male, n, (%)	41 (55.4)	3 (60)
White - British, n, (%)	52 (70.3)	4 (80)
Genotype, n, (%)		
Homozygous F508	37 (50)	2 (40)
Heterozygous F508	20 (27)	2 (40)
Other	17 (23)	1 (20)
Current BMI, kg/m², (IQR)	22.20 (20.58-25.11)	22.2 (18.5-23.92)
Lowest BMI, kg/m², (IQR)	20.73 (18.82-23.62)	19.85 (16.08-22.5)
Meconium ileus, n, (%)	5 (6.8)	0
Pancreatic insufficiency, n, (%)	58 (78.4)	5 (100)
UDCA, n, (%)	26 (35.1)	4 (80)
Diabetes, n, (%)	30 (40.5)	5 (100)
Previous Decompensation, n, (%)	0	3 (60)
Hepatomegaly on examination, n (%)	2 (2.7)	1 (20)
Splenomegaly on ultrasound, n (%)	1 (1.4)	4 (80)
Organism, n, (%)		
Pseudomonas aeruginosa	23 (31.1)	4 (80)
NPA	40 (54.1)	0
Multidrug-resistant Pseudomonas aeruginosa	7 (9.5)	0
Other	4 (5.4)	1 (20)
Median FEV1, L, (IQR)	2560 (1870-3540)	2000 (940-3095)
Median FVC, L, (IQR)	3650 (2810-4705)	3800 (2010-4235)
Median FEV1/FVC, %, (IQR)	69 (60-79)	51 (45-74)
Median predicted FEV1, %, (IQR)	76 (59-95)	55 (40-67)
Median Hb, g/dl, (IQR)	139 (127-149)	121 (115-133.5)
Median Platelets, x10⁹/L, (IQR)	287 (229-354.5)	98 (91.5-185.5)
Median Sodium, mmol/L, (IQR)	140 (139-142)	140 (136.5-142.5)
Median Creatinine, µmol/L, (IQR)	64 (56-79)	58 (47-115)
Median Bilirubin, µmol/L, (IQR)	7 (4-9)	9 (5-15)
Median ALP, IU/L, (IQR)	99 (76-132)	34 (90.5-257.5)
Median ALT, units/L, (IQR)	20 (14-29)	24 (16.5-29)
Median γGT, U/L, (IQR)	18 (13.8-28.8)	57 (42.5-123)
Median albumin, g/L, (IQR)	45 (43-48)	45 (42.5-46.5)
APRI score, (IQR)	0.2	0.3

BMI: Body mass index, UDCA: Ursodeoxycholic acid, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, NPA: Non-Pseudomonas Aeruginosa, Hb: Haemoglobin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase concentration, γGT: Gamma-glutamyl transferase, APRI: AST to platelet ratio index, n: Number, yrs: Years, %: Percentage, IQR: Inter-quartile range, m: Metre, dl: Decilitre, L: Litre, fL: Femtoliters, kg: Kilograms, g: Grams, mg: Milligram, µg: Micrograms, mmol: Millimole, µmol: Micromole, U: Units, IU: International units.

3.4.3.3.1 Univariate analysis

We investigated clinically relevant factors to ascertain those that would highlight patients with advanced fibrosis or cirrhosis.

Several factors were important, with correlations significant for age, lowest BMI, predicted FEV1 and ALP. Comparative differences are shown for UDCA use, diabetes mellitus, pseudomonas infection, platelets, albumin and US abnormalities (Table 3.27).

Table 3.27: Univariate analysis of factors differentiating normal and non-severe fibrosis (TE ≤8.9kPa) or the presence of advanced fibrosis or cirrhosis (TE≥9.0kPa)

Characteristic	p-value
Age	0.944
Lowest BMI	0.579
UDCA use	0.060
Diabetes mellitus	*0.013
Pseudomonas aerogenosa infection	*0.049
US abnormalities	*<0.001
Haemoglobin	0.059
Platelet concentration	*0.001
Alkaline phosphatase concentration	0.253
ɣ-glutamyl transpeptidase concentration	*0.004
Albumin	0.083
Predictive FEV1	*0.048
APRI score	0.176

Significance for continuous variables calculated via Mann-Whitney U and categorical with Chi sq or Fisher's exact test where appropriate. Significant taken as $p<0.05$, *denotes significant results. BMI: Body mass index, UDCA: Ursodeoxycholic acid, US: Ultrasound, FEV1: Forced expiratory volume in 1 second, APRI: AST to platelet ratio index

3.4.3.3.2 Multivariate Analysis

Due to the small number of patients with advanced fibrosis or cirrhosis, proceeding to a multivariate analysis was deemed futile as there would be an increased risk for low precision modelling and, therefore, incorrect conclusions.

3.4.3.4 Established scoring systems

The inherent difficulty for CFLD has been the production of a unifying diagnostic criteria. Several attempts have been made, two without non-invasive tests and one with the incorporation of TE and the scoring systems of APRI, Fib4 and AST/ALT ratio (AAR). The Flass criteria utilises clinical examination, liver function tests, imaging, histology or direct visualisation of the liver via laparoscopy and splits the cohort into three groups, with the most extreme being cirrhotic with portal hypertension. We assessed our cohort according to these criteria.

The Debray criteria which relies upon examination, the elevation of transaminases and US findings, diagnosed 16 patients (20.3%) as having CFLD. One patient with cirrhosis was diagnosed on ultrasound but had normal blood tests and examination and therefore was missed by this criterion. Koh criteria denoted 32 patients (40.5%) as CFLD. Flass identified 34 patients (43%) with liver involvement without cirrhosis and a further nine (11.4%) as cirrhotic with portal hypertension. For comparison of the scoring systems see appendix 6.1.

Table 3.28: Patients detected with fibrosis via Transient Elastography vs those detected as CFLD by scoring criteria.

Criteria		Transient elastography		Concordance
		Fibrosis	No Fibrosis	
Debray	CFLD, n	10	6	0.426
	No CFLD, n	9	54	
Koh	CFLD, n	12	20	0.244
	No CFLD, n	7	40	
Flass	CFLD, n	14	29	0.332
	No CFLD, n	5	31	

Spearman correlation test applied for concordance. Significant taken as $p < 0.05$, *denotes significant results. CFLD: Cystic fibrosis liver disease, n: Number

Regarding TE, all scoring systems agreed upon 24 patients without CFLD and 10 with CFLD (43%) (see Table 3.28). Six patients (7.6%) were identified by all criteria but not TE. Two

patients with cirrhosis are already described, three showed a derangement in a transaminase with a coarsened echotexture on US and the final patient showed a raised AST and hepatomegaly on US but not examination. Four patients (5%) were identified by TE only, all of whom had normal blood tests, with two displaying increased heterogeneity on the US. Of these, three had transient elastographies showing early fibrosis (6.3-6.4kPa), which did not reach the threshold for Koh criteria. The remaining patient with a positive liver of 6.8kPa only showed fatty liver changes on the US with a BMI of 24.5kg/m².

Debray criteria agreed with TE for 81% (correlation=0.426, p<0.001) of patients, with the bulk of this agreement for patients with no CFLD. Of the six with CFLD via Debray but not TE, these were agreed upon by the other criteria and described above. Of the nine picked up by TE and not Debray, four were not identified by any criteria as above. The other systems identified one due to cirrhosis shown on the US. They had normal serum tests despite a liver stiffness of 44.7kPa. Otherwise, Koh identified one due to deranged ALP and AAR, while Flass recognised the other three due to only a derangement of a single transaminase.

Koh criteria, which is the only one including TE, agreed on 12 patients having CFLD and 40 patients without CFLD (correlation=0.244, p=0.03). Koh did not agree upon seven patients identified by TE. Four of these were not picked up by the other systems and are described above, though, of note, three had a raised AAR despite individual blood tests being within the normal range. Flass identified the remaining three due to a deranged transaminase. Koh identified a further 20 patients as CFLD, which were not indicated by TE, six of which were agreed upon by the other systems and described above. Flass also identified seven, three had normal blood tests but abnormalities on the US, two with coarsened echotexture and one with hepatomegaly which was not noted clinically and the remaining four showed two with raised

AST and two with raised γGT though normal US throughout. All of these had a raised AAR. Seven were identified only on Koh due to a raised ALP and AAR, which are not included by the other scoring systems, with three of these only showing a fatty liver on the US.

Flass criteria agreed with TE for 57% of patients (correlation=0.332, p=0.003), though had the highest conformity for patients with a raised TE, as 14 patients were agreed upon. Of the remaining five, four were not picked up by any other criteria and the final only by Koh due to a raised ALP and AAR, which are not included in Flass criteria. No CFLD was also shown in 31 patients on both Flass and TE. Of the remaining 29 patients who were identified by Flass but had normal transient elastographies, six were picked up by all the other criteria as described above and seven were in agreement only with Koh, also described above. The remaining 16 were detected by abnormal transaminase or γGT, with the US only showing fatty liver in five cases. AAR was also negative for all these cases.

Regarding advanced fibrosis or cirrhosis, Flass and Koh identified all five, with Debray missing one as explained above. Koh and Flass detected the four cases not identified on TE due to their use of US or histology as described above.

In summary our work shows that, as yet, there is no reliable agreed diagnostic criteria for the non-invasive diagnosis of cirrhosis in patients with adult CFLD. Although TE is often regarded as the 'gold standard' for non-invasive diagnosis of cirrhosis it failed to identify two patients with very clear evidence of cirrhosis. As liver biopsies were rarely performed in these patients it is impossible to determine whether the remaining patients who did not have cirrhosis diagnosed by TE did, in reality, have advanced fibrosis. Our analysis of the different scoring systems for CFLD, albeit with a small cohort, showed marked heterogeneity with no

clear consensus as to which patient had cirrhosis. Our data highlights the challenges in

establishing the diagnosis of cirrhosis in patients with CFLD and indicates a need for further

studies in this area.

3.4.4 <u>Discussion</u>

Cystic fibrosis liver disease (CFLD) in adults is an ill-defined, though growing issue. To gain a better understanding we performed an audit of all CF patients treated at a tertiary centre to initially find the prevalence of CFLD and used this information to identify factors indicating a deterioration in liver health. TE scores were used to determine the degree of fibrosis and accept that this may be suboptimal. We also assessed the need for a Fibroscan®/liver service embedded within the respiratory department.

3.4.4.1 <u>Summary of findings</u>

CFLD was suspected on clinical grounds for 30 patients, however, TE only identified 19 of these. Importantly, TE missed two patients with known cirrhosis; one with portal hypertension, diagnosed either via ultrasound or liver biopsy and misclassified a further two as normal or non-severe fibrosis. TE also identified a further six patients with CFLD which were not recognised clinically. This is a concerning disparity; however, we went on to identify factors which may be associated with a raised TE and utilising the cut-off of 6kPa to denote any form of fibrosis (a very conservative value) we found a prevalence of 14%.

Looking at factors that may indicate patients that are at risk of CFLD, several were identified with only UDCA and albumin indicating severe fibrosis, though albumin is within normal limits making its usefulness doubtful. Due to a lack of power, any conclusion for those with the most advanced disease is difficult to conclude.

Diagnostic criteria for CFLD has been proposed but agreement was weak, as was concordance with TE, though for advanced fibrosis or cirrhosis, Flass matched with TE for

all patients and identified the four others also. These results highlight the need for better diagnostic criteria in conjunction with TE, as well as vigilance by clinicians.

3.4.4.2 <u>Interpretation</u>

Diagnostic values for CFLD diagnosis by TE have varied widely, a recent meta-analysis suggested a value of 5.95kPa but could not comment on advanced disease [413]. We, therefore, have used a cut-off of 6-8.9kPa for fibrosis and ≥9kPa for advanced fibrosis or cirrhosis.

Our primary objective was to ascertain the prevalence of CFLD within our population. We found a prevalence of 14% for fibrosis and 6.3% for severe fibrosis or cirrhosis. If we included the patients not identified by TE, we increased the prevalence to 26.6% for patients with fibrosis and 12.2% for advanced fibrosis or cirrhosis. A UK registry study investigated 3417 cases, with 2355 of these being adults (68.9%), showed an overall prevalence of 22.8% for CFLD and 13.8% for cirrhosis; however, this number is inclusive for all age groups [414] which had increased from 20.3% five years earlier. A French study utilised the Debray criteria to find a prevalence of 18% with CFLD and advanced CFLD as 5%, diagnosed as cirrhosis on imaging or signs of portal hypertension again, this is regardless of age, though they did find a continual increase in incidence with age, with a maximum of 32.2% for CFLD and 10.2% for severe CFLD at 25 years old [317]. Koh et al. specifically investigated adult disease, following 36 patients for a median of 24.5 years and found a prevalence of 22% according to Debray criteria, they also proposed a new criterion incorporating TE and found a prevalence of 47% utilising this [319]. Taken together, our prevalence agrees with national and international data.

Pancreatic insufficiency is a more severe phenotype and therefore, CFLD patients have been selected from these cohorts in certain studies [317]. We found pancreatic insufficiency in 80% of our cohort and in all patients with cirrhosis. A major clinical effect of pancreatic insufficiency is diabetes which is associated with cirrhosis as well as a deleterious effect on prognosis [414, 415]. We found diabetes indicated a raised liver stiffness. Possible causes for this may be a similar pathological effect, either due to reduction in ductal secretions causing cellular damage, the effect of a build-up in more toxic bile acids or a choline deficiency [416-418].

The role of UDCA in CFLD is controversial. It may be used to treat pancreatic insufficiency with a proposed mechanism of encouraging biliary flow and replacing bile acids within the intestinal tract. However, it has also been used as a treatment for CFLD, though the mechanism for action here is less evident. The use of UDCA has been encouraged due to a reduction in transaminases following initiation and an improvement in histology two years following the commencement of treatment, mainly secondary to a reduction in inflammation [419]. A possible mechanism may be an anti-inflammatory effect by stabilising CFTR and in turn may allow a reduction in enteric and biliary inflammation [420, 421]. However, a Cochrane study identified only three randomised controlled studies, comprising of a total 118 patients and only short follow-up, hindering any conclusions. Within these constraints, they concluded no obvious overall benefit or hindrance from UDCA [422] and has been supported by follow up studies [317]. Paired TE evaluation on patients taking UDCA compared with historical and contemporary controls shows patients with fibrosis had a reduction in follow-up TE whilst patients with cirrhosis worsened [423]. These findings are supported both histologically [419] and prognostically [414]. This would support early use of UDCA for patients suspected of CFLD and as many of these patients are pancreatic insufficient also,

there is a possible dual benefit. We found that many of our patients with fibrosis were taking UDCA, thereby displaying an active approach to CFLD within our department.

Chronic bacterial colonisation has been shown to increase the risk of cirrhosis [414] and may increase the risk of liver-related death [415]. We found a low degree of significance with pseudomonas infection in cirrhosis. This link may be accounted for by the pro-inflammatory state of CF patients and the possible effects associated with chronic infections. Another theory would be increased exposure to regular antibiotic therapy causing hepatic damage.

Platelets are indicative of cirrhosis and portal hypertension; therefore, we recommend surveillance endoscopy in line with the Baveno VII recommendations [424].

With the uncertainty given by single serum tests, attention has been given to algorithms, namely APRI and AST to ALT ratio (AAR) and therefore have been incorporated into the Koh diagnostic criteria [319]. It should be noted, the quoted cut-offs for severe fibrosis or cirrhosis are much lower than quoted in other diseases, possibly due their validation occurring in paediatric cohorts [425, 426]. We found the APRI score to be discriminatory for fibrosis whilst using adult cut-offs; we lost significance when the lower paediatric value was applied. We would suggest utilising the adult value.

With the diagnosis of CFLD remaining controversial, several scoring systems have been proposed, though each criterion had weaknesses. Debray missed a patient in opposition to the other criteria and TE. Koh criteria utilised a higher cut-off for TE (6.8kPa) than we did (6kPa). The cut-off values for APRI were the lower values used in the paediatric cohort and used to delineate F1 fibrosis, whilst using a Fib4 cut-off for F4 fibrosis. They also used AAR,

again with a cut-off for cirrhosis, though the reliability of this ratio itself has been brought into question [427]. Within their validation cohort, only APRI demonstrated an abnormal result between groups, whilst all other median values were within normal limits, including TE. However, this study has a long follow-up of its patients and includes stringent inclusion criteria. However, the Koh criteria has been externally validated [428]. Flass et al. encourage the use of platelets for screening, but this is not included within their criteria. The incumbent difficulty for validating any of these criteria is the lack of a gold standard and the higher rate of diagnosis is uncertain clinically.

As shown, the use of TE alone is not safe and requires the addition of other parameters to ensure that patients are not missed or misclassified. The decisions of which parameters to include need further investigation with larger cohorts and the possibility of histology being incorporated, though may include APRI, platelet concentration, US abnormalities and any previous histology.

3.4.4.3 <u>Limitations</u>

This was an audit within a tertiary centre and thus has a selected patient cohort, with access to patients treated in satellite clinics being limited. These may impact in several ways, including differing treatment for CF as a whole, though this is likely minimal, bacterial exposure may differ by area and thus vary antibiotic resistance, as well as the different treatment regimens involved, with several antibiotics noted to affect the liver, e.g. Co-amoxiclav. Other potential confounders may include the local management of associated diseases such as pancreatic insufficiency and the use of UDCA. We offered TE to all patients

regardless of pre-existing clinical suspicion to avoid selection bias, however, only half of patients attended for TE despite three cycles of appointment offers.

CF clinics are inherently difficult due to patients having chronic colonisation of bacteria within their respiratory tract. To avoid cross-contamination, patients are kept within a clinic room with all instruments used for a single patient, with clinicians rotating to minimise risk. These difficulties, alongside the clinics taking place at separate site mean non-participation bias is a possibility and to a degree, will undoubtedly remain. To mitigate this, we invited patients to a non-dedicated TE clinic with the precaution of keeping patients in separate waiting rooms, on days with patients with similar colonisation, whilst spreading out appointments for CF patients with non-CF patients. Though this allowed tests to be performed safely, it also meant an extra clinic appointment and potentially a long journey to the appointment and may partly explain the low uptake of TE. In addition, the three patients identified as likely to have cirrhosis did not attend their appointments; we, therefore, lost a valuable cohort due to this.

The study was carried out prospectively to avoid recall and reporting bias, logged in real-time and designed to keep data collection as objective as possible. To avoid systematic bias, patients were seen on different days and throughout the day.

As described within the Introduction, cut-off values for CFLD diagnosis have been controversial and much lower than accepted values in other diseases. Therefore, we set our cut-off at 8.9kPa for advanced fibrosis or cirrhosis; however, had we utilised a lower value of 8.5kPa, we would have identified extra cases identified with CFLD by other means.

Newer Fibroscan® machines have the added ability to display a Controlled Attenuation Parameter (CAP) score, indicating fatty liver, however, the machine used for this audit did not have this functionality. It was originally thought that this result would not be required for this cohort, however, several patients with raised liver stiffness were found to have fatty liver on US scanning. Therefore, it is unclear whether raised stiffness was secondary to fatty liver or fibrosis or both.

With audits being inherently observational in nature, the obvious difficulty of unavailable data was encountered, possibly as these were not deemed important for certain patients. These were particularly prevalent for tests that needed to be individually requested, particularly AST and GGT. The consequence is limited ability to utilise non-invasive CFLD diagnostic scoring systems. This is particularly prescient for scoring systems that do not incorporate TE, hindering our ability to compare against these. To overcome this, we could take a set of blood tests on all patients when they attend their appointment, however, this is incongruent with the observational design of this audit.

An inherent concern with ultrasound scanning is the difficulty in interpreting images, then misinterpretation of the reports produced. This is a leading reason for the introduction of TE, the presence of an objective score to diagnose patients. To overcome this, we identified any abnormality on US scanning to be positive to avoid this error. This allowed us to contrast US reports with TE results and illuminated the fact of several cases of missed cirrhosis on TE as opposed to US.

We have investigated several markers for patients and considered whether Bonferroni correction was needed, however, as simple tests were performed and overall testing was performed by regression analysis, significance correction is not required.

Though this cohort represents one of the largest studied for cystic fibrosis liver disease, the relative paucity of cases of CFLD, whilst being re-assuring, significantly affects the conclusions which may be drawn from the data. To overcome this, multi-centre or international study will allow for better understanding.

3.4.4.4 Implementation and future work

We have shown the results of an audit of CF services at Barts Health NHS Trust, following the introduction of TE. We aimed to investigate the effect of introducing TE to our diagnostic yield, investigate the prevalence of liver fibrosis as diagnosed by TE and investigate any causative factors which may be associated with the development of an increased liver stiffness. TE either misclassified or completely missed the diagnosis of four patients with cirrhosis while diagnosing a further five patients. This rate is unacceptable to rely on TE as a sole diagnostic test. All current scoring systems have inherent drawbacks, as described above. From our data, the addition of a form of pancreatic dysfunction, either with the inclusion of the use of UDCA or the presence of diabetes mellitus and employing platelets to differentiate patients with cirrhosis. Overall, an amalgamation of all scoring systems would be the most useful to incorporate TE, such as Koh criteria [319], and to allow a staged diagnosis, such as Flass criteria [323], to allow those with the most advanced disease to be treated first. A higher-powered study most likely requiring multi-centre or international collaboration would

be needed to produce and validate this. This was highlighted by the inability of our study to perform multivariate analysis for patients as they shift from severe fibrosis to cirrhosis.

To allow the use of all modalities within a proposed scoring system, a TE service would need to be run alongside routine CF clinic appointments. The obvious difficulty would be equipment availability, particularly in a manner to avoid cross-contamination. Dividing clinic appointments into four patients per clinic to allow them to attend for an elastography reading would be a possible way to overcome this and provide an efficient service. However, this would require a large degree of planning and coordination to ensure patients were not overbooked and TE slots were not wasted; whilst also not interfering with the running of the CF clinic.

The lack of correlated liver biopsies is the obvious limitation of our study and this would be the gold standard for validating any scoring system produced overall. CF patients requiring liver biopsies have a background of underlying respiratory compromise and the introduction of a potentially exacerbating procedure may add an unacceptable level of risk to patients for research purposes.

The use of TE to monitor patients serially is an interesting avenue to examine, especially in conjunction with US and routine blood tests, to understand disease progression and elucidate which investigations give the earliest indication of derangement. Alternatively, the effect of treatment adaption would aid better understanding of the effects on liver prognosis, particularly with increasing evidence for recovery of liver architecture and function following removal of the causative insult.

3.4.5 <u>Conclusion</u>

We performed an audit to understand the impact of introducing transient elastography as a screening tool for all of our patients with cystic fibrosis to understand the diagnostic impact of introducing this service to our patients. Unfortunately, 44.4% of cirrhotic patients were misclassified or missed entirely, an unacceptably high rate. Incorporating TE into a scoring system may allow these patients to be identified appropriately, allowing for early recognition. Current scoring systems show discrepancy in their diagnostic yield and possibly accuracy and an amalgamation and refinement of these would be the best course of action. We identified pancreatic insufficiency, whether this is UDCA treatment or diabetes mellitus, to be indicative of fibrosis or cirrhosis, respectively.

Overall conclusion

Within this thesis, we have looked at non-invasive functional liver tests and their application to various liver diseases both in extension to knowledge already gained as well as in novel ways, with the aim to understand their usefulness and efficacy within different pathologies. The overarching benefit of dynamic liver tests are their ease to perform, speed, low morbidity and reproducibility. We thereby reduce inter-operator variability whilst performing a safe test that can be performed serially, providing a better understanding than current static tests provide.

We have utilised ICG excretion tests in conjunction with transient elastography to monitor patients' excretory liver function before and after treatment for HCV-induced cirrhosis. We show that once the pathogenic agent is removed and SVR is achieved, there is an improvement in liver function alongside reduction in liver stiffness within 12 months. Our understanding of the improvement in liver function was previously based on indirect serum markers or direct invasive measures of portal hypertension. This is the first study to show this functional improvement for patients following DAA treatment. This work needs larger cohort validation, over a longer timeframe, possibly in patients with more advanced disease.

We then investigated patients nationally, being treated for hepatitis C cirrhosis with DAA therapy to understand whether there was an increased rate of de novo HCCs, whether this occurred during or after treatment, whether this was viewable on routine imaging, whether DAA treatment affected HCC progression and if there were any other markers which indicated at-risk patients. We found non-malignant lesions on imaging, in addition to diabetes and a lower platelet count, to be indicative of HCC development. DAA treatment did not

affect HCC development or progression. This is an important series of observations indicating no increased risk of HCC in patients receiving DAA therapy and that pre-existing, presumably benign lesions, may indicate a cohort of patients at high risk of liver cancer following viral clearance.

We also introduced TE to both our adult sickle cell disease and adult cystic fibrosis services and audited the effects for adult-onset disease, which were dissimilar. The diagnostic yield and accuracy for patients with SCLD were high, proving to be a useful screening tool whilst not being hindered by iron deposition within the liver. For CFLD, the sole use of TE is unsatisfactory and therefore may be used as part of a scoring system with other markers of liver disease.

Overall, safety for all non-invasive functional liver test modalities was high, with no patient reacting to the tests with low morbidity and only one patient withdrawing from ICG testing due to difficulty gaining venous access.

We have shown the use and safety of dynamic non-invasive liver tests, either those we have directly investigated or those detailed within the introduction, are overall safe, efficacious, and provide useful information for patient screening, monitoring, and ongoing treatment and management.

References

1.   Williams, R., et al., *Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis.* Lancet, 2014. **384**(9958): p. 1953-97.

2.   *Cirrhosis - NICE CKS.* 2018; Available from: https://cks.nice.org.uk/cirrhosis#!backgroundsub:1.

3.   Ratib, S., J. West, and K.M. Fleming, *Liver cirrhosis in England-an observational study: are we measuring its burden occurrence correctly?* BMJ Open, 2017. **7**(7): p. e013752.

4.   Tsochatzis, E.A., J. Bosch, and A.K. Burroughs, *Liver cirrhosis.* Lancet, 2014. **383**(9930): p. 1749-61.

5.   Lozano, R., et al., *Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010.* Lancet, 2012. **380**(9859): p. 2095-128.

6.   Blachier, M., et al., *The burden of liver disease in Europe: a review of available epidemiological data.* J Hepatol, 2013. **58**(3): p. 593-608.

7.   Ballinger, A.P., S., *Kumar & Clark's Pocket Essentials of Clinical Medicine*. 4 ed, ed. P.C. Kumar, M. 2007: Elsevier.

8.   Nalpas, B., et al., *Interferon gamma receptor 2 gene variants are associated with liver fibrosis in patients with chronic hepatitis C infection.* Gut, 2010. **59**(8): p. 1120-6.

9.   Trepo, E., et al., *Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C.* Hepatology, 2011. **54**(1): p. 60-9.

10.   Trepo, E., et al., *Common polymorphism in the PNPLA3/adiponutrin gene confers higher risk of cirrhosis and liver damage in alcoholic liver disease.* J Hepatol, 2011. **55**(4): p. 906-12.

11.   Schuppan, D. and N.H. Afdhal, *Liver cirrhosis.* Lancet, 2008. **371**(9615): p. 838-51.

12.   Schaffner, F. and H. Poper, *Capillarization of hepatic sinusoids in man.* Gastroenterology, 1963. **44**: p. 239-42.

13.   Popper, H., H. Elias, and D.E. Petty, *Vascular pattern of the cirrhotic liver.* Am J Clin Pathol, 1952. **22**(8): p. 717-29.

14.   Friedman, S.L., *Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury.* J Biol Chem, 2000. **275**(4): p. 2247-50.

15.   Ishak, K., et al., *Histological grading and staging of chronic hepatitis.* J Hepatol, 1995. **22**(6): p. 696-9.

16.   Bedossa, P. and T. Poynard, *An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group.* Hepatology, 1996. **24**(2): p. 289-93.

17.   Garcia-Pagan, J.C., J. Gracia-Sancho, and J. Bosch, *Functional aspects on the pathophysiology of portal hypertension in cirrhosis.* J Hepatol, 2012. **57**(2): p. 458-61.

18.   Bolognesi, M., et al., *Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis.* World J Gastroenterol, 2014. **20**(10): p. 2555-63.

19.   Heim, M.H. and R. Thimme, *Innate and adaptive immune responses in HCV infections.* J Hepatol, 2014. **61**(1 Suppl): p. S14-25.

20.   *EASL Recommendations on Treatment of Hepatitis C 2016.* J Hepatol, 2017. **66**(1): p. 153-194.

21.   *AASLD-IDSA. Recommendations for testing, managing, and treating hepatitis C.* 2017; Available from: http://www.hcvguidelines.org/node/141.

22.   Knodell, R.G., et al., *Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis.* Hepatology, 1981. **1**(5): p. 431-5.

23.   Batts, K.P. and J. Ludwig, *Chronic hepatitis. An update on terminology and reporting.* Am J Surg Pathol, 1995. **19**(12): p. 1409-17.

24.   Rockey, D.C., et al., *Liver biopsy.* Hepatology, 2009. **49**(3): p. 1017-44.

25.   Scheuer, P.J., *Classification of chronic viral hepatitis: a need for reassessment.* J Hepatol, 1991. **13**(3): p. 372-4.

26.   Child, C.G. and J.G. Turcotte, *Surgery and portal hypertension.* Major Probl Clin Surg, 1964. **1**: p. 1-85.

27.   Cholongitas, E., et al., *Systematic review: The model for end-stage liver disease--should it replace Child-Pugh's classification for assessing prognosis in cirrhosis?* Aliment Pharmacol Ther, 2005. **22**(11-12): p. 1079-89.

28.   Kamath, P.S. and W.R. Kim, *The model for end-stage liver disease (MELD).* Hepatology, 2007. **45**(3): p. 797-805.

29.   Kamath, P.S., et al., *A model to predict survival in patients with end-stage liver disease.* Hepatology, 2001. **33**(2): p. 464-70.

30.   Tian, Z., Y. Chen, and B. Gao, *Natural killer cells in liver disease.* Hepatology, 2013. **57**(4): p. 1654-62.

31.   Ramachandran, P., et al., *Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis.* Proc Natl Acad Sci U S A, 2012. **109**(46): p. E3186-95.

32.   Jiao, J., et al., *Dendritic cell regulation of carbon tetrachloride-induced murine liver fibrosis regression.* Hepatology, 2012. **55**(1): p. 244-55.

33.   Hammerich, L., et al., *Chemokine receptor CCR6-dependent accumulation of gammadelta T cells in injured liver restricts hepatic inflammation and fibrosis.* Hepatology, 2014. **59**(2): p. 630-42.

34.   Tacke, F. and C. Trautwein, *Mechanisms of liver fibrosis resolution.* J Hepatol, 2015. **63**(4): p. 1038-9.

35.   Yang, L., et al., *Vascular endothelial growth factor promotes fibrosis resolution and repair in mice.* Gastroenterology, 2014. **146**(5): p. 1339-50.e1.

36.   Kantari-Mimoun, C., et al., *Resolution of liver fibrosis requires myeloid cell-driven sinusoidal angiogenesis.* Hepatology, 2015. **61**(6): p. 2042-55.

37.   Wanless, I.R., E. Nakashima, and M. Sherman, *Regression of human cirrhosis. Morphologic features and the genesis of incomplete septal cirrhosis.* Arch Pathol Lab Med, 2000. **124**(11): p. 1599-607.

38.   Sun, Y., et al., *New classification of liver biopsy assessment for fibrosis in chronic hepatitis B patients before and after treatment.* Hepatology, 2017. **65**(5): p. 1438-1450.

39.   Lo, R.C. and H. Kim, *Histopathological evaluation of liver fibrosis and cirrhosis regression*, in *Clin Mol Hepatol*. 2017. p. 302-7.

40.   Mathiesen, U.L., et al., *Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases.* Dig Liver Dis, 2002. **34**(7): p. 516-22.

41.   Heimbach, J.K., et al., *AASLD guidelines for the treatment of hepatocellular carcinoma.* Hepatology, 2018. **67**(1): p. 358-380.

42. *EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma.* J Hepatol, 2018. **69**(1): p. 182-236.

43. Allan, R., K. Thoirs, and M. Phillips, *Accuracy of ultrasound to identify chronic liver disease.* World J Gastroenterol, 2010. **16**(28): p. 3510-20.

44. Kudo, M., et al., *Diagnostic accuracy of imaging for liver cirrhosis compared to histologically proven liver cirrhosis. A multicenter collaborative study.* Intervirology, 2008. **51 Suppl 1**: p. 17-26.

45. Huber, A., et al., *Computed tomography findings in liver fibrosis and cirrhosis.* Swiss Med Wkly, 2014. **144**: p. w13923.

46. Albiin, N., *MRI of Focal Liver Lesions*, in *Curr Med Imaging Rev*. 2012. p. 107-16.

47. Venkatesh, S.K., et al., *Non-invasive detection of liver fibrosis: MR imaging features vs. MR elastography.* Abdom Imaging, 2015. **40**(4): p. 766-75.

48. Rustogi, R., et al., *Accuracy of MR elastography and anatomic MR imaging features in the diagnosis of severe hepatic fibrosis and cirrhosis.* J Magn Reson Imaging, 2012. **35**(6): p. 1356-64.

49. Dietrich, C.F., et al., *EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, Update 2017 (Long Version).* Ultraschall Med, 2017. **38**(4): p. e16-e47.

50. Gennisson, J.L., et al., *Ultrasound elastography: principles and techniques.* Diagn Interv Imaging, 2013. **94**(5): p. 487-95.

51. Sarvazyan, A.P., et al., *Biophysical Bases of Elasticity Imaging*, in *Acoustical Imaging*, J.P. Jones, Editor. 1995, Springer US: Boston, MA. p. 223-240.

52. Shiina, T., et al., *WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: basic principles and terminology.* Ultrasound Med Biol, 2015. **41**(5): p. 1126-47.

53. Bamber, J., et al., *EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology.* Ultraschall Med, 2013. **34**(2): p. 169-84.

54. Britannica, T.E.o.E., *encyclopaedia Britannica*, in *Encyclopaedia Britannica*. 2018, Encyclopædia Britannica, inc.

55. Sandrin, L., et al., *Transient elastography: a new noninvasive method for assessment of hepatic fibrosis.* Ultrasound Med Biol, 2003. **29**(12): p. 1705-13.

56. Sandrin, L., et al., *Shear modulus imaging with 2-D transient elastography.* IEEE Trans Ultrason Ferroelectr Freq Control, 2002. **49**(4): p. 426-35.

57. Oudry, J., et al., *Comparison of four different techniques to evaluate the elastic properties of phantom in elastography: is there a gold standard?* Phys Med Biol, 2014. **59**(19): p. 5775-93.

58. Sandrin, L., et al., *Time-resolved pulsed elastography with ultrafast ultrasonic imaging.* Ultrason Imaging, 1999. **21**(4): p. 259-72.

59. Sandrin, L., et al., *Shear elasticity probe for soft tissues with 1-D transient elastography.* IEEE Trans Ultrason Ferroelectr Freq Control, 2002. **49**(4): p. 436-46.

60. Foucher, J., et al., *Prevalence and factors associated with failure of liver stiffness measurement using FibroScan in a prospective study of 2114 examinations.* Eur J Gastroenterol Hepatol, 2006. **18**(4): p. 411-2.

61. de Ledinghen, V., et al., *Feasibility of liver transient elastography with FibroScan using a new probe for obese patients.* Liver Int, 2010. **30**(7): p. 1043-8.

62. Fraquelli, M., et al., *Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease.* Gut, 2007. **56**(7): p. 968-73.

63.   Audiere, S., et al., *Measurement of the skin-liver capsule distance on ultrasound RF data for 1D transient elastography.* Med Image Comput Comput Assist Interv, 2010. **13**(Pt 2): p. 34-41.

64.   Myers, R.P., et al., *Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients.* Hepatology, 2012. **55**(1): p. 199-208.

65.   Catheline, S., et al., *Diffraction field of a low frequency vibrator in soft tissues using transient elastography.* IEEE Trans Ultrason Ferroelectr Freq Control, 1999. **46**(4): p. 1013-9.

66.   Catheline, S., F. Wu, and M. Fink, *A solution to diffraction biases in sonoelasticity: the acoustic impulse technique.* J Acoust Soc Am, 1999. **105**(5): p. 2941-50.

67.   Lucidarme, D., et al., *Factors of accuracy of transient elastography (fibroscan) for the diagnosis of liver fibrosis in chronic hepatitis C.* Hepatology, 2009. **49**(4): p. 1083-9.

68.   Ziol, M., et al., *Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C.* Hepatology, 2005. **41**(1): p. 48-54.

69.   Lupsor Platon, M., et al., *Performance of unidimensional transient elastography in staging chronic hepatitis C. Results from a cohort of 1,202 biopsied patients from one single center.* J Gastrointestin Liver Dis, 2013. **22**(2): p. 157-66.

70.   Colletta, C., et al., *Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases.* Hepatology, 2005. **42**(4): p. 838-45.

71.   Friedrich-Rust, M., et al., *Performance of transient elastography for the staging of liver fibrosis: a meta-analysis.* Gastroenterology, 2008. **134**(4): p. 960-74.

72.   Castera, L., et al., *Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C.* Gastroenterology, 2005. **128**(2): p. 343-50.

73.   Afdhal, N.H., et al., *Accuracy of fibroscan, compared with histology, in analysis of liver fibrosis in patients with hepatitis B or C: a United States multicenter study.* Clin Gastroenterol Hepatol, 2015. **13**(4): p. 772-9.e1-3.

74.   Tsochatzis, E.A., et al., *Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy.* J Hepatol, 2011. **54**(4): p. 650-9.

75.   Talwalkar, J.A., et al., *Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and meta-analysis.* Clin Gastroenterol Hepatol, 2007. **5**(10): p. 1214-20.

76.   Singh, S., et al., *American Gastroenterological Association Institute Technical Review on the Role of Elastography in Chronic Liver Diseases.* Gastroenterology, 2017. **152**(6): p. 1544-1577.

77.   de Franchis, R., *Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension.* J Hepatol, 2015. **63**(3): p. 743-52.

78.   Kim, S.U., K.H. Han, and S.H. Ahn, *Transient elastography in chronic hepatitis B: an Asian perspective.* World J Gastroenterol, 2010. **16**(41): p. 5173-80.

79.   Horowitz, J.M., et al., *Evaluation of Hepatic Fibrosis: A Review from the Society of Abdominal Radiology Disease Focus Panel.* Abdom Radiol (NY), 2017. **42**(8): p. 2037-53.

80.   Neukam, K., et al., *Impact of observer experience on the reproducibility of transient elastometry in HIV/HCV co-infected patients.* HIV Clin Trials, 2009. **10**(4): p. 276-81.

81.   Armstrong, M.J., et al., *Operator training requirements and diagnostic accuracy of Fibroscan in routine clinical practice*, in *Postgrad Med J.* 2013. p. 685-92.

82.   Pang, J.X., et al., *The feasibility and reliability of transient elastography using Fibroscan(R): a practice audit of 2335 examinations.* Can J Gastroenterol Hepatol, 2014. **28**(3): p. 143-9.

83.   Castera, L., et al., *Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations.* Hepatology, 2010. **51**(3): p. 828-35.

84.   Boursier, J., et al., *Learning curve and interobserver reproducibility evaluation of liver stiffness measurement by transient elastography.* Eur J Gastroenterol Hepatol, 2008. **20**(7): p. 693-701.

85.   Fabrellas, N., et al., *Using transient elastography to detect chronic liver diseases in a primary care nurse consultancy.* Nurs Res, 2013. **62**(6): p. 450-4.

86.   Deleanu, A., et al., *Feasability, accuracy and reproducibility of transient elastography.* 2009, 2009. **11**(4): p. 5.

87.   Boursier, J., et al., *Reproducibility of liver stiffness measurement by ultrasonographic elastometry.* Clin Gastroenterol Hepatol, 2008. **6**(11): p. 1263-9.

88.   van Katwyk, S., et al., *Transient elastography for the diagnosis of liver fibrosis: a systematic review of economic evaluations.* Liver Int, 2017. **37**(6): p. 851-861.

89.   Vigano, M., et al., *Transient elastography assessment of the liver stiffness dynamics during acute hepatitis B.* Eur J Gastroenterol Hepatol, 2010. **22**(2): p. 180-4.

90.   Sagir, A., et al., *Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage.* Hepatology, 2008. **47**(2): p. 592-5.

91.   Millonig, G., et al., *Liver stiffness is directly influenced by central venous pressure.* J Hepatol, 2010. **52**(2): p. 206-10.

92.   Coco, B., et al., *Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases.* J Viral Hepat, 2007. **14**(5): p. 360-9.

93.   Arena, U., et al., *Acute viral hepatitis increases liver stiffness values measured by transient elastography.* Hepatology, 2008. **47**(2): p. 380-4.

94.   Colli, A., et al., *Decompensated chronic heart failure: increased liver stiffness measured by means of transient elastography.* Radiology, 2010. **257**(3): p. 872-8.

95.   Millonig, G., et al., *Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis.* Hepatology, 2008. **48**(5): p. 1718-23.

96.   Arena, U., et al., *Liver stiffness is influenced by a standardized meal in patients with chronic hepatitis C virus at different stages of fibrotic evolution.* Hepatology, 2013. **58**(1): p. 65-72.

97.   Mederacke, I., et al., *Food intake increases liver stiffness in patients with chronic or resolved hepatitis C virus infection.* Liver Int, 2009. **29**(10): p. 1500-6.

98.   Friedrich-Rust, M., et al., *Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis.* J Viral Hepat, 2012. **19**(2): p. e212-9.

99.   Nightingale, K.R., et al., *On the feasibility of remote palpation using acoustic radiation force.* J Acoust Soc Am, 2001. **110**(1): p. 625-34.

100. Nierhoff, J., et al., *The efficiency of acoustic radiation force impulse imaging for the staging of liver fibrosis: a meta-analysis.* Eur Radiol, 2013. **23**(11): p. 3040-53.

101. Sporea, I., et al., *Acoustic Radiation Force Impulse elastography for fibrosis evaluation in patients with chronic hepatitis C: an international multicenter study.* Eur J Radiol, 2012. **81**(12): p. 4112-8.

102. Herrmann, E., et al., *Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: An individual patient data-based meta-analysis.* Hepatology, 2018. **67**(1): p. 260-272.

103.   Tang, A., et al., *Ultrasound Elastography and MR Elastography for Assessing Liver Fibrosis: Part 1, Principles and Techniques.* AJR Am J Roentgenol, 2015. **205**(1): p. 22-32.

104.   Low, G., S.A. Kruse, and D.J. Lomas, *General review of magnetic resonance elastography.* World J Radiol, 2016. **8**(1): p. 59-72.

105.   Singh, S., et al., *Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data.* Clin Gastroenterol Hepatol, 2015. **13**(3): p. 440-451.e6.

106.   Yin, M., et al., *Assessment of hepatic fibrosis with magnetic resonance elastography.* Clin Gastroenterol Hepatol, 2007. **5**(10): p. 1207-1213.e2.

107.   Volpi, N., et al., *Role, metabolism, chemical modifications and applications of hyaluronan.* Curr Med Chem, 2009. **16**(14): p. 1718-45.

108.   Poynard, T., et al., *Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C.* Clin Chem, 2004. **50**(8): p. 1344-55.

109.   Piton, A., et al., *Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group.* Hepatology, 1998. **27**(5): p. 1213-9.

110.   Wai, C.T., et al., *A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C.* Hepatology, 2003. **38**(2): p. 518-26.

111.   Lin, Z.H., et al., *Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis.* Hepatology, 2011. **53**(3): p. 726-36.

112.   Chou, R. and N. Wasson, *Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection: a systematic review.* Ann Intern Med, 2013. **158**(11): p. 807-20.

113.   *EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis.* J Hepatol, 2015. **63**(1): p. 237-64.

114.   Wong, G.L., *Non-invasive assessments for liver fibrosis: The crystal ball we long for.* J Gastroenterol Hepatol, 2018. **33**(5): p. 1009-1015.

115.   Popescu, A., et al., *The influence of food intake on liver stiffness values assessed by acoustic radiation force impulse elastography-preliminary results.* Ultrasound Med Biol, 2013. **39**(4): p. 579-84.

116.   Bota, S., et al., *Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis.* Liver Int, 2013. **33**(8): p. 1138-47.

117.   Friedrich-Rust, M., et al., *Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography.* Radiology, 2009. **252**(2): p. 595-604.

118.   Rizzo, L., et al., *Comparison of transient elastography and acoustic radiation force impulse for non-invasive staging of liver fibrosis in patients with chronic hepatitis C.* Am J Gastroenterol, 2011. **106**(12): p. 2112-20.

119.   Cassinotto, C., et al., *Liver fibrosis: noninvasive assessment with acoustic radiation force impulse elastography--comparison with FibroScan M and XL probes and FibroTest in patients with chronic liver disease.* Radiology, 2013. **269**(1): p. 283-92.

120.   Kircheis, G., et al., *Evaluation of acoustic radiation force impulse imaging for determination of liver stiffness using transient elastography as a reference.* World J Gastroenterol, 2012. **18**(10): p. 1077-84.

121. Ebinuma, H., et al., *Evaluation of liver fibrosis by transient elastography using acoustic radiation force impulse: comparison with Fibroscan((R)).* J Gastroenterol, 2011. **46**(10): p. 1238-48.

122. Rifai, K., et al., *Clinical feasibility of liver elastography by acoustic radiation force impulse imaging (ARFI).* Dig Liver Dis, 2011. **43**(6): p. 491-7.

123. Vermehren, J., et al., *Comparison of acoustic radiation force impulse imaging with transient elastography for the detection of complications in patients with cirrhosis.* Liver Int, 2012. **32**(5): p. 852-8.

124. Poynard, T., et al., *Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C.* J Hepatol, 2013. **59**(4): p. 675-83.

125. Elkrief, L., et al., *Prospective comparison of spleen and liver stiffness by using shear-wave and transient elastography for detection of portal hypertension in cirrhosis.* Radiology, 2015. **275**(2): p. 589-98.

126. Sporea, I., et al., *Acoustic radiation force impulse and supersonic shear imaging versus transient elastography for liver fibrosis assessment.* Ultrasound Med Biol, 2013. **39**(11): p. 1933-41.

127. Degos, F., et al., *Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study).* J Hepatol, 2010. **53**(6): p. 1013-21.

128. Boursier, J., et al., *A new combination of blood test and fibroscan for accurate non-invasive diagnosis of liver fibrosis stages in chronic hepatitis C.* Am J Gastroenterol, 2011. **106**(7): p. 1255-63.

129. Bende, F., et al., *Performance of 2D-SWE.GE for predicting different stages of liver fibrosis, using Transient Elastography as the reference method.* Med Ultrason, 2017. **19**(2): p. 143-149.

130. Guo, Y., et al., *Magnetic resonance elastography and acoustic radiation force impulse for staging hepatic fibrosis: a meta-analysis.* Abdom Imaging, 2015. **40**(4): p. 818-34.

131. Yoon, J.H., et al., *Hepatic fibrosis: prospective comparison of MR elastography and US shear-wave elastography for evaluation.* Radiology, 2014. **273**(3): p. 772-82.

132. Toguchi, M., et al., *Magnetic resonance elastography in the assessment of hepatic fibrosis: a study comparing transient elastography and histological data in the same patients.* Abdom Radiol (NY), 2017. **42**(6): p. 1659-1666.

133. Imajo, K., et al., *Magnetic Resonance Imaging More Accurately Classifies Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease Than Transient Elastography.* Gastroenterology, 2016. **150**(3): p. 626-637.e7.

134. De Gasperi, A., E. Mazza, and M. Prosperi, *Indocyanine green kinetics to assess liver function: Ready for a clinical dynamic assessment in major liver surgery?* World J Hepatol, 2016. **8**(7): p. 355-67.

135. SE, P.M.S., *ICG-Pulsion - physicians prescribing information*, P.M.S. SE, Editor. 2015.

136. Faybik, P. and H. Hetz, *Plasma disappearance rate of indocyanine green in liver dysfunction.* Transplant Proc, 2006. **38**(3): p. 801-2.

137. de Graaf, W., et al., *Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green.* J Hepatol, 2011. **54**(4): p. 738-45.

138. Baker, K.J., *Binding of sulfobromophthalein (BSP) sodium and indocyanine green (ICG) by plasma alpha-1 lipoproteins.* Proc Soc Exp Biol Med, 1966. **122**(4): p. 957-63.

139. Leevy, C.M., et al., *ESTIMATION OF HEPATIC BLOOD FLOW WITH INDOCYANINE GREEN**. J Clin Invest, 1962. **41**(5): p. 1169-79.

140. Paumgartner, G., et al., *KINETICS OF INDOCYANINE GREEN REMOVAL FROM THE BLOOD**. Annals of the New York Academy of Sciences, 1970. **170**(1): p. 134-147.

141. Leevy, C.M., et al., *PHYSIOLOGY OF DYE EXTRACTION BY THE LIVER: COMPARATIVE STUDIES OF SULFOBROMOPHTHALEIN AND INDOCYANINE GREEN**. Annals of the New York Academy of Sciences, 1963. **111**(1): p. 161-175.

142. Vos, J.J., et al., *Green light for liver function monitoring using indocyanine green? An overview of current clinical applications.* Anaesthesia, 2014. **69**(12): p. 1364-76.

143. de Liguori Carino, N., et al., *Perioperative use of the LiMON method of indocyanine green elimination measurement for the prediction and early detection of post-hepatectomy liver failure.* Eur J Surg Oncol, 2009. **35**(9): p. 957-62.

144. Huet, P.M., et al., *Assessment of liver microcirculation in human cirrhosis.* J Clin Invest, 1982. **70**(6): p. 1234-44.

145. Kawasaki, S., et al., *Hepatic clearances of antipyrine, indocyanine green, and galactose in normal subjects and in patients with chronic liver diseases.* Clin Pharmacol Ther, 1988. **44**(2): p. 217-24.

146. Kawasaki, S., et al., *Pharmacokinetic study on the hepatic uptake of indocyanine green in cirrhotic patients.* Am J Gastroenterol, 1985. **80**(10): p. 801-6.

147. Shinohara, H., et al., *Direct measurement of hepatic indocyanine green clearance with near-infrared spectroscopy: separate evaluation of uptake and removal.* Hepatology, 1996. **23**(1): p. 137-44.

148. Sheng, Q.S., et al., *Indocyanine green clearance test and model for end-stage liver disease score of patients with liver cirrhosis.* Hepatobiliary Pancreat Dis Int, 2009. **8**(1): p. 46-9.

149. Corradi, F., et al., *Assessment of liver fibrosis in transplant recipients with recurrent HCV infection: usefulness of transient elastography.* Dig Liver Dis, 2009. **41**(3): p. 217-25.

150. Castera, L., X. Forns, and A. Alberti, *Non-invasive evaluation of liver fibrosis using transient elastography.* J Hepatol, 2008. **48**(5): p. 835-47.

151. Wilder, J. and K. Patel, *The clinical utility of FibroScan((R)) as a noninvasive diagnostic test for liver disease.* Med Devices (Auckl), 2014. **7**: p. 107-14.

152. Gupta, S., et al., *Indocyanine green clearance test (using spectrophotometry) and its correlation with model for end stage liver disease (MELD) score in Indian patients with cirrhosis of liver.* Trop Gastroenterol, 2012. **33**(2): p. 129-34.

153. Imai, T., et al., *Measurement of blood concentration of indocyanine green by pulse dye densitometry--comparison with the conventional spectrophotometric method.* J Clin Monit Comput, 1998. **14**(7-8): p. 477-84.

154. von Spiegel, T., et al., *Perioperative monitoring of indocyanine green clearance and plasma disappearance rate in patients undergoing liver transplantation.* Anaesthesist, 2002. **51**(5): p. 359-66.

155. Sakka, S.G. and N. van Hout, *Relation between indocyanine green (ICG) plasma disappearance rate and ICG blood clearance in critically ill patients.* Intensive Care Med, 2006. **32**(5): p. 766-9.

156. Omagari, K., et al., *Comparison of the grade evaluated by "Liver damage" of Liver Cancer Study Group of Japan and Child-Pugh classification in patients with hepatocellular carcinoma.* Hepatol Res, 2006. **34**(4): p. 266-72.

157. Albers, I., et al., *Superiority of the Child-Pugh classification to quantitative liver function tests for assessing prognosis of liver cirrhosis.* Scand J Gastroenterol, 1989. **24**(3): p. 269-76.

158. Merkel, C., et al., *Indocyanine green intrinsic hepatic clearance as a prognostic index of survival in patients with cirrhosis.* J Hepatol, 1989. **9**(1): p. 16-22.

159. Herold, C., et al., *Quantitative testing of liver function in patients with cirrhosis due to chronic hepatitis C to assess disease severity.* Liver, 2001. **21**(1): p. 26-30.

160. Mukherjee, S., M.A. Rogers, and B. Buniak, *Comparison of indocyanine green clearance with Child's-Pugh score and hepatic histology: a multivariate analysis.* Hepatogastroenterology, 2006. **53**(67): p. 120-3.

161. Stauber, R.E., et al., *Evaluation of indocyanine green clearance and model for end-stage liver disease for estimation of short-term prognosis in decompensated cirrhosis.* Liver Int, 2009. **29**(10): p. 1516-20.

162. Cholongitas, E. and A.K. Burroughs, *The evolution in the prioritization for liver transplantation*, in *Ann Gastroenterol*. 2012. p. 6-13.

163. Zipprich, A., et al., *Incorporating indocyanin green clearance into the Model for End Stage Liver Disease (MELD-ICG) improves prognostic accuracy in intermediate to advanced cirrhosis.* Gut, 2010. **59**(7): p. 963-8.

164. Lisotti, A., et al., *Indocyanine green retention test as a noninvasive marker of portal hypertension and esophageal varices in compensated liver cirrhosis.* Hepatology, 2014. **59**(2): p. 643-50.

165. Pind, M.L., et al., *Indocyanine green retention test (ICG-r15) as a noninvasive predictor of portal hypertension in patients with different severity of cirrhosis.* Eur J Gastroenterol Hepatol, 2016. **28**(8): p. 948-54.

166. Pind, M.L., et al., *Predictive value of indocyanine green retention test and indocyanine green clearance in Child-Pugh class A patients.* Hepatology, 2015. **61**(6): p. 2112-3.

167. Everson, G.T., et al., *Quantitative tests of liver function measure hepatic improvement after sustained virological response: results from the HALT-C trial.* Aliment Pharmacol Ther, 2009. **29**(5): p. 589-601.

168. Everson, G.T., et al., *Quantitative liver function tests improve the prediction of clinical outcomes in chronic hepatitis C: results from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis Trial.* Hepatology, 2012. **55**(4): p. 1019-29.

169. Everson, G.T., et al., *The spectrum of hepatic functional impairment in compensated chronic hepatitis C: results from the Hepatitis C Anti-viral Long-term Treatment against Cirrhosis Trial.* Aliment Pharmacol Ther, 2008. **27**(9): p. 798-809.

170. Di Bisceglie, A.M., et al., *Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon.* N Engl J Med, 2008. **359**(23): p. 2429-41.

171. Addario, L., et al., *Prognostic value of quantitative liver function tests in viral cirrhosis: a prospective study.* Eur J Gastroenterol Hepatol, 2006. **18**(7): p. 713-20.

172. Botta, F., et al., *MELD scoring system is useful for predicting prognosis in patients with liver cirrhosis and is correlated with residual liver function: a European study*, in *Gut*. 2003. p. 134-9.

173. Forestier, J., et al., *Noninvasive diagnosis and prognosis of liver cirrhosis: a comparison of biological scores, elastometry, and metabolic liver function tests.* Eur J Gastroenterol Hepatol, 2010. **22**(5): p. 532-40.

174. Choo, Q.L., et al., *Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome.* Science, 1989. **244**(4902): p. 359-62.

175. Abdel-Ghaffar, T.Y., M.M. Sira, and S. El Naghi, *Hepatitis C genotype 4: The past, present, and future.* World J Hepatol, 2015. **7**(28): p. 2792-810.

176.   *WHO | Hepatitis C.* WHO, 2016.

177.   Frank, C., et al., *The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt.* Lancet, 2000. **355**(9207): p. 887-91.

178.   Larney, S., et al., *Incidence and prevalence of hepatitis C in prisons and other closed settings: results of a systematic review and meta-analysis.* Hepatology, 2013. **58**(4): p. 1215-24.

179.   Midgard, H., et al., *Hepatitis C reinfection after sustained virological response.* J Hepatol, 2016. **64**(5): p. 1020-6.

180.   Mecci, A.J.a.C., M., *Treatment of Hepatitis C.* eLS, 2017.

181.   Yanagi, M., et al., *Hepatitis C virus in fulminant hepatic failure.* N Engl J Med, 1991. **324**(26): p. 1895-6.

182.   Chen, S.L. and T.R. Morgan, *The Natural History of Hepatitis C Virus (HCV) Infection*, in *Int J Med Sci.* 2006. p. 47-52.

183.   co-ordinator), A.C.R., et al., *Hepatitis C in England 2017 report.* PHE publications gateway number: 2016667, 2017: p. 57.

184.   Martinot-Peignoux, M., et al., *Twelve weeks posttreatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin.* Hepatology, 2010. **51**(4): p. 1122-6.

185.   van der Meer, A.J. and M. Berenguer, *Reversion of disease manifestations after HCV eradication.* J Hepatol, 2016. **65**(1 Suppl): p. S95-s108.

186.   van der Meer, A.J., et al., *Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication.* J Hepatol, 2017. **66**(3): p. 485-493.

187.   Younossi, Z.M., et al., *Ribavirin-Free Regimen With Sofosbuvir and Velpatasvir Is Associated With High Efficacy and Improvement of Patient-Reported Outcomes in Patients With Genotypes 2 and 3 Chronic Hepatitis C: Results From Astral-2 and -3 Clinical Trials.* Clin Infect Dis, 2016. **63**(8): p. 1042-8.

188.   Innes, H., et al., *The risk of hepatocellular carcinoma in cirrhotic patients with hepatitis C and sustained viral response: role of the treatment regimen.* Journal of Hepatology, 2017. **0**(0).

189.   Innes, H., et al., *Mortality in hepatitis C patients who achieve a sustained viral response compared to the general population.* J Hepatol, 2017. **66**(1): p. 19-27.

190.   Cheung, M.C., et al., *Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis.* J Hepatol, 2016. **65**(4): p. 741-7.

191.   Simmons, R., et al., *Causes of death among persons diagnosed with hepatitis C infection in the pre- and post-DAA era in England: A record linkage study.* J Viral Hepat, 2019. **26**(7): p. 873-880.

192.   Harris, M., *Hepatitis C virus*, in *eLS.* 2001, John Wiley & Sons, Ltd.

193.   Rehermann, B., *Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence.* J Clin Invest, 2009. **119**(7): p. 1745-54.

194.   Poordad, F., et al., *Boceprevir for untreated chronic HCV genotype 1 infection.* N Engl J Med, 2011. **364**(13): p. 1195-206.

195.   Sherman, K.E., et al., *Response-guided telaprevir combination treatment for hepatitis C virus infection.* N Engl J Med, 2011. **365**(11): p. 1014-24.

196.   Lesburg, C.A., et al., *Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site.* Nat Struct Biol, 1999. **6**(10): p. 937-43.

197. Curry, M.P., et al., *Sofosbuvir and Velpatasvir for HCV in Patients with Decompensated Cirrhosis.* N Engl J Med, 2015. **373**(27): p. 2618-28.

198. Foster, G.R., et al., *Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection.* N Engl J Med, 2015. **373**(27): p. 2608-17.

199. GR Foster, J.M., W. Irving, M. Cheung, B. Hudson, S. Verma, K. Agarwal, HCV Research UK EAP Group, *Treatment of decompensated HCV cirrhosis in patients with diverse genotypes: 12 weeks sofosbuvir and NS5A inhibitors with/without ribavirin is effective in HCV Genotypes 1 and 3.* EASL, 2015.

200. Nelson, D.R., et al., *All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study.* Hepatology, 2015. **61**(4): p. 1127-35.

201. Asselah, T., et al., *High SVR Rates in Patients with Genotype 4 Chronic Hepatitis C Infection and Compensated Cirrhosis with Ombitasvir/Paritaprevir/Ritonavir Co-Administered with Ribavirin (AGATE-I).* Journal of Hepatology, 2016. **64**(2).

202. Sperl, J., et al., *Efficacy and safety of elbasvir/grazoprevir and sofosbuvir/pegylated interferon/ribavirin: A phase III randomized controlled trial.* J Hepatol, 2016. **65**(6): p. 1112-1119.

203. Zeuzem, S., et al., *Glecaprevir-Pibrentasvir for 8 or 12 Weeks in HCV Genotype 1 or 3 Infection.* N Engl J Med, 2018. **378**(4): p. 354-369.

204. Smith, D.A., et al., *Real world SOF/VEL/VOX retreatment outcomes and viral resistance analysis for HCV patients with prior failure to DAA therapy.* J Viral Hepat, 2021. **28**(9): p. 1256-1264.

205. Heim, M.H., *25 years of interferon-based treatment of chronic hepatitis C: an epoch coming to an end*, in *Nat Rev Immunol.* 2013: England. p. 535-42.

206. Fernandez-Rodriguez, C.M., et al., *Peginterferon plus ribavirin and sustained virological response in HCV-related cirrhosis: outcomes and factors predicting response.* Am J Gastroenterol, 2010. **105**(10): p. 2164-72; quiz 2173.

207. Manns, M., et al., *Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial.* Lancet Infect Dis, 2016. **16**(6): p. 685-97.

208. Afdhal, N., et al., *Effect of viral suppression on hepatic venous pressure gradient in hepatitis C with cirrhosis and portal hypertension.* J Viral Hepat, 2017. **24**(10): p. 823-831.

209. *EASL Recommendations on Treatment of Hepatitis C 2018.* J Hepatol, 2018. **69**(2): p. 461-511.

210. Transplant., N.B.a., *Annual Report on Liver Transplantation 2016/2017.* 2017.

211. Belli, L.S., et al., *Delisting of liver transplant candidates with chronic hepatitis C after viral eradication: A European study.* J Hepatol, 2016. **65**(3): p. 524-31.

212. Poynard, T., et al., *A comparison of three interferon alfa-2b regimens for the long-term treatment of chronic non-A, non-B hepatitis. Multicenter Study Group.* N Engl J Med, 1995. **332**(22): p. 1457-62.

213. Beccarello, A., et al., *Monoethylglycinexylide kinetics and galactose elimination capacity during treatment with interferon-alfa for hepatitis C virus infection: Possible predictors of response?* Current Therapeutic Research, 2002. **63**(11): p. 772-785.

214. Hezode, C., et al., *Liver stiffness diminishes with antiviral response in chronic hepatitis C.* Aliment Pharmacol Ther, 2011. **34**(6): p. 656-63.

215. Wang, J.H., et al., *Liver stiffness decrease after effective antiviral therapy in patients with chronic hepatitis C: Longitudinal study using FibroScan.* J Gastroenterol Hepatol, 2010. **25**(5): p. 964-9.

216. D'Ambrosio, R., et al., *The diagnostic accuracy of Fibroscan for cirrhosis is influenced by liver morphometry in HCV patients with a sustained virological response.* J Hepatol, 2013. **59**(2): p. 251-6.

217. Crisan, D., et al., *Prospective non-invasive follow-up of liver fibrosis in patients with chronic hepatitis C.* J Gastrointestin Liver Dis, 2012. **21**(4): p. 375-82.

218. Arima, Y., et al., *Reduction of liver stiffness by interferon treatment in the patients with chronic hepatitis C.* Hepatol Res, 2010. **40**(4): p. 383-92.

219. Martinez, S.M., et al., *Longitudinal liver stiffness assessment in patients with chronic hepatitis C undergoing antiviral therapy.* PLoS One, 2012. **7**(10): p. e47715.

220. D'Ambrosio, R., et al., *Serological Tests Do Not Predict Residual Fibrosis in Hepatitis C Cirrhotics with a Sustained Virological Response to Interferon.* PLoS One, 2016. **11**(6): p. e0155967.

221. Elsharkawy, A., et al., *Changes in liver stiffness measurements and fibrosis scores following sofosbuvir based treatment regimens without interferon.* J Gastroenterol Hepatol, 2017. **32**(9): p. 1624-1630.

222. Sporea, I., et al., *Dynamics of liver stiffness values by means of transient elastography in patients with HCV liver cirrhosis undergoing interferon free treatment.* J Gastrointestin Liver Dis, 2017. **26**(2): p. 145-150.

223. Olveira, A., et al., *Persistently altered liver test results in hepatitis C patients after sustained virological response with direct-acting antivirals.* J Viral Hepat, 2018. **25**(7): p. 818-824.

224. Mandorfer, M., et al., *Sustained virologic response to interferon-free therapies ameliorates HCV-induced portal hypertension.* J Hepatol, 2016. **65**(4): p. 692-9.

225. Lens, S., et al., *Effects of All-Oral Anti-Viral Therapy on HVPG and Systemic Hemodynamics in Patients With Hepatitis C Virus-Associated Cirrhosis.* Gastroenterology, 2017. **153**(5): p. 1273-1283.e1.

226. Chung, R.T. and T.F. Baumert, *Curing chronic hepatitis C--the arc of a medical triumph.* N Engl J Med, 2014. **370**(17): p. 1576-8.

227. Reig, M., et al., *Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy.* J Hepatol, 2016.

228. Conti, F., et al., *Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals.* J Hepatol, 2016. **65**(4): p. 727-733.

229. Kozbial, K., et al., *Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment.* J Hepatol, 2016. **65**(4): p. 856-8.

230. Cardoso, H., et al., *High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis.* J Hepatol, 2016. **65**(5): p. 1070-1071.

231. Ravi, S., et al., *Unusually High Rates of Hepatocellular Carcinoma After Treatment With Direct-Acting Antiviral Therapy for Hepatitis C Related Cirrhosis.* Gastroenterology, 2017. **152**(4): p. 911-912.

232. Bielen, R., et al., *The risk of early occurrence and recurrence of hepatocellular carcinoma in hepatitis C-infected patients treated with direct-acting antivirals with and without pegylated interferon: A Belgian experience.* J Viral Hepat, 2017. **24**(11): p. 976-981.

233. Waziry, R., et al., *Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression.* J Hepatol, 2017. **67**(6): p. 1204-1212.

234. Ioannou, G.N., P.K. Green, and K. Berry, *HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma.* J Hepatol, 2017.

235. Romano, A., et al., *Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: A prospective population study.* J Hepatol, 2018. **69**(2): p. 345-352.

236. Calvaruso, V., et al., *Incidence of Hepatocellular Carcinoma in Patients with HCV-associated Cirrhosis Treated with Direct-Acting Antiviral Agents.* Gastroenterology, 2018.

237. Hallager, S., et al., *Hepatocellular carcinoma in patients with chronic hepatitis C and cirrhosis in Denmark: A nationwide cohort study.* J Viral Hepat, 2018. **25**(1): p. 47-55.

238. Masetti, C., et al., *Lack of reduction of serum alphafetoprotein during treatment with direct antiviral agents predicts hepatocellular carcinoma development in a large cohort of patients with hcv-related cirrhosis.* Journal of Viral Hepatitis. **0**(ja).

239. van der Meer, A.J., et al., *Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis.* Jama, 2012. **308**(24): p. 2584-93.

240. Noh, R., et al., *Clinical Impact of Viral Load on the Development of Hepatocellular Carcinoma and Liver-Related Mortality in Patients with Hepatitis C Virus Infection.* Gastroenterol Res Pract, 2016. **2016**: p. 7476231.

241. Toyoda, H., et al., *The emergence of non-hypervascular hypointense nodules on Gd-EOB-DTPA-enhanced MRI in patients with chronic hepatitis C.* Aliment Pharmacol Ther, 2019. **50**(11-12): p. 1232-1238.

242. Prenner, S.B., et al., *Hepatocellular carcinoma decreases the chance of successful hepatitis C virus therapy with direct-acting antivirals.* J Hepatol, 2017. **66**(6): p. 1173-1181.

243. He, S., et al., *Systematic review with meta-analysis: effectiveness of direct-acting antiviral treatment for hepatitis C in patients with hepatocellular carcinoma.* Aliment Pharmacol Ther, 2020. **51**(1): p. 34-52.

244. Alavi, M., et al., *Trends in hepatocellular carcinoma incidence and survival among people with hepatitis C: An international study.* J Viral Hepat, 2017.

245. Llovet, J.M., et al., *Sorafenib in advanced hepatocellular carcinoma.* N Engl J Med, 2008. **359**(4): p. 378-90.

246. Kielar, A.Z., et al., *LI-RADS 2017: An update.* J Magn Reson Imaging, 2018.

247. Alessandro, F. and B.A. A., *Problematic lesions in cirrhosis.* Clinical Liver Disease, 2018. **11**(2): p. 43-47.

248. Johnson, P.J., et al., *Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade.* J Clin Oncol, 2015. **33**(6): p. 550-8.

249. Llovet, J.M., C. Bru, and J. Bruix, *Prognosis of hepatocellular carcinoma: the BCLC staging classification.* Semin Liver Dis, 1999. **19**(3): p. 329-38.

250. Forner, A., M. Reig, and J. Bruix, *Hepatocellular carcinoma.* The Lancet, 2018. **391**(10127): p. 1301-1314.

251. Mazzaferro, V., et al., *Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis.* N Engl J Med, 1996. **334**(11): p. 693-9.

252. Schwartz, L.H., et al., *RECIST 1.1 – Update and Clarification: From the RECIST Committee.* Eur J Cancer, 2016. **62**: p. 132-7.

253. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.

254.   Herrick, J.B., *Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia.* Archives of Internal Medicine, 1910. **VI**(5): p. 517-521.

255.   Organisation, W.H., *Sickle-Cell anaemia.* 2006: p. 5.

256.   Dormandy, E., et al., *How many people have sickle cell disease in the UK?* J Public Health (Oxf), 2017.

257.   Telfer, P., et al., *Clinical outcomes in children with sickle cell disease living in England: a neonatal cohort in East London.* Haematologica, 2007. **92**(7): p. 905-12.

258.   Powars, D.R., et al., *Outcome of sickle cell anemia: a 4-decade observational study of 1056 patients.* Medicine (Baltimore), 2005. **84**(6): p. 363-76.

259.   Gardner, K., et al., *Survival in adults with sickle cell disease in a high-income setting*, in *Blood*. 2016: United States. p. 1436-8.

260.   Rees, D.C., T.N. Williams, and M.T. Gladwin, *Sickle-cell disease.* Lancet, 2010. **376**(9757): p. 2018-31.

261.   Bunn, H.F., *Pathogenesis and treatment of sickle cell disease.* N Engl J Med, 1997. **337**(11): p. 762-9.

262.   Brittenham, G.M., A.N. Schechter, and C.T. Noguchi, *Hemoglobin S polymerization: primary determinant of the hemolytic and clinical severity of the sickling syndromes.* Blood, 1985. **65**(1): p. 183-9.

263.   Howard, J.A., K.; Atkin, K.; Atoyebi, W.; Awogbade; M.; Cho, C.; Davis,V.; James, J.; Lewis, N.; Okocho, J.; Pancham, S.; Ryan, K.; Salter, M.; Telfer, P.; Yardumian, A., *Standards for Clinical Care of Adults with Sickle Cell Disease in the UK.* The Sickle Cell Society, UK, 2018. **2**: p. 310.

264.   Ware, R.E., et al., *Sickle cell disease.* Lancet, 2017. **390**(10091): p. 311-323.

265.   Platt, O.S., et al., *Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia.* J Clin Invest, 1984. **74**(2): p. 652-6.

266.   Charache, S., et al., *Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia.* N Engl J Med, 1995. **332**(20): p. 1317-22.

267.   Nevitt, S.J., A.P. Jones, and J. Howard, *Hydroxyurea (hydroxycarbamide) for sickle cell disease.* Cochrane Database Syst Rev, 2017. **4**: p. Cd002202.

268.   Eckman, J.R., *Techniques for blood administration in sickle cell patients.* Semin Hematol, 2001. **38**(1 Suppl 1): p. 23-9.

269.   Shah, R., C. Taborda, and S. Chawla, *Acute and chronic hepatobiliary manifestations of sickle cell disease: A review.* World J Gastrointest Pathophysiol, 2017. **8**(3): p. 108-16.

270.   Ebert, E.C., M. Nagar, and K.D. Hagspiel, *Gastrointestinal and hepatic complications of sickle cell disease.* Clin Gastroenterol Hepatol, 2010. **8**(6): p. 483-9; quiz e70.

271.   Berry, P.A., et al., *Hepatic dysfunction in sickle cell disease: a new system of classification based on global assessment.* Clin Gastroenterol Hepatol, 2007. **5**(12): p. 1469-76; quiz 1369.

272.   Banerjee, S., C. Owen, and S. Chopra, *Sickle cell hepatopathy.* Hepatology, 2001. **33**(5): p. 1021-8.

273.   Manci, E.A., et al., *Causes of death in sickle cell disease: an autopsy study.* Br J Haematol, 2003. **123**(2): p. 359-65.

274.   Omata, M., et al., *Pathological spectrum of liver diseases in sickle cell disease.* Dig Dis Sci, 1986. **31**(3): p. 247-56.

275.   Charlotte, F., et al., *Vascular lesions of the liver in sickle cell disease. A clinicopathological study in 26 living patients.* Arch Pathol Lab Med, 1995. **119**(1): p. 46-52.

276. Darbari, D.S., et al., *Circumstances of death in adult sickle cell disease patients.* Am J Hematol, 2006. **81**(11): p. 858-63.

277. Shah, P., et al., *Pattern of mortality in sickle cell disease: an autopsy study.* 2017, 2017. **5**(5): p. 5.

278. Bauer, T.W., G.W. Moore, and G.M. Hutchins, *The liver in sickle cell disease. A clinicopathologic study of 70 patients.* Am J Med, 1980. **69**(6): p. 833-7.

279. Teixeira, A.L., et al., *Sickle cell disease: a clinical and histopathologic study of the liver in living children.* J Pediatr Hematol Oncol, 2002. **24**(2): p. 125-9.

280. Perronne, V., et al., *Patterns of mortality in sickle cell disease in adults in France and England.* Hematol J, 2002. **3**(1): p. 56-60.

281. Feld, J.J., et al., *Liver injury is associated with mortality in sickle cell disease.* Aliment Pharmacol Ther, 2015. **42**(7): p. 912-21.

282. Hamideh, D. and O. Alvarez, *Sickle cell disease related mortality in the United States (1999-2009).* Pediatr Blood Cancer, 2013. **60**(9): p. 1482-6.

283. Buchanan, G.R. and B.E. Glader, *Benign course of extreme hyperbilirubinemia in sickle cell anemia: analysis of six cases.* J Pediatr, 1977. **91**(1): p. 21-4.

284. Green, T.W., C.L. Conley, and M. Berthrong, *[The liver in sickle cell anemia].* Bull Johns Hopkins Hosp, 1953. **92**(2): p. 99-127.

285. Kotila, T., et al., *Liver dysfunction in steady state sickle cell disease.* Ann Hepatol, 2005. **4**(4): p. 261-3.

286. Prieto, J., M. Barry, and S. Sherlock, *Serum ferritin in patients with iron overload and with acute and chronic liver diseases.* Gastroenterology, 1975. **68**(3): p. 525-33.

287. Angelucci, E., et al., *Hepatic iron concentration and total body iron stores in thalassemia major.* N Engl J Med, 2000. **343**(5): p. 327-31.

288. Inati, A., et al., *Iron overload indices rise linearly with transfusion rate in patients with sickle cell disease*, in *Blood*. 2010: United States. p. 2980-1; author reply 2981-2.

289. Adamkiewicz, T.V., et al., *Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion are nonlinear and are associated with iron load and liver injury.* Blood, 2009. **114**(21): p. 4632-8.

290. Mazza, P., et al., *Iron overload in thalassemia: comparative analysis of magnetic resonance imaging, serum ferritin and iron content of the liver.* Haematologica, 1995. **80**(5): p. 398-404.

291. Karam, L.B., et al., *Liver biopsy results in patients with sickle cell disease on chronic transfusions: poor correlation with ferritin levels.* Pediatr Blood Cancer, 2008. **50**(1): p. 62-5.

292. Files, B., et al., *Longitudinal changes in ferritin during chronic transfusion: a report from the Stroke Prevention Trial in Sickle Cell Anemia (STOP).* J Pediatr Hematol Oncol, 2002. **24**(4): p. 284-90.

293. Brownell, A., S. Lowson, and M. Brozovic, *Serum ferritin concentration in sickle cell crisis.* J Clin Pathol, 1986. **39**(3): p. 253-5.

294. St Pierre, T.G., et al., *Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance.* Blood, 2005. **105**(2): p. 855-61.

295. Wolfe, J.L., et al., *Longitudinal changes in liver fibrosis in children with sickle cell disease undergoing chronic transfusion therapy.* Acta Gastroenterol Belg, 2012. **75**(4): p. 419-24.

296. Drasar, E., et al., *Interim assessment of liver damage in patients with sickle cell disease using new non-invasive techniques.* Br J Haematol, 2017. **176**(4): p. 643-650.

297. Pinto, V.M., et al., *Noninvasive monitoring of liver fibrosis in sickle cell disease: Longitudinal observation of a cohort of adult patients.* Am J Hematol, 2017. **92**(12): p. E666-e668.

298.   Voskaridou, E., et al., *Liver Transient Elastography (FibroScan) Correlates with Liver Iron Concentration and Reflects Liver Fibrosis In Patients with Sickle Cell Disease.* Blood, 2010. **116**(21): p. 1646-1646.

299.   Koh, C., et al., *Liver stiffness increases acutely during sickle cell vaso-occlusive crisis.* Am J Hematol, 2013. **88**(11): p. E250-4.

300.   Mirault, T., et al., *Non-invasive assessment of liver fibrosis by transient elastography in post transfusional iron overload.* Eur J Haematol, 2008. **80**(4): p. 337-40.

301.   Ou, G., et al., *Utility of Transient Elastography in Estimating Hepatic Iron Concentration in Comparison to Magnetic Resonance Imaging in Patients Who are Transfusion-Dependent: A Canadian Center Experience.* Hemoglobin, 2017. **41**(1): p. 21-25.

302.   Di Marco, V., et al., *Noninvasive assessment of liver fibrosis in thalassaemia major patients by transient elastography (TE) - lack of interference by iron deposition.* Br J Haematol, 2010. **148**(3): p. 476-9.

303.   Adhoute, X., et al., *Diagnosis of liver fibrosis using FibroScan and other noninvasive methods in patients with hemochromatosis: a prospective study.* Gastroenterol Clin Biol, 2008. **32**(2): p. 180-7.

304.   Sinakos, E., et al., *Is liver stiffness really unrelated to liver iron concentration?*, in *Br J Haematol*. 2010: England. p. 247-8.

305.   Pipaliya, N., et al., *Comparison of Tissue Elastography With Magnetic Resonance Imaging T2* and Serum Ferritin Quantification in Detecting Liver Iron Overload in Patients With Thalassemia Major.* Clin Gastroenterol Hepatol, 2017. **15**(2): p. 292-298.e1.

306.   Sarigianni, M., et al., *Accuracy of magnetic resonance imaging in diagnosis of liver iron overload: a systematic review and meta-analysis.* Clin Gastroenterol Hepatol, 2015. **13**(1): p. 55-63.e5.

307.   DELICOU, S., *TRANSIENT ELASTOGRAPHY (TE) IS A USEFUL TOOL FOR ASSESSING THE RESPONSE OF LIVER IRON CHELATION IN SICKLE CELL DISEASE PATIENTS.* 2018, 2018. **10**.

308.   Gardner, K., et al., *How we treat sickle hepatopathy and liver transplantation in adults.* Blood, 2014. **123**(15): p. 2302-7.

309.   Charman, S.C., R.; Cosgriff. R.; Lee, A.; Carr, S.; The UK CF Registry Steering Committee, *UK Cystic Fibrosis Registry 2017 Annual Data Report.* 2018, Cystic Fibrosis.org.uk.

310.   Elborn, J.S., *Cystic fibrosis.* Lancet, 2016. **388**(10059): p. 2519-2531.

311.   Castellani, C., et al., *Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice.* J Cyst Fibros, 2008. **7**(3): p. 179-96.

312.   Potter, C.J., et al., *Can the histologic changes of cystic fibrosis-associated hepatobiliary disease be predicted by clinical criteria?* J Pediatr Gastroenterol Nutr, 1997. **25**(1): p. 32-6.

313.   Woodruff, S.A., et al., *Prevalence of elevated liver enzymes in children with cystic fibrosis diagnosed by newborn screen.* J Cyst Fibros, 2017. **16**(1): p. 139-145.

314.   Lamireau, T., et al., *Epidemiology of liver disease in cystic fibrosis: a longitudinal study.* J Hepatol, 2004. **41**(6): p. 920-5.

315.   Williams, S.M., et al., *Ultrasound evaluation of liver disease in cystic fibrosis as part of an annual assessment clinic: a 9-year review.* Clin Radiol, 2002. **57**(5): p. 365-70.

316.   Colombo, C., et al., *Liver disease in cystic fibrosis: A prospective study on incidence, risk factors, and outcome.* Hepatology, 2002. **36**(6): p. 1374-82.

317.   Boelle, P.Y., et al., *Cystic Fibrosis Liver Disease: Outcomes and Risk Factors in a Large Cohort of French Patients.* Hepatology, 2018.

318. Nash, K.L., et al., *A single centre experience of liver disease in adults with cystic fibrosis 1995-2006.* J Cyst Fibros, 2008. **7**(3): p. 252-7.

319. Koh, C., et al., *Adult-onset cystic fibrosis liver disease: Diagnosis and characterization of an underappreciated entity.* Hepatology, 2017. **66**(2): p. 591-601.

320. Stonebraker, J.R., et al., *Features of Severe Liver Disease With Portal Hypertension in Patients With Cystic Fibrosis.* Clin Gastroenterol Hepatol, 2016. **14**(8): p. 1207-1215.e3.

321. Debray, D., et al., *Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease.* J Cyst Fibros, 2011. **10 Suppl 2**: p. S29-36.

322. Parisi, G.F., et al., *Liver Disease in Cystic Fibrosis: an Update.* Hepat Mon, 2013. **13**(8).

323. Flass, T. and M.R. Narkewicz, *Cirrhosis and other liver disease in cystic fibrosis.* J Cyst Fibros, 2013. **12**(2): p. 116-24.

324. Davison, S., *Assessment of liver disease in cystic fibrosis.* Paediatr Respir Rev, 2018.

325. Bartlett, J.R., et al., *GENETIC MODIFIERS OF LIVER DISEASE IN CYSTIC FIBROSIS.* JAMA, 2009. **302**(10): p. 1076-83.

326. Ciuca, I.M., et al., *Cystic fibrosis liver disease - from diagnosis to risk factors.* Rom J Morphol Embryol, 2014. **55**(1): p. 91-5.

327. Karlas, T., et al., *Non-invasive evaluation of cystic fibrosis related liver disease in adults with ARFI, transient elastography and different fibrosis scores.* PLoS One, 2012. **7**(7): p. e42139.

328. Karlas, T., et al., *ARFI and transient elastography for characterization of cystic fibrosis related liver disease: first longitudinal follow-up data in adult patients.* J Cyst Fibros, 2013. **12**(6): p. 826-7.

329. Kitson, M.T., et al., *Utility of transient elastography in the non-invasive evaluation of cystic fibrosis liver disease.* Liver Int, 2013. **33**(5): p. 698-705.

330. Sadler, M.D., et al., *Noninvasive methods, including transient elastography, for the detection of liver disease in adults with cystic fibrosis*, in *Can J Gastroenterol Hepatol*. 2015. p. 139-44.

331. Hillaire, S., et al., *Liver transplantation in adult cystic fibrosis: Clinical, imaging, and pathological evidence of obliterative portal venopathy.* Liver Transpl, 2017. **23**(10): p. 1342-1347.

332. Hillaire, S., et al., *Cystic fibrosis liver disease in adults: Limits of noninvasive tests of fibrosis.* Hepatology, 2017.

333. Witters, P., et al., *Liver disease in cystic fibrosis presents as non-cirrhotic portal hypertension.* J Cyst Fibros, 2017. **16**(5): p. e11-e13.

334. Lam, S., et al., *Transient Elastography in the Evaluation of Cystic Fibrosis Associated Liver Disease: Systematic Review and Meta-Analysis.* Gastroenterology, 2017. **152**(5): p. S1106-S1107.

335. Debray, D., et al., *Cystic Fibrosis-related Liver Disease: Research Challenges and Future Perspectives.* J Pediatr Gastroenterol Nutr, 2017. **65**(4): p. 443-448.

336. Lewindon, P.J., et al., *Importance of hepatic fibrosis in cystic fibrosis and the predictive value of liver biopsy.* Hepatology, 2011. **53**(1): p. 193-201.

337. guidelines, N., *Cystic fibrosis: diagnosis and management (NG78).* 2017. p. 43.

338. Leung, D.H. and M.R. Narkewicz, *Cystic Fibrosis-related cirrhosis.* J Cyst Fibros, 2017. **16 Suppl 2**: p. S50-s61.

339. Forner, A., et al., *Current strategy for staging and treatment: the BCLC update and future prospects.* Semin Liver Dis, 2010. **30**(1): p. 61-74.

340. Altman, D., *Practical statistics for medical research.* 1991, London: Chapman & Hall.

341. Kaplan, E.L. and P. Maier, *Nonparametric Estimation from Incomplete Observations.* Journal of the American Statistical Association, 1958. **53**(282): p. 457-481.

342. Wang, J., et al., *Albumin-Bilirubin (ALBI) as an accurate and simple prognostic score for chronic hepatitis B-related liver cirrhosis.* Dig Liver Dis, 2019. **51**(8): p. 1172-1178.

343. Chan, A.W., et al., *New simple prognostic score for primary biliary cirrhosis: Albumin-bilirubin score.* J Gastroenterol Hepatol, 2015. **30**(9): p. 1391-6.

344. Fragaki, M., et al., *Comparative evaluation of ALBI, MELD, and Child-Pugh scores in prognosis of cirrhosis: is ALBI the new alternative?* Ann Gastroenterol, 2019. **32**(6): p. 626-632.

345. Kanwal, F., et al., *Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents.* Gastroenterology, 2017. **153**(4): p. 996-1005.e1.

346. Nahon, P., et al., *Incidence of Hepatocellular Carcinoma After Direct Antiviral Therapy for HCV in Patients With Cirrhosis Included in Surveillance Programs.* Gastroenterology, 2018. **155**(5): p. 1436-1450.e6.

347. Singer, A.W., et al., *Direct-acting antiviral treatment for hepatitis C virus infection and risk of incident liver cancer: a retrospective cohort study.* Aliment Pharmacol Ther, 2018. **47**(9): p. 1278-1287.

348. Huang, Y.W., et al., *Increased risk of hepatocellular carcinoma in chronic hepatitis C patients with new onset diabetes: a nation-wide cohort study.* Aliment Pharmacol Ther, 2015. **42**(7): p. 902-11.

349. Ogawa, E., et al., *Short-term risk of hepatocellular carcinoma after hepatitis C virus eradication following direct-acting anti-viral treatment.* Aliment Pharmacol Ther, 2018. **47**(1): p. 104-113.

350. Ioannou, G.N., et al., *Development of models estimating the risk of hepatocellular carcinoma after antiviral treatment for hepatitis C.* J Hepatol, 2018. **69**(5): p. 1088-1098.

351. Toyoda, H., et al., *The impact of HCV eradication by direct-acting antivirals on the transition of precancerous hepatic nodules to HCC: A prospective observational study.* Liver Int, 2019. **39**(3): p. 448-454.

352. Marino, Z., et al., *Time association between hepatitis C therapy and hepatocellular carcinoma emergence in cirrhosis: Relevance of non-characterized nodules.* J Hepatol, 2019. **70**(5): p. 874-884.

353. Sangiovanni, A., et al., *Undefined/non-malignant hepatic nodules are associated with early occurrence of HCC in DAA-treated patients with HCV-related cirrhosis.* J Hepatol, 2020. **73**(3): p. 593-602.

354. Ji, F., et al., *Sustained virologic response to direct-acting antiviral therapy in patients with chronic hepatitis C and hepatocellular carcinoma: A systematic review and meta-analysis.* J Hepatol, 2019. **71**(3): p. 473-485.

355. Knop, V., et al., *Regression of fibrosis and portal hypertension in HCV-associated cirrhosis and sustained virologic response after interferon-free antiviral therapy.* J Viral Hepat, 2016. **23**(12): p. 994-1002.

356. Everson, G.T., et al., *Quantitative tests of liver function measure hepatic improvement after sustained virological response: results from the HALT-C trial.* Aliment Pharmacol Ther, 2009. **29**(5): p. 589-601.

357. Ocker, M., et al., *Improvement of quantitative testing of liver function in patients with chronic hepatitis C after installment of antiviral therapy.* World J Gastroenterol, 2005. **11**(35): p. 5521-4.

358. Stintzing, S., et al., *Liver function under interferon/ribavirin therapy of chronic hepatitis C.* Hepatogastroenterology, 2009. **56**(90): p. 462-5.

359. Shiratori, Y., et al., *Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy.* Ann Intern Med, 2000. **132**(7): p. 517-24.

360. Manns, M.P., et al., *Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial.* Lancet, 2001. **358**(9286): p. 958-65.

361. Singh, S., et al., *Magnitude and Kinetics of Decrease in Liver Stiffness After Antiviral Therapy in Patients With Chronic Hepatitis C: A Systematic Review and Meta-analysis.* Clin Gastroenterol Hepatol, 2018. **16**(1): p. 27-38 e4.

362. Poynard, T., et al., *Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C.* J Hepatol, 2013. **59**(4): p. 675-83.

363. Shiffman, M.L., et al., *Long term changes in liver histology following treatment of chronic hepatitis C virus.* Ann Hepatol, 2014. **13**(4): p. 340-9.

364. Pons, M., et al., *Rapid liver and spleen stiffness improvement in compensated advanced chronic liver disease patients treated with oral antivirals.* Therap Adv Gastroenterol, 2017. **10**(8): p. 619-629.

365. Pons, M., et al., *Non-invasive prediction of liver-related events in patients with HCV-associated compensated advanced chronic liver disease after oral antivirals.* J Hepatol, 2020. **72**(3): p. 472-480.

366. Knop, V., et al., *Dynamics of liver stiffness by transient elastography in patients with chronic hepatitis C virus infection receiving direct-acting antiviral therapy-Results from the German Hepatitis C-Registry.* J Viral Hepat, 2020. **27**(7): p. 690-698.

367. McPhail, J., et al., *Fibrosis improvement in patients with HCV treated with direct-acting antivirals.* Eur J Gastroenterol Hepatol, 2021. **33**(7): p. 996-1000.

368. Facciorusso, A., et al., *Long-term liver stiffness assessment in hepatitis C virus patients undergoing antiviral therapy: Results from a 5-year cohort study.* J Gastroenterol Hepatol, 2018. **33**(4): p. 942-949.

369. Pan, J.J., et al., *Morphometry Confirms Fibrosis Regression From Sustained Virologic Response to Direct-Acting Antivirals for Hepatitis C.* Hepatol Commun, 2018. **2**(11): p. 1320-1330.

370. Martinez-Camprecios, J., et al., *Transient elastography in DAA era. Relation between post-SVR LSM and histology.* J Viral Hepat, 2020. **27**(4): p. 453-455.

371. European Association for the Study of the Liver. Electronic address, e.e.e., et al., *EASL recommendations on treatment of hepatitis C: Final update of the series().* J Hepatol, 2020. **73**(5): p. 1170-1218.

372. European Association for the Study of the Liver. Electronic address, e.e.e., et al., *EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update.* J Hepatol, 2021. **75**(3): p. 659-689.

373. Broquetas, T., et al., *Elastography is unable to exclude cirrhosis after sustained virological response in HCV-infected patients with advanced chronic liver disease.* Liver Int, 2021. **41**(11): p. 2733-2746.

374. Herold, C., et al., *Quantitative testing of liver function in relation to fibrosis in patients with chronic hepatitis B and C.* Liver, 2001. **21**(4): p. 260-5.

375. Moller, S., et al., *Indocyanine green retention test in cirrhosis and portal hypertension: Accuracy and relation to severity of disease.* J Gastroenterol Hepatol, 2019. **34**(6): p. 1093-1099.

376. Laursen, T.L., et al., *Time-dependent improvement of liver inflammation, fibrosis and metabolic liver function after successful direct-acting antiviral therapy of chronic hepatitis C.* J Viral Hepat, 2020. **27**(1): p. 28-35.

377. Huang, R., et al., *Histopathology and the predominantly progressive, indeterminate and predominately regressive score in hepatitis C virus patients after direct-acting antivirals therapy.* World J Gastroenterol, 2021. **27**(5): p. 404-415.

378. Enomoto, M., et al., *Short-term histological evaluations after achieving a sustained virologic response to direct-acting antiviral treatment for chronic hepatitis C.* United European Gastroenterol J, 2018. **6**(9): p. 1391-1400.

379. de Franchis, R. and V.I.F. Baveno, *Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension.* J Hepatol, 2015. **63**(3): p. 743-52.

380. Lens, S., et al., *Clinical outcome and hemodynamic changes following HCV eradication with oral antiviral therapy in patients with clinically significant portal hypertension.* J Hepatol, 2020. **73**(6): p. 1415-1424.

381. Mandorfer, M., et al., *Changes in Hepatic Venous Pressure Gradient Predict Hepatic Decompensation in Patients Who Achieved Sustained Virologic Response to Interferon-Free Therapy.* Hepatology, 2020. **71**(3): p. 1023-1036.

382. Semmler, G., et al., *Noninvasive Risk Stratification After HCV Eradication in Patients With Advanced Chronic Liver Disease.* Hepatology, 2021. **73**(4): p. 1275-1289.

383. Verna, E.C., et al., *DAA therapy and long-term hepatic function in advanced/decompensated cirrhosis: Real-world experience from HCV-TARGET cohort.* J Hepatol, 2020. **73**(3): p. 540-548.

384. Hsu, W.F., et al., *Rapid decline of noninvasive fibrosis index values in patients with hepatitis C receiving treatment with direct-acting antiviral agents.* BMC Gastroenterol, 2019. **19**(1): p. 63.

385. Vergniol, J., et al., *Changes of non-invasive markers and FibroScan values during HCV treatment.* J Viral Hepat, 2009. **16**(2): p. 132-40.

386. Nakajima, T., et al., *Factors affecting the recovery of hepatic reserve after sustained virologic response by direct-acting antiviral agents in chronic hepatitis C virus-infected patients.* J Gastroenterol Hepatol, 2021. **36**(2): p. 367-375.

387. Ghany, M.G., et al., *Predicting clinical outcomes using baseline and follow-up laboratory data from the hepatitis C long-term treatment against cirrhosis trial.* Hepatology, 2011. **54**(5): p. 1527-37.

388. Johnson, P.J., et al., *Impact of direct-acting antiviral agents on liver function in patients with chronic hepatitis C virus infection.* J Viral Hepat, 2021. **28**(1): p. 168-176.

389. Salerno, F., et al., *Prognostic value of the galactose test in predicting survival of patients with cirrhosis evaluated for liver transplantation. A prospective multicenter Italian study. AISF Group for the Study of Liver Transplantation. Associazione Italiana per lo Studio del Fegato.* J Hepatol, 1996. **25**(4): p. 474-80.

390. Botta, F., et al., *MELD scoring system is useful for predicting prognosis in patients with liver cirrhosis and is correlated with residual liver function: a European study.* Gut, 2003. **52**(1): p. 134-9.

391. Bernardi, M., et al., *Albumin in decompensated cirrhosis: new concepts and perspectives.* Gut, 2020. **69**(6): p. 1127-1138.

392. Mehta, G. and R. Jalan, *The "Alter Ego" of Albumin in Cirrhosis.* Hepatology, 2021. **74**(4): p. 1734-1736.

393. Harris, M., et al., *Understanding hepatitis C intervention success-Qualitative findings from the HepCATT study.* J Viral Hepat, 2018. **25**(7): p. 762-770.

394. Pascasio, J.M., et al., *Clinical outcomes of patients undergoing antiviral therapy while awaiting liver transplantation.* J Hepatol, 2017. **67**(6): p. 1168-1176.

395. Vaziri, A., et al., *Liver transplant listing for hepatitis C-associated cirrhosis and hepatocellular carcinoma has fallen in the United Kingdom since the introduction of direct-acting antiviral therapy.* J Viral Hepat, 2019. **26**(2): p. 231-235.

396. Young, K., et al., *Improved liver transplant waitlist mortality and lower risk of disease progression among chronic hepatitis C patients awaiting liver transplantation after the introduction of direct-acting antiviral therapies in the United States.* J Viral Hepat, 2019. **26**(3): p. 350-361.

397. Krassenburg, L.A.P., et al., *Clinical outcomes following DAA therapy in patients with HCV-related cirrhosis depend on disease severity.* J Hepatol, 2021. **74**(5): p. 1053-1063.

398. McDonald, S.A., et al., *Real-world impact following initiation of interferon-free hepatitis C regimens on liver-related outcomes and all-cause mortality among patients with compensated cirrhosis.* J Viral Hepat, 2020. **27**(3): p. 270-280.

399. Calvaruso, V. and A. Craxi, *Hepatic benefits of HCV cure.* J Hepatol, 2020. **73**(6): p. 1548-1556.

400. Rodriguez-Tajes, S., et al., *Hepatitis C-related cirrhosis will be a marginal cause of hospital admissions by 2025.* J Hepatol, 2020. **73**(6): p. 1360-1367.

401. Vergara, M., et al., *Use of healthcare resources and drug expenditure before and after treatment of chronic hepatitis C with direct antiviral agents.* J Viral Hepat, 2021. **28**(5): p. 728-738.

402. Backus, L.I., et al., *Impact of Sustained Virologic Response with Direct-Acting Antiviral Treatment on Mortality in Patients with Advanced Liver Disease.* Hepatology, 2019. **69**(2): p. 487-497.

403. Carrat, F., et al., *Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study.* Lancet, 2019. **393**(10179): p. 1453-1464.

404. Hamdane, N., et al., *HCV-Induced Epigenetic Changes Associated With Liver Cancer Risk Persist After Sustained Virologic Response.* Gastroenterology, 2019. **156**(8): p. 2313-2329.e7.

405. McKerrell, T.D., H.W. Cohen, and H.H. Billett, *The older sickle cell patient.* Am J Hematol, 2004. **76**(2): p. 101-6.

406. Brody, J.I., W.N. Ryan, and M.A. Haidar, *Serum Alkaline Phosphatase Isoenzymes In Sickle Cell Anemia.* JAMA, 1975. **232**(7): p. 738-741.

407. Rosenblate, H.J., R. Eisenstein, and A.W. Holmes, *The liver in sickle cell anemia. A clinical-pathologic study.* Arch Pathol, 1970. **90**(3): p. 235-45.

408. Schubert, T.T., *Hepatobiliary system in sickle cell disease.* Gastroenterology, 1986. **90**(6): p. 2013-21.

409. Hilkovitz, G. and A. Jacobson, *Hepatic dysfunction and abnormalities of the serum proteins and serum enzymes in sickle-cell anemia.* J Lab Clin Med, 1961. **57**: p. 856-67.

410. Harmatz, P., et al., *Severity of iron overload in patients with sickle cell disease receiving chronic red blood cell transfusion therapy.* Blood, 2000. **96**(1): p. 76-9.

411. Costa, P., et al., *Liver Stiffness Measurement by Vibration Controlled Transient Elastography Does Not Correlate to Hepatic Iron Overload in Children With Sickle Cell Disease.* J Pediatr Hematol Oncol, 2020. **42**(3): p. 214-217.

412. Ben Yakov, G., et al., *Vibration Controlled Transient Elastography (Fibroscan®) in sickle cell liver disease - could we strike while the liver is hard?* British Journal of Haematology, 2019. **187**(1): p. 117-123.

413.	Lam, S., et al., *Transient Elastography in the Evaluation of Cystic Fibrosis-Associated Liver Disease: Systematic Review and Meta-analysis.* J Can Assoc Gastroenterol, 2019. **2**(2): p. 71-80.

414.	Toledano, M.B., et al., *The emerging burden of liver disease in cystic fibrosis patients: A UK nationwide study.* PLoS One, 2019. **14**(4): p. e0212779.

415.	Ye, W., et al., *Variceal Hemorrhage and Adverse Liver Outcomes in Patients With Cystic Fibrosis Cirrhosis.* J Pediatr Gastroenterol Nutr, 2018. **66**(1): p. 122-127.

416.	Dana, J., M. Girard, and D. Debray, *Hepatic manifestations of cystic fibrosis.* Curr Opin Gastroenterol, 2020. **36**(3): p. 192-198.

417.	Staufer, K., *Current Treatment Options for Cystic Fibrosis-Related Liver Disease.* Int J Mol Sci, 2020. **21**(22).

418.	Bernhard, W., *Choline in cystic fibrosis: relations to pancreas insufficiency, enterohepatic cycle, PEMT and intestinal microbiota.* Eur J Nutr, 2021. **60**(4): p. 1737-1759.

419.	Lindblad, A., H. Glaumann, and B. Strandvik, *A two-year prospective study of the effect of ursodeoxycholic acid on urinary bile acid excretion and liver morphology in cystic fibrosis-associated liver disease.* Hepatology, 1998. **27**(1): p. 166-74.

420.	Fiorotto, R., et al., *The cystic fibrosis transmembrane conductance regulator controls biliary epithelial inflammation and permeability by regulating Src tyrosine kinase activity.* Hepatology, 2016. **64**(6): p. 2118-2134.

421.	Fiorotto, R. and M. Strazzabosco, *Pathophysiology of Cystic Fibrosis Liver Disease: A Channelopathy Leading to Alterations in Innate Immunity and in Microbiota.* Cell Mol Gastroenterol Hepatol, 2019. **8**(2): p. 197-207.

422.	Cheng, K., D. Ashby, and R.L. Smyth, *Ursodeoxycholic acid for cystic fibrosis-related liver disease.* Cochrane Database Syst Rev, 2017. **9**: p. Cd000222.

423.	van der Feen, C., et al., *Ursodeoxycholic acid treatment is associated with improvement of liver stiffness in cystic fibrosis patients.* J Cyst Fibros, 2016. **15**(6): p. 834-838.

424.	de Franchis, R., et al., *Baveno VII - Renewing consensus in portal hypertension.* J Hepatol, 2022. **76**(4): p. 959-974.

425.	Leung, D.H., et al., *Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease.* Hepatology, 2015. **62**(5): p. 1576-83.

426.	Karnsakul, W., et al., *A longitudinal assessment of non-invasive biomarkers to diagnose and predict cystic fibrosis-associated liver disease.* J Cyst Fibros, 2020. **19**(4): p. 546-552.

427.	Aberg, F., et al., *A Dynamic Aspartate-to-Alanine Aminotransferase Ratio Provides Valid Predictions of Incident Severe Liver Disease.* Hepatol Commun, 2021. **5**(6): p. 1021-1035.

428.	Alexopoulou, A., et al., *Evaluation of noninvasive markers for the diagnosis of cystic fibrosis liver disease.* Scand J Gastroenterol, 2018. **53**(12): p. 1547-1552.

Appendix

Table of Contents

1   Elastography physics

Both ultrasound and shear elastic waves cause a small amount of movement of the liver tissue as they pass through and it is these which are being measured. Ultrasound waves are a short wavelength and pass faster through the liver and therefore cause a much smaller amount of movement. This change is shown as a change in position frame to frame in a graphical manner.

Shear wave movement, on the other hand, uses a much lower frequency which also travels much slower within the liver. With ultrasound waves moving much faster, this change in liver tissue placement can be measured. A certain pressure or stress is applied when a shear wave is pushed into the liver. This causes a movement and slight deformation or strain within the liver tissue.

The elastic properties of a material are determined via various physical rules, which are based upon Hooke's Law, which states that a spring's change in length is proportional to a force applied. This is true until the plastic limit, at which point this becomes irreversible. As the movements associated with elastography are small, we may use certain moduli, such as Young's modulus. Two imperative concepts are:

Stress - force applied per unit area

Strain - tissue displacement per unit sample length

Young's and shear modulus are the most important elastic properties within elastography.

Young's modulus (E) is a specific type of Hooke's law and describes the ability of a material to resist changes in length when a force is placed longitudinally. This is true for both stretching and compression. This is equal to stress/strain.

Shear modulus (may be referred to as G or μ) describes the ability of a material to resist a deformation in shape whilst maintaining volume when opposing forces are applied. This is equal to shear stress/shear strain.

These properties are tested by determining the speed a shear wave (Vs) passes through a material. The manner with which these are produced and the speed determined are measured via different methods, which we will describe subsequently. It must also be noted that the different techniques report only one of these moduli, although the output units are the same (kPa) and are not always directly comparable. We shall now explore how each is arrived at and how they are related.

Once the speed that the shear wave (Vs) passes through a homogenous, isotropic and linearly elastic medium (i.e. liver) has been measured, the shear modulus can be determined by the equation

$\mu = G = \rho Vs2$

ρ is the material density and is assumed to be 1g cm-3 for soft tissues. This means the shear modulus equals the square of shear wave speed.

$\mu = G = Vs2$

this outputs a measure of stiffness in kPa.

Also, within homogenous, isotropic materials, the above elastic constants of Young's modulus and shear modulus are related by the equation.

$E = 2\mu(1+v) = 2G(1+v)$

When a material is lengthened, there is a decrease in lateral width, which constitutes a transverse strain, and this ratio is Poisson's ratio (v). This is usually taken as incompressible for soft tissues and thus assigned the value of 0.5. This finally leaves the equation as:

$E=3G=3\mu$

It is due to this relationship that Young's modulus may also be stated for the stiffness, again in kPa. It must be noted that with the discrepancy in values (with shear modulus being three times smaller than Young's modulus) with the same units, the moduli used must be stated to avoid confusion [1-5].

This all eventually distils down to three types of measurement, which are reported by different machines.

1. Shear wave speed = m/s

2. Young's modulus = square of shear wave speed = kPa

3. Bulk modulus = Young's modulus X 3 = kPa

The basis for TE the system was first described by Catheline et al. in 1999. These initial experiments described the production and propagation of a shear wave within a medium with the use of an ultrasound transducer placed on the opposite surface to the side of the vibration production. A shear wave was produced, as defined above and displacement observed. When looked at before and after a low-frequency vibration is applied, four phases are seen (See

Figure 1.1). Zone a shows the tissues at rest and, therefore, nil displacement. Line X represents the point at which the vibration is applied at 35ms following the start of measuring. Zone b is the region showing compressional wave displacement. Compressional waves move much faster than shear waves and so this is to be expected. Line Y indicates where the shear wave first transitions through the medium, as can be seen with the displacement throughout all layers. Zone c continues to show displacement by the shear waves until line z. this region is the transient area used for calculating TE. Line z delineates the region where non-monochromatic displacement is seen. Thus, zone d displacements show reflections from boundaries, termed backscatter, and are therefore unsuitable for measurements of tissue elasticity as it is not constant. The induction of a shear wave in discrete periods allows the elimination of backscatter and thus the sole use of the transient period to give the most accurate results with the lowest amount of artefact {Catheline, 1999 #960;Catheline, 1999 #959}.
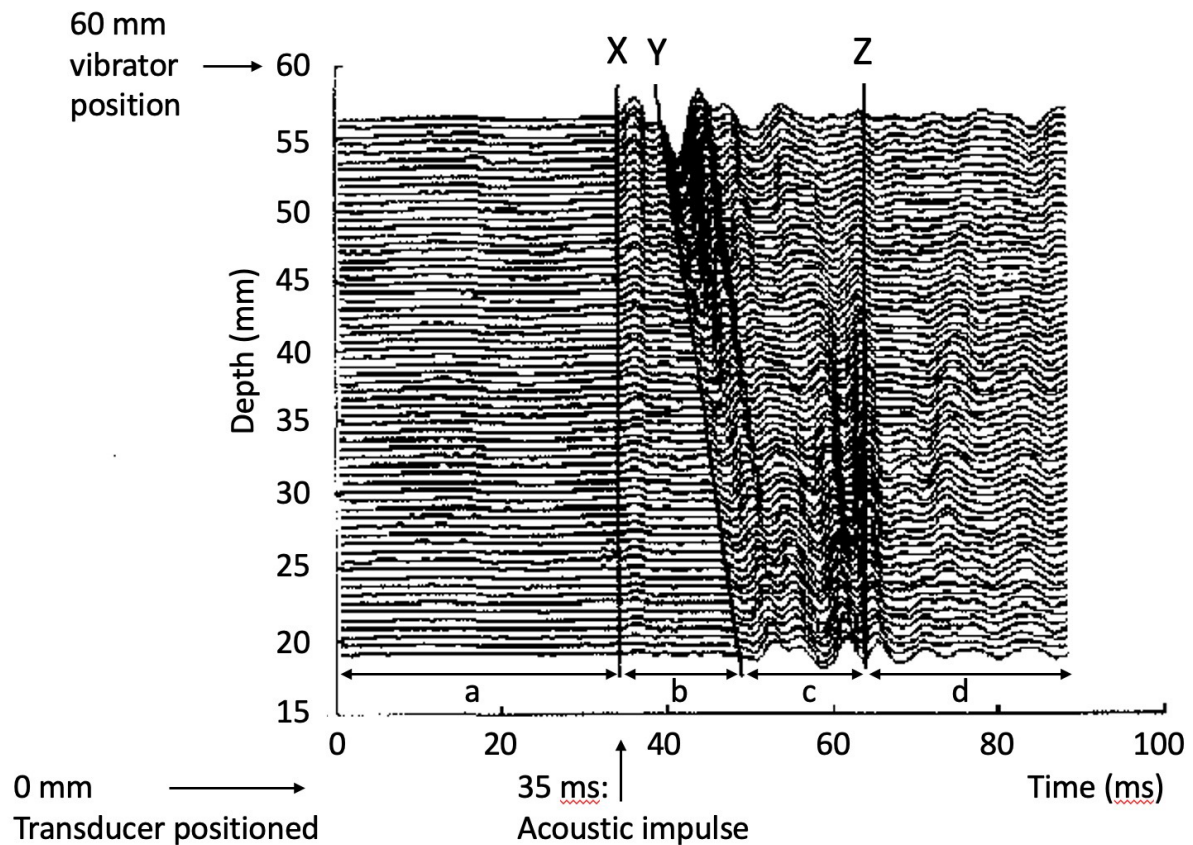
Figure 1.1: Shear wave propagation through tissue shown as displacement at varying depths against time. Zone a - the transducer is placed on the patient with the tissues at rest. At 35ms, the vibration is introduced to the tissue. Zone b is the region showing compressional wave displacement. Line Y indicates where the shear wave first transitions through the medium, as can be seen with the displacement throughout all layers. Zone c shows displacement by the shear waves and is the transient area used for calculating transient elastography. Line z delineates the region where measurements can no longer be taken due to reflections from boundaries, as seen within zone d, termed backscatter. mm = millimetre, ms: millisecond {Catheline, 1999 #960;Catheline, 1999 #959}.

By using an ultrafast ultrasound system, this work was advanced upon by Sandrin et al. {Sandrin, 1999 #796}. The standard ultrasound production is 100 frames/sec, but ultrafast ultrasound does not focus the image and instead concentrates on gathering as much information as possible to be gathered in the smallest amount of time. This involves the production of 10,000 frames per second and post-acquisition processing by the attached computer. This means sequential images can measure the displacement produced by shear waves. Thus, two points of a known distance separating them may be chosen and the time taken for shear wave propagation between these points will be used to give the shear wave speed (i.e. speed=distance/time). This calculation is made slightly more complicated with the

use of Green's function to ensure the correct displacement (i.e. the shear wave and not the compression wave) is used for this calculation.

These tests were initially performed with a separate ultrasound transducer placed at a separate location to the shear wave vibrator. The natural final step was taken in 2002 when Sandrin et al. described the use of a single probe with incorporated vibrator and transducer within a single unit. This led to the potential bias of the transducer physically moving with the vibrator and causing inherent measurement inconsistencies. This was overcome by designing the reflection mode, which uses motion compensation techniques to work out the movement of the vibrator and remove this from the absolute displacement of tissue and thus leaves only the motion due to the shear wave {Sandrin, 2002 #797}. Finally, as the waves are parabolic in nature, due to the vibrator area being smaller than the wavelength produced, two vibrators are included within the probe to allow an increased signal and, therefore, sensitivity in the overlapping central, measured area {Sandrin, 1999 #796;Sandrin, 2002 #794}.

2 The association between hepatocellular carcinoma and direct anti-viral treatment in patients with decompensated cirrhosis – ethics clearance
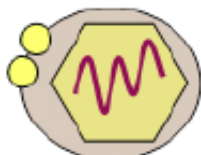
HCV Research UK

Tue, 21 Mar 2017

Will Irving
Queen's Medical Centre

Dear Will Irving,

> **RE: Study of hepatocellular carcinoma development in patients with decompensated liver disease following onset of DAA therapy.**

> The Tissue Access Committee has approved your application and someone from HCV Research UK will be in contact shortly.
>
> This application has been accepted pending completion of a suitable MTA and identification of the appropriate patients and samples.

Regards,

Sarah McDonald
HCV Research UK Biobank Manager

3   Changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy

3.5   JRMO Non-CTIMP Protocol Template

1.

Full Title	Changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy.
Short Title/Acronym	Dynamic liver tests in liver disease
Sponsor	Queen Mary, University of London
	Representative of the Sponsor:
	Dr Sally Burtles
	Director of Research Services and Business Development
	Queen Mary University of London
	Joint Research Management Office (JRMO)
	Queen Mary Innovation Centre
	Lower Ground Floor
	5 Walden Street
	London, E1 2EF
	Tel: **020 7882 7260**
	Office fax: **020 7882 7276** (Internal: 13-7276)
	E-mail: sponsorsrep@bartshealth.nhs.uk
REC Reference	16/WA/0082

Chief Investigator

Professor Graham Foster

The Blizard Institute

Queen Mary, University of London

4 Newark Street

London

E1 2AT

Tel 020 7882 7241

g.r.foster@qmul.ac.uk

Principal Investigators

Ali Jibran Mecci

The Blizard Institute

Queen Mary, University of London

4 Newark Street

London

E1 2AT

a.j.mecci@qmul.ac.uk

alij16@hotmail.com

Mr. Robert Hutchins,

The Royal London Hospital

Whitechapel Rd,

London

E1 1BB

robert.hutchins@bartshealth.nhs.uk

roberthutchins@btinternet.com

Research Sites   The Blizard Institute

Bart's & The London School of Medicine

4 Newark Street

London

E1 2AT

The Royal London Hospital

Whitechapel Rd,

London

E1 1BB

CONTENTS PAGE

2.   GLOSSARY of Terms and Abbreviations

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
AST	*Aspartate Aminotransferase*
ATP	Adenosine Tri-Phosphate
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Computerised Tomography
DLT	Dynamic Liver Tests
DMC	Data Monitoring Committee
GAfREC	Governance Arrangements for NHS Research Ethics Committees
GCP	Good Clinical Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
ICF	Informed Consent Form
ICG	Indocyanine green
ICG15	ICG plasma retention rate at 15 minutes
IQR	Interquartile range
IV	Intravenous
JRMO	Joint Research Management Office
LFT	Liver Function Test
MELD	Model for End-Stage Liver Disease

NHS	National Health Service
NHS REC	National Health Service Research Ethics Committee
NHS R&D	National Health Service Research & Development
NICE	The National Institute for Health and Care Excellence
NTCP	Na+-taurocholate co-transporting polypeptides transporter
OGD	Oesophago-Gastro- Duodenoscopy
Participant	An individual who takes part in a clinical trial
PDR_{ICG}	Plasma Disappearance Rate
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
QMUL	Queen Mary, University of London
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RLH	The Royal London hospital
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIPS	Transjugular intrahepatic portosystemic shunt
TMG	Trial Management Group
TSC	Trial Steering Committee
UKELD	United Kingdom Model for End-Stage Liver Disease

3.   SIGNATURE PAGE

Chief Investigator Agreement

The clinical study as detailed within this research protocol (Version 4.1, dated 02/11/2015), or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Chief Investigator Name: **Graham R Foster**

Chief Investigator Site: **Department of Hepatology,**

                                    Centre for Immunobiology

                                    Blizard Institute

                                    Queen Mary University of London

                                    4 Newark St

                                    London

                                    E1 2AT

Signature and Date: **24/02/17**

4. SUMMARY/SYNOPSIS

Short Title	Dynamic liver tests in liver disease
Methodology	Pilot study, investigating the role of dynamic liver testing in predicting patient outcome pre and post treatment for patients with liver disease.
Research Sites	Bart's Health NHS Trust, The Royal London Hospital, Whitechapel Road Blizard Institute, Queen Mary University, University of London
Objectives/Aims	The primary aim of the study is to validate the use of liver testing in various disease processes, in order to predict patients who would benefit from closer monitoring and early, more intensive treatment.
Number of Participants/Patients	This is a proof of concept study. We will enrol ~60 patients with chronic hepatitis C infection and cirrhosis who are planning to start antiviral therapy, ~60 patients with chronic HCV and cirrhosis who have been successfully treated in the past and ~60 patients with chronic hepatitis B and cirrhosis.
Main Inclusion Criteria	The following cohorts will be recruited: a) All patients attending The Royal London Hospital with liver cirrhosis secondary to chronic HCV infection will be considered for the study. All patients with cirrhosis and active viral replication are currently offered therapy under the NHSE access scheme and patients will be studied prior to therapy, one month following therapy, one year and five years after treatment. In addition we will study patients with previously

	treated HCV cirrhosis to determine whether any changes observed continue medium term. b) All patients attending The Royal London Hospital with liver cirrhosis secondary to chronic HBV infection will be considered for the study. All patients with cirrhosis and active viral replication are currently offered therapy and patients will be studied prior to therapy and one year after treatment. In addition we will study patients with previously treated HBV cirrhosis to determine whether any changes observed continue medium term.
Statistical Methodology and Analysis	This is a pilot project to examine changes in liver function following antiviral therapy in chronic HCV. We will present descriptive statistics of the magnitude of changes in liver function, the proportion of patients who improve and we will perform exploratory analyses of factors that predispose to improvement or deterioration. These factors will include age, sex, viral genotype, response to therapy, pre-treatment MELD score, pre-treatment synthetic liver function (albumin, bilirubin, INR) and ICG and fibroscan reports.
Proposed Start Date	16/11/15
Proposed End Date	24/11/ 21
Study Duration	6 years

5. INTRODUCTION

5.1 Background

Many diseases effect the liver and induce hepatic scarring. In many, the liver fibrosis progresses, leading eventually to liver cirrhosis that is associated with both an increase in portal pressure (portal hypertension) and a reduction in liver synthetic function (liver failure). Removal of the causative agent underlying liver inflammation (e.g. stopping alcohol consumption, eradicating viral infections) leads to an improvement in liver function and a reduction in portal hypertension in many patients but in some there is no, or limited, recovery. It is unclear why some patients benefit and some do not. The availability of effective treatments for viral hepatitis (see later) has led to discussions as to which patients should be treated prior to transplantation in the hope of improving liver function sufficiently to avoid the need for surgery and which patients would benefit from early transplantation followed by postoperative antiviral therapy. We hypothesise that pre-treatment assessment of liver function and liver stiffness may allow early identification of those who are likely to benefit from viral eradication.

There are multiple scoring systems currently available including static (United Kingdom Model for End-Stage Liver Disease (UKELD), Model for End-Stage Liver Disease (MELD) or Childs-Pugh scores) which look at a snapshot of laboratory blood tests with or without symptoms or functional methods, such as Indocyanine green (ICG). MELD score was initially derived for stratification of patients requiring transjugular intrahepatic portosystemic shunt (TIPS) procedure but has since been validated in multiple liver pathologies and is widely used to stratify and prioritise patients for liver transplantation[6, 7]. Childs-Pugh score uses clinical symptoms in addition to blood tests and provides one year prognoses for chronic liver diseases, particularly cirrhosis. Transient elastography is widely used to assess the degree of hepatic fibrosis and recent studies [8] indicate that it may be useful in assessing the degree of portal hypertension. Its use in Hepatitis C has been investigated with guide values of – no liver dysfunction < 9.6 kPa, significant fibrosis 9.6-11.4kPa, cirrhosis >11.5kPa. Significant portal hypertension is probable in patients with fibrosis scores of >20.

Indocyanine green (ICG) is a functional liver test which was developed over 50 years ago. The test uses non-invasive pulse spectrophotometry to monitor clearance of a compound, ICG, which is exclusively metabolised in the liver and the test therefore measures synthetic liver function. Indocyanine green is

injected intravenously and is then excreted exclusively by the liver in an ATP dependant manner into bile. The rate of excretion is determined peripherally by pulse spectrophotometry and liver function inferred.

Chronic viral hepatitis with either the Hepatitis C virus (HCV) or the Hepatitis B virus (HBV) are infections which lead to chronic inflammation and fibrosis of the liver. As fibrosis progresses this may lead to cirrhosis associated with complications. The most serious of these complications include haemorrhage secondary to portal hypertension, encephalopathy, the formation of ascites and premature death. Once a person develops these complications the liver is not able to undertake its normal processes and functions and the patient is termed as having decompensated disease.

Patients with decompensated liver disease secondary to viral hepatitis are likely to improve their liver function upon eradication of the virus. For HCV recently licensed therapies – such as sofosbuvir and NS5A inhibitors (e.g. ledipasvir and daclatasvir) achieve a virological response in approximately 90% of patients. Many treated patients benefit from therapy with an improvement in MELD scores but some do not and the liver disease continues to advance. Identification of such patients prior to the introduction of therapy would allow early prioritisation for transplantation with antiviral therapy provided post transplantation. We speculate that measuring ICG clearance and Fibroscan® scores in such patients will allow better stratification of patients who may, or may not, benefit from therapy.

For patients with chronic HBV infection highly effective antiviral agents are available and widely used. These include the nucleotide analogues tenofovir and entecavir. In patients with cirrhosis a reduction and improvement in liver fibrosis has been observed. However, once again, it is not clear which patients will derive maximum benefit from therapy and we speculate that pre-treatment assessment of liver function with ICG clearance may improve identification of patients who are most likely to benefit from treatment.

5.2 Preclinical Data

Indocyanine green was first described in 1961 [9]. The initial clinical science was established in baboons and noted that direct measurements would be useful in clinical medicine [10]. Upon IV injection ICG attaches to lipoproteins and is then passively taken up by the liver parenchymal cells, by the Na-taurocholate co-transporting polypeptide. This is then excreted in an ATP-dependent manner into bile [11-14]. Originally direct blood sampling was used to perform this test but the recent introduction of near infra-red spectrophotometry has made ICG testing into a bedside test as it is non-invasive with instantaneous results.

Initial testing of this technology was performed on New Zealand white rabbits with the probe being placed directly on to liver surface. Measurements with the infra-red probe correlated well with serum values [15]. This correlation was confirmed in rabbits with varying degrees of dietary induced cirrhosis, with accurate reduction in blood flow and excreted ICG shown [16].

5.3 Clinical Data

The use of Indocyanine green in cirrhotic patients has been well described and the indication for its use, further strengthened by the confirmation that peripheral levels found in serum and via near infra-red testing in humans are similar [17]. This functional measurement correlates well with the degree of fibrosis as analysed by transient elastography and the current standard, model of end stage liver disease (MELD) scoring system [3, 18-23].

We recently employed the MELD scoring system to assess the use of NS5a inhibitors in association with sofosbuvir in decompensated cirrhotic patients. This is a new treatment option which has been ratified by NICE in a draft proposal this year. We found this treatment regime to be effective in eliminating the virus in 70% of patients with decompensated HCV cirrhosis [24]. While 40% show an early improvement in liver function following HCV therapy, unfortunately the remainder showed minimal improvement and 10% showed worsening liver function.

Figure 1. The effect of new medication regimes on cure rate and MELD scores 4 weeks post
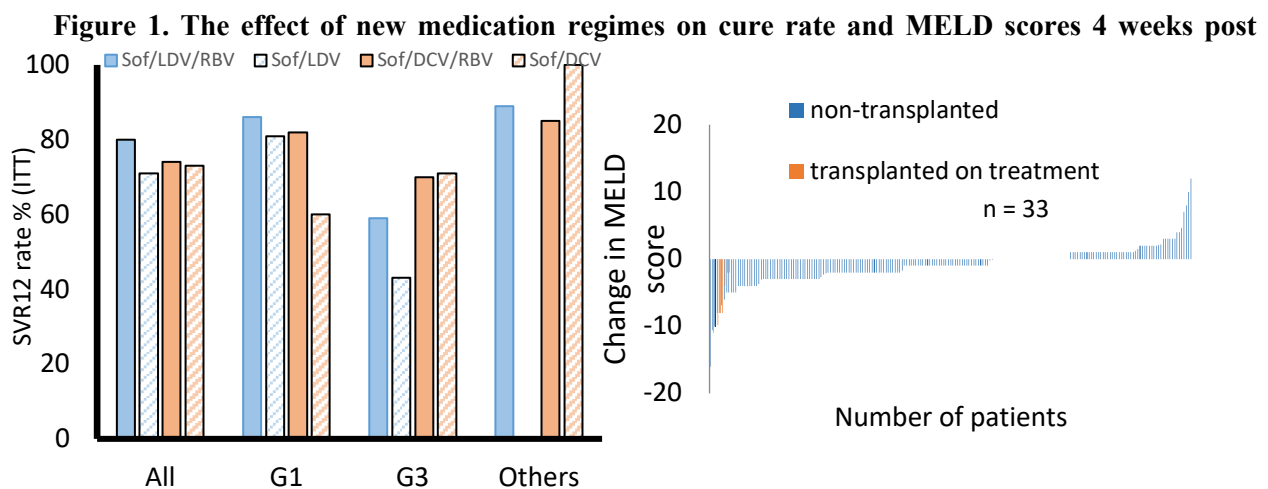
treatment. (SVR12 defined as HCV RNA at 12 weeks post-treatment < 30 IU/ml) Genotype 3 shows 70% viral cure rate. MELD scores show functional improvement in 40% (defined as a decrease of >2) though static/worsening in 60%.

The ability to distinguish which patients would benefit from therapy and which would not would clearly be highly advantageous. Indocyanine green and transient elastography.

Hepatitis B treatment is via suppression of viral replication. Marcellin et al. followed-up patients taking tenofovir for 5 years and found regression of cirrhosis on liver biopsy, in 71 of the 96 patients (74%) enrolled. They found body mass index to be an independent predictor for cirrhotic regression [25] but unfortunately, predicting individuals who will benefit with a reduction in liver cirrhosis is still lacking. We hypothesis that the use of Indocyanine green and transient elastography will allow us to delineate patients who will benefit most from treatment. This will be achieved by comparing the pre-treatment

and post treatment values for each and using multi-variate analysis to determine if a cut-off value can be determined to predict whether dynamic liver tests (DLT) will improve post-treatment and therefore patients successfully treated or alternatively DLT worsens or stagnates and thus patients will be better served by transplantation initially.

5.4 Rationale and Risks/Benefits

5.4.1 Justification

For patients with chronic viral hepatitis who do not regain liver function following effective antiviral therapy, liver transplantation is required. Given that patients receiving antiviral therapy may be suspended from the transplantation waiting list, potentially delaying access to organs, identification of patients who will not benefit from therapy and therefore should be prioritised for transplantation is important. Conversely identification of patients who will probably benefit from treatment may allow re-prioritisation of their transplantation status thereby maximising the use of scarce donated organs.

5.4.2 Risks

As there is no change to patient management and therefore minimal risks are involved. ICG clearance and Fibroscan® are well established techniques in widespread use and the known risks are very rare and include allergy to ICG, discomfort, bruising and infection at cannulation site. To minimise the risks from allergic reactions and complications of cannulation these tests will be performed by experienced operators in a hospital setting with full resuscitation facilities available.

Patients will also be asked to attend additional appointments as stipulated in the PIS and agreed to in the consent form.

5.4.3 Benefits

Though this study will not benefit the participant we hope the results of this study will lead to more effective treatments for patients in the future.

6. TRIAL OBJECTIVES

6.1 Primary Objective

To determine the changes in liver fibrosis and function (assessed by Fibroscan® and ICG clearance) in patients with cirrhosis undergoing antiviral therapy for chronic HCV and HBV.

6.2 Secondary Objective

To determine whether pre-treatment Fibroscan® and ICG testing allows stratification of patients into those at high or low risk of post treatment recovery of liver function.

To determine the medium-term effects of viral eradication on liver fibrosis and function in patients with cirrhosis who have undergone viral eradication in the past.

6.3 Primary Endpoint

This is a pilot study to examine changes in liver function in patients undergoing antiviral therapy for chronic hepatitis B and C infection. The primary endpoint is changes in Fibroscan® and ICG clearance 12 months after antiviral therapy in patients with viral hepatitis induced cirrhosis undergoing antiviral therapy or point estimates of Fibroscan and ICG clearance in patients treated 1, 3 or 5 years ago.

NB: This study (Protocol V5) has been classified as falling outside MHRA CTIMP regulations remit as confirmed from the email dated 14.01.2016 by the MHRA. The administration and assessment of Indocyanine Green tests are not the focus of this study.

7. METHODOLOGY

7.1 Inclusion Criteria

- Patients attending The Royal London Hospital with cirrhosis (defined as fibroscan score >11.5 OR APRI score >2 OR liver biopsy or imaging report of cirrhosis) who are planning to commence antiviral therapy for either chronic hepatitis B or chronic hepatitis C.

- Patients attending The Royal London Hospital with cirrhosis (defined as above) due to chronic HCV infection who have undergone successful antiviral therapy in the past.

- Patients attending The Royal London Hospital with cirrhosis (defined as above) due to chronic HBV infection who are taking antiviral medication

- Age 18 or above

- Willing and able to provide Informed consent

7.2 Exclusion Criteria

- Any inclusion criteria not met

- Pregnancy or breast feeding, checked with pregnancy test if needed

- Known allergy to ICG

7.3 Study Design / Plan – Study Visits

Potential patients will be seen in clinic by their clinical team and will come to an agreement with regard to the treatment options available to them. Those that meet the above criteria will then be offered the opportunity to partake in research with the understanding these tests will not affect their treatment in any way. They will be referred to the research team for informed consent to be taken.

Using the patient's medical notes, baseline patient demographics will be recorded in addition to past medical history (including co-aetiologies such as alcohol consumption / nash / autoimmune / iron etc), clinical examination, biochemical tests (LFT, AST, clotting studies), genotype if available. oesophago-gastro-duodenoscopy (*OGD*), MELD score, Childs-Pugh score and transient elastography as these are routine clinical tests.

For patients with chronic HCV or HBV who are planning to undergo antiviral therapy (short-term patients), baseline Indocyanine green testing will take place within 3 months prior to starting treatment. This will be repeated 4 weeks (+/- 2 weeks) following therapy and again at 1 and 5 calendar years after treatment initiation.

Patients who are 3 or 5 years (+/- 3 months) post viral eradication (medium-term patients) will also have a similar one-off appointment with all above tests and scores recorded with the addition of Indocyanine green testing.

7.3.1 MELD and Childs-Pugh score.

These will be ascertained by the clinical team along standard protocols (see table 1) and extracted from patient notes.

Measure – MELD			
MELD=(0.957 x ln(creatinine µmol/L) + 0.378 x ln(bilirubin µmol/L) + 1.12 x ln(INR) + 0.643)			
Measure – Childs-Pugh	**1 point**	**2 points**	**3 points**
Total bilirubin,	<34 µmol/l	34-50 µmol/l	>50 µmol/l
Serum albumin,	>35 g/L	28-35 g/L	<28 g/L
INR	<1.7	1.7-2.3	>2.3
Ascites	None	Mild	Moderate to Severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

Table 1. Current static liver function scoring. MELD score shows 3-month mortality rate, 40 or more points= 71.3%, 30-39 points =52.6%, 20-29 points =19.6%, 10-19 points =6.0%, <9 points =1.9%. Childs-Pugh score A= 5-6 points, B= 7-9 points, C= 10-15

7.3.2 Transient elastography.

Patients will be asked to have fasted for 3 hours prior to procedure. Transient Elastography will be performed with 10 validated measurements with results expressed in kilopascals (kPa) along with the number of attempts and the interquartile range (IQR).

7.3.3 Indocyanine green.

Patients will be asked to have fasted for 3 hours prior to procedure, cannulated and bloods taken for routine tests if required. Indocyanine green will be injected at a dose of 0.3mg/kg with the tourniquet on. The arm will be raised and tourniquet released ensuring rapid transport of dye. Testing will be by pulse spectrophotometry using a near infra-red finger clip sensor measuring output wavelengths of between, 805-905nm. Measurements will be calculated automatically by the machine for plasma disappearance rate (PDRICG %/min) and ICG plasma retention rate at 15 minutes (ICG15 %/min).

7.4 Study Scheme Diagram
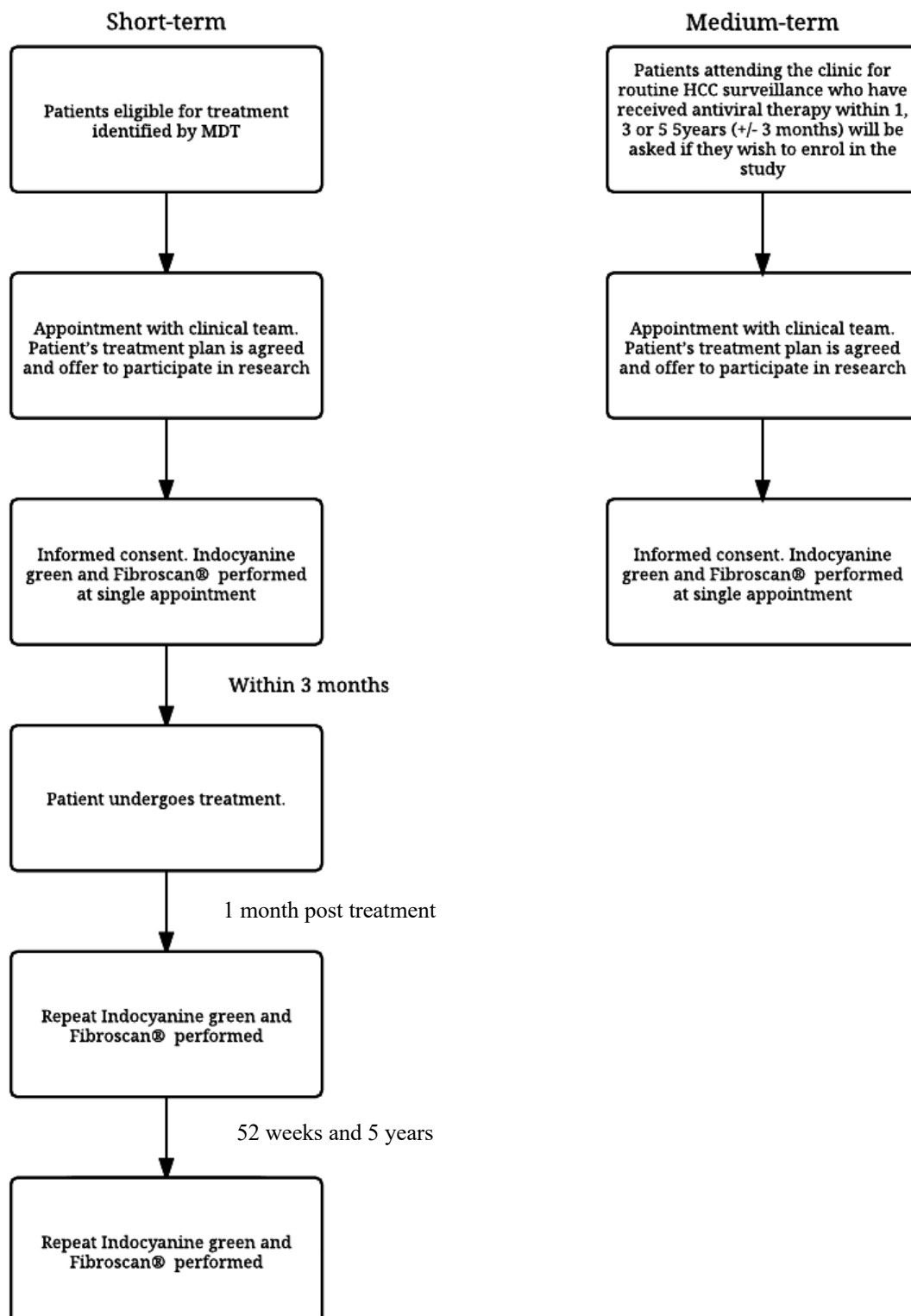
Figure 2. Protocol outline. Patients attending for HCV treatment are termed short term and will thus be enrolled into this arm with pre and post treatment testing. Patients having surveillance testing are termed medium term and receive a single test. HBV patients currently

begin life-long treatment and thus will be tested in either of these 2 arms with the same protocols applied.

## 8.	STUDY PROCEDURES

8.1 Informed Consent Procedures

Ultimately the Principal Investigator (PI) is responsible for the conduct of all aspects of the study.

Patients will be identified via Multi-Disciplinary Team (MDT) or using chronic viral hepatitis databases and those deemed suitable for the study by their consultant, will be contacted during their appointment with the clinical team and verbally informed of the study by their clinical team and asked if they wish to participate in a study. Members of the research team will be available should further information be requested. Willing participants will be provided with a patient information sheet and consent form and asked to consider this at their leisure for a minimum of 24 hours.

Following this, patients wishing to enroll for the study will meet with a member of the study team to discuss and sign the consent form. This will be either the PI, or appropriately GCP trained person as delegated. Following explanation of the study including aims, methods, anticipated benefits and potential hazards, the PI should be available to answer any questions or concerns the patient may have before consent is signed and any participation/study specific procedures performed. The PI will reassure participants, they are completely free to refuse to enter the study or to withdraw at any time during and for any reason.

The consent form and patient information sheet will be reviewed and updated, should new information pertaining to significant changes in risk/benefit assessment becomes available. These should be disseminated to all participants, including those already enrolled and consent to continue in the study attained.

An independent translator will be provided for patients who do not speak or read English. The translator will study the patient information leaflet with the patient, to ensure comprehension of the information. Both the patient and the interpreter will sign the informed consent form

8.2 Screening, Enrollment

The patients will be screened by their clinical teams to ascertain which treatment they should undergo. All patient, physician discussions and decisions for treatment remain unaltered. A clinical examination, routine blood tests, genotype if appropriate, MELD, Childs-Pugh with or without a Fibroscan® will be performed.

Once this has been performed, patients will have the nature of the study, the procedures and the risks fully explained both verbally and as part of the Patient Information Sheet. There are no specific screening procedures within the study. Patients will sign an Informed Consent Form in which they acknowledge that they wish to be enrolled in the study and the above tests retrieved for the purposes of the study. All patients who satisfy these requirements will enter the study.

8.3 Randomization Procedures

Not applicable

8.4 Schedule of intervention or treatment for each visit – see below

Once treatment regime is established by the clinical team, eligible patients will be informed of the research and if willing referred to the research team and contact details given to the patient. If they are willing to participate an appointment will be scheduled within three months prior to the start of therapy.

At this appointment patients will be given an opportunity to (re-)review the patient information sheet and have any remaining related questions answered (up to 15 minutes). Specifically, it will be highlighted, their participation is voluntary and will by no means affect their proposed clinical management. Written consent will then be gained (5 minutes). Patients will then undergo Fibroscan® if not already performed (10-15 minutes) and finally intravenous cannulation followed by Indocyanine green test (15-20 minutes).

One month (+/- 2 weeks) following completion of treatment a repeat appointment will be organised for post treatment tests. Patients will be invited to return again 12 months and 5 years after starting your treatment, so will be asked to attend for a maximum of 4 extra visits.

For patients in the medium-term section (i.e. 3 or 5 years (+/- 3 months) post eradication), only one appointment will be organised.

8.5 Criteria for Discontinuation

The patient's best interests will always take precedence over this study. If the patients' health is affected by the study the patient may be withdrawn and reasons clearly documented in the registry. If this is due to an adverse event, this must be disclosed on an Adverse Event Form and patient followed up to the satisfaction of the Chief Investigator.

8.6 Subject withdrawal (including data collection / retention for withdrawn participants)

If patients have any concerns or following giving consent these will be fully discussed with the research team and will be reminded they are free to withdraw consent at any time and for any reason. Any data already collected will be analysed but no further information will be collected unless the patient specifically states otherwise.

8.7 Electronic Case Report Forms (eCRFs) and storage

Electronic case report forms will be used to record all study related data according to GCP standards and Queen Mary, University of London policies.

These are kept on the central electronic database within Bart's health NHS trust. The eCRF consists participants initials, birth date within the header. The electronic forms with patient identifiers will be stored on NHS computers, protected by standard NHS security systems and no patient identifiable data will be transferred to any other computer.

All eCRFs must be reviewed, signed and dated by the PI. The completed original eCRFs are to be returned to the Sponsor as soon as it is practicable after completion and review. Forms for registration of possible adverse events and for suspension of the study will be available for inclusion.

8.8 Follow-up Procedures

Beyond the fourth appointment for short term and first for the medium-term patients there is no further formal follow up of patients within the study. The patient's clinical team will follow up patients as appropriate.

8.9 Schedule of Assessment (in Diagrammatic Format)

Table 2. Diagrammatic schedule for short term hepatitis B & C patients

Assessment	Details of requirements	1st visit within 3 months of starting treatment	2nd visit following completion of treatment (+/- 2 weeks)	3rd visit after 52 weeks after treatment initiation	4th visit after 5 years after treatment initiation
Informed consent		✓			
	Age	✓			
	Ethnicity	✓			
Recording demographics	Sex	✓			
Record past medical history		✓			
Recording clinical examination		✓	✓	✓	✓
	FBC	✓	✓	✓	✓
	LFT, AST	✓	✓	✓	✓
Recording Routine tests (NB no additional tests will be performed and tests not performed will not be repeated for the study)	Clotting screen	✓	✓	✓	✓
	Genotype if available	✓			
	US	✓			
	OGD	✓			
	Model for end stage liver disease (MELD)	✓	✓	✓	✓
Recording scores	Childs-Pugh	✓	✓	✓	✓
Transient elastography		✓	✓	✓	✓
Indocyanine green testing		✓	✓	✓	✓

Table 3. Diagrammatic schedule for medium term patients 3 or 5 years (+/- 3 months) after either hepatitis C eradication or initiation of therapy for hepatitis B.

Assessment	Details of requirements	Single appointment either 3 or 5 years (+/- 3 months) following either HCV eradication or initiation of HBV treatment
Informed consent		✓
Recording demographics	Age	✓
	Ethnicity	✓
	Sex	✓
Record past medical history		
Recording clinical examination		✓
Recording Routine tests (NB no additional tests will be performed and tests not performed will not be repeated for the study)	FBC	✓
	LFT, AST	✓
	Clotting screen	✓
	Genotype if available	✓
	US	✓
	OGD	✓
Recording scores	Model for end stage liver disease (MELD)	✓
	Childs-Pugh	✓
Transient elastography		✓
Indocyanine green testing		✓

8.10 End of Study Definition

The end of the study will be defined as the day of completion of the last patient final study visit

9 STATISTICAL CONSIDERATIONS

9.1 Sample Size

As this is initially a proof of concept study we shall use the number of variables as 5 (biochemical liver function tests, MELD score, Childs Pugh score, Indocyanine green and transient elastography) and multiply this by 10 thereby aiming for 50 patients. To allow for a 15% drop out, we are aiming to recruit 50 + 15%, which is 8 participants. This higher aim will allow us to recruit sufficient numbers early and mean if people fall out we shall have sufficient numbers to prevent the study from being under powered.

This early data will then be used to calculate the effect size and thus allow for a larger more definitive study to be performed. Data will be calculated with a median level to a 95% confidence interval.

9.2 Method of Analysis

Univariate analysis of continuous and categorical variables will be undertaken using Mann-Whitney U tests and Chi squared analyses respectively. Sensitivity and specificity analysis will be undertaken using receiver operator curves (ROC) to determine their respective optimum cut off levels. Kaplan-Meier curves for survival analysis using cox proportional hazards model will then be used to determine if Indocyanine green and/or transient elastography, at their pre-determined optimum cut offs, predict best treatment course. Logistic predictive modelling will be undertaken to identify the most important baseline factors for response to treatment.

A significant proportion of patients with hepatitis and cirrhosis will be very unwell and will often be admitted to intensive care. We therefore expect some of the recruited patients to die; this would reflect their general overall poor health. Their deaths would not be linked to being included in this study. As a result, we are hoping not to have to report participant deaths as serious adverse events unless they are due to the direct actions of the research team (which we feel is extremely unlikely).

10 ETHICS

The Principal Investigator must ensure that the study will be carried out in accordance with the ethical principles in the Research Governance Framework for Health and Social Care, Second Edition, 2005 and its subsequent amendments as applicable and applicable legal and regulatory requirements.

As such, this project will be submitted for review from a formal research ethics committee and more in-depth analysis is included below.

All clinical decisions and care will be reviewed by a multidisciplinary team in conjunction with the patient and in line with current clinical practice. The additional tests are either side of this treatment plan and thus does not affect this.

The protocol will be conducted in accordance with the principles of the Declaration of Helsinki 2000.

This protocol will be conducted in accordance with the requirements of the Data Protection Act 1998. Data will only be collected and utilised for projects which have ethical, scientific and information governance endorsement. All data will be protected within the requirements of the Bart's Health NHS Trust and Connecting for health.

Specific Ethical considerations:

- Informed consent is voluntary.

- Patients will be free to withdraw consent at any time and without giving a reason.

- Their withdrawal or discontinuation from the study will not affect their planned or future treatment and care in any way.

- Their consent does not affect or influence their rights to decline future approaches or contacts related to participation in clinical research.

- Efforts to prevent "intrusive" approaches to patient volunteers will be made.

- We will inform patients should we identify any clinically relevant findings, according to our duty of care.

The best interests (clinical, mental and personal) of the patient will always take precedence over this protocols.

This protocol, along with any accompanying material provided to the patient in addition to any advertising material and any subsequent amendments will be submitted by the PI to a Research Ethics

Committee. Once written approval is obtained it will be submitted to the JRMO to obtain Final R&D approval.

11 SAFETY CONSIDERATIONS:

Safety considerations for patients are reviewed in section 15. Equipment safety is discussed in section 14.

12 DATA HANDLING AND RECORD KEEPING:

In line with GCP guidelines the study is subject to monitoring by the Sponsor and their elected representative. All auditors and study monitors will be co-operated with. The Sponsor is responsible for monitoring, organisation and quality assurance throughout this study. Direct access to source documents by the representatives of the Sponsor is mandatory in order to ensure the accuracy of data.

12.1 Confidentiality

The PI will be responsible to ensure that patient anonymity is protected and maintained as well as information provided by the Bart's Health NHS Trust and all data and records generated in the course of conducting the study. This information, data, or records will not be used for any purpose other than conducting the study.

The PI will also ensure that patient identities are protected from any unauthorised parties. Information with regards to study patients will be kept confidential and managed in accordance with the eight core principals of the Data Protection Act (1998), NHS Caldicott Guardian provisions, The Research Governance Framework for Health and Social Care (Second Edition 2005), NRES Research Ethics Committee Approval and the regulations of the Bart's Health NHS Trust. The confidentiality of records that could identify participants will be protected, respecting the privacy and confidentiality rules

The study will use participant initials and participant number to refer to patients on all study related documentation. These pseudo-anonymised details will also be used for study correspondence with third parties (sponsor and study funder).

A main participant ID log will be kept in the Investigator Site File that can be located by the Principal Investigator and study team only.

The Chief Investigator is the 'Custodian' of the data. Subjects have the right to revoke their authorisation for the use of their private health information. The patients will be anonymised with regards to any future publications relating to this study.

This study is subject to audit as part of the Sponsor SOPs and policies. In addition regulatory authorities or the REC may request access to all source documents, data capture records, informed consent forms, and other study documentation for on-site audit or inspection. The patient consent form includes information that this will happen and patients are asked to give to consent for these third parties to have supervised access to their patient identifiable data. This should only be accessed for the purposes of audit and inspection and cannot be copied or removed from the study site.

12.2 Record Retention and Archiving

The Chief Investigator is responsible for all records during this research. These will be kept in secure conditions throughout. At the completion of the trial records will be kept for a further 20 years as is required by the Research Governance Framework and Trust Policy that the. For trials involving Bart's Health NHS Trust patients, or Sponsored by Bart's Health NHS Trust or QMUL, the approved repository for long-term storage of local records is the Trust Modern Records Centre.

13. PRODUCTS, DEVICES, TECHNIQUES AND TOOLS

13.1 Devices

Transient elastography will be measured using a Fibroscan® machine which is manufactured by echosens®. It has a CE mark of 0459 and is already in use and available within the hepatology department at The Royal London Hospital. All electrical equipment connected to the mains supply within the hospital is safety tested.

It is used as a non-invasive method to accurately measure fibrosis of a patient's liver without the need for a liver biopsy. This is measured by an externally placed ultrasound probe which creates an elastic shear wave at 50Hz, and measures the velocity of the echo through the liver tissue. Ten measurements are taken and the median average is then expressed as the liver stiffness measurement in kilopascals (kPa). It has been shown to be 99% effective in detecting cirrhosis in hepatitis C and has been shown to correlate with hepatoma production in both hepatitis C and hepatitis B. Values range from – normal < 9.6 kPa, significant fibrosis 9.6-11.5kPa, cirrhosis >11.5kPa. It cannot be used in patients with ascites.

The machine to monitor the elimination of Indocyanine green is manufactured by PULSION medical systems (Munich, Germany) and consists of the PulsioFlex monitor and LiMON module. The LiMON module is a finger probe which measure near-infrared wavelengths between 805nm and 905nm. The absorption maximum for ICG is 800nm and emission at 830nm. This is then used to produce two calculations. The first is PDR_{ICG} which is a measure of the plasma disappearance rate of ICG. This is based on working out the constant and backward extrapolation and expressed as a percentage per minute. The other is the retention ratio after 15 minutes ICG15.

13.2 Techniques and interventions

Please see methods.

Tests will be conducted following local guidelines and SOPs and will either be performed by an appropriately trained research team-member or PI and.

13.3 Medicinal product

Indocyanine green is a green dye and is a monosodium salt of 1-[sulfo-butyl)-3.3-dimethyl-2-(7-[-(4-sulfo-butyl)-3.3-dimethyl-4.5-benzoindoliny-liden-(2)]-heptatrien(1.3.5)-yl)4.5 benzoindolium iodide and a molecular formula of $C_{43}H_{47}N_2NO_6S_2$. The molecular weight is 774.97 Daltons. 1-2 seconds following intravenous injection the dye binds almost completely to globulins, preferentially α1-lipoproteins. This binding means peripheral excretion is negligible and is solely by the liver. The liver's parenchymal cells take up the ICG, bound by organic anion transporting polypeptide 1B3, by the Na+-taurocholate co-transporting polypeptide (NTCP) transporter and it is eliminated in bile in an ATP dependant process. This is very efficient with more than 99% being eliminated via this route with only a small fraction detectable peripherally after 10 minutes. The dye undergoes no chemical change in the liver and does not undergo enterohepatic circulation.

ICG is supplied in powder form and is reconstituted with water only and is administered at a dose of 0.3mg/kg. It should be stored at less than +25˚C and out of direct sunlight. It shows little decomposition once reconstituted for the first few hours. Its effect in pregnancy and on breast milk is unknown and therefore caution advised.

Side–effects are urticarial or anaphylactic reactions including patients allergic to iodine and are rare. Certain drugs may alter absorption of ICG in the liver, these include drugs that reduce absorption – anti-convulsants, disulphite compounds, haloperidol, heroin, pethidine, methamizol, methadone, morphine, nitrofurantoin, opium alkaloids, phenobarbitone, phenylbutazone and drugs that increase absorption – cyclopropane, probenecid, and rifamycin. Due to the compound containing iodine, thyroid uptake studies should not take place within a week of the test due to interference.

14 SAFETY REPORTING

14.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with study activities.

14.2 Notification and reporting Adverse Events or Reactions

If the AE is not defined as SERIOUS, the AE is recorded in the study file and the participant is followed up by the research team. The AE is documented in the participants' medical notes (where appropriate) and the CRF.

14.3 Serious Adverse Event (SAE)

In other research other than CTIMPs, a serious adverse event (SAE) is defined as an untoward occurrence that:

(a) Results in death;

(b) is life-threatening;

(c) requires hospitalisation or prolongation of existing hospitalisation;

(d) Results in persistent or significant disability or incapacity;

(e) Consists of a congenital anomaly or birth defect; or

(f) Is otherwise considered medically significant by the PI.

An SAE occurring to a research participant should be reported to the main REC where in the opinion of the Chief Investigator the event was:

- Related – that is, it resulted from administration of any of the research procedures, and

- Unexpected – that is, the type of event is not listed in the protocol as an expected occurrence.

14.4 Notification and Reporting of Serious Adverse Events

As the sponsor of the Study, Queen Mary, University of London (QMUL) and Professor GR Foster shall be responsible for complying, within the required timelines, with any safety reporting obligation towards the competent Health Authorities, the Ethics Committee(s) and CI/PI, as defined in the applicable laws and regulations, or elsewhere. Serious Adverse Event (SAEs) that are considered to be 'related' and 'unexpected' are to be reported to the sponsor within 24 hours of learning of the event and to the Main REC within 15 days in line with the required timeframe.

This includes:

- The retrieval and assessment of all serious adverse events as reported by the PI in the study;
- Submission of expedited serious single case reports to Health Authorities (by electronic means where applicable), the Ethics committee(s) and where applicable to the distribution of these to all participating investigators in the concerned study;
- The preparation and submission of annual safety reports (ASR) of the concerned study;
- The submission of periodic listings of expedited serious unexpected adverse events as appropriate;
- The submission of any updated documents as required.

QMUL and GR Foster will submit to LAPresearch UK, the following safety information:

- All Serious Adverse Events regardless of whether causality of the study is suspected by the PI as well as transmission of an infectious agent and medication errors. QMUL and GR Foster will transmit these **SAE reports by facsimile in English within 24 hours of becoming aware of the event(s)**. Follow-up information will be transmitted within the same timelines;
- Copies of all expedited serious single case reports sent to the Health Authorities and Ethics Committees (following causality and expectedness assessments made as applicable for the current study sponsored by the institution and/or Principal Investigator);

QMUL and GR Foster shall notify LAPresearch UK immediately in case of a suspension of recruitment or premature cessation of the concerned clinical study because of a safety concern; preferably by means of

telephone contact, alternatively by fax within 24 hours of the decision; at the end of the treatment phase (= "last patient off treatment") as well as the end of any follow-up phase (= "last patient out") of the Study, QMUL and GR Foster shall provide all Adverse Events, both serious and non-serious, in report format within 90 days after completion of the treatment or follow-up respectively.
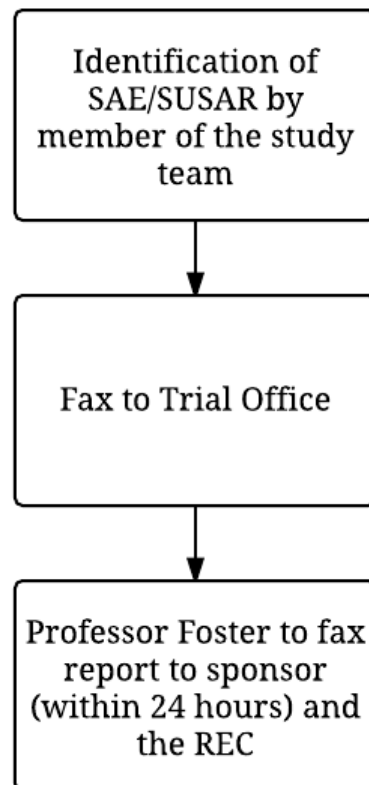
Figure 3. Diagrammatic representation of protocol for reporting serious adverse effect and Suspected Unexpected Serious Adverse Reaction

14.5 Investigators Assessment

14.5.1 Seriousness

The Chief/Principal Investigator responsible for the care of the patient, or in his absence an authorised medic within the research team, is responsible for assessing whether the event is serious according to the definitions given in section 7.1.

14.5.2 Causality

The PI must assess the causality of all serious adverse events/reactions in relation to the trial treatment according to the definition given. If the SAE is assessed as having a reasonable causal relationship, then it is defined as a SAR.

14.5.3 Expectedness

The PI must assess the expectedness of all SARs according to the definition given. If the SAR is unexpected, then it is a SUSAR.

14.5.4 Severity

The PI must assess the severity of the event according to the following terms and assessments. The intensity of an event should not be confused with the term "serious" which is a regulatory definition based on patient/event outcome criteria.

Mild: Some discomfort noted but without disruption of daily life

Moderate: Discomfort enough to affect/reduce normal activity

Severe: Complete inability to perform daily activities and lead a normal life

14.5.5 Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical trial subjects from any immediate hazard to their health and safety. The measures should be taken immediately. In this instance, the approval of the REC prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor and Main Research Ethics Committee (via telephone) of this event immediately.

The CI has an obligation to inform both the Main REC in writing within 3 days, in the form of a substantial amendment. The sponsor (Joint Research Management Office [JRMO]) must be sent a copy of the correspondence with regards to this matter. For further guidance on this matter, please refer to NRES website and JRMO SOPs.

14.5.6 Annual Safety Reporting

The CI will send the Annual Progress Report to the main REC using the NRES template (the anniversary date is the date on the MREC "favourable opinion" letter from the MREC) and to the sponsor. Please see NRES website and JRMO SOP for further information

14.5.7 Overview of the Safety Reporting responsibilities

The CI/PI has the overall pharmacovigilance oversight responsibility. The CI/PI has a duty to ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

15 MONITORING &AUDITING

15.1 Monitoring

This Study will be monitored as per sponsors SOPs and study specific monitoring plan and to ensure safety of Patients, Integrity of data and compliance with the Protocol and regulations.

15.2 Audit

Definition:

"A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analysed and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s)."

A study may be identified for audit by any method listed below:

1. A project may be identified via the risk assessment process.

2. An individual investigator or department may request an audit.

3. A project may be identified via an allegation of research misconduct or fraud or a suspected breach of regulations.

4. Projects may be selected at random. The Department of Health states that Trusts should be auditing a minimum of 10% of all research projects.

5. Projects may be randomly selected for audit by an external organisation.

This project is subject to audit as part of the Sponsor SOPs and policies. In addition regulatory authorities, the REC and/or the Funder LAPresearch UK may request access to all source documents, data capture records, informed consent forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the PI, who must provide support at all times for these activities. In addition the study may be audited as part of the Sites Quality Management requirements.

Any non-compliance as highlighted by these processes will be logged by the Sponsor. These will then be assessed and a timeline put in place to correct these individually and dependent on severity. If these are not achieved satisfactorily the JRMO will agree on an appropriate action.

16 TRIAL COMMITTEES

The project will be reviewed by independent medical peers who will review the safety data and will have the authority to terminate the study if they believe that its continuation poses unacceptable risks to patients.

It will also be reviewed by an independent ethical review committee via the National Research Ethics Service (NRES) and includes the Protocol, Consent Forms and Subject Information Sheet as well as any subsequent amendments. The PI will notify the Sponsor immediately, if approval is suspended or terminated by the Ethics Committee. The PI is responsible to report study progress to the Ethics Committee at intervals not greater than one year.

All conduct and progress will be monitored by the trial management group who will ensure the protocol is adhered to and participant safety is maintained throughout. This team will consist of the CI and PI and will meet monthly.

In addition the study may be audited as part of the Sites Quality Management requirements.

Serious adverse events will be reported to the Ethics Committee as soon as possible and in any event within 72 hours by the PI.

Written approval from the Committee must be obtained and subsequently submitted to the JRMO to obtain Final R&D approval.

17 FINANCE AND FUNDING

The study is funded by the charity LAPresearch UK (Registered Charity No 1130523). The LiMON system and consumables will be supplied by them. The Fibroscan machine is already owned by The Royal London Hospital.

18 INDEMNITY

Queen Mary University of London have arranged for suitable indemnity concerning negligent harm to be in place for this study. Indemnity will be provided by Queen Mary, University of London. The insurance that Queen Mary, University of London has in place provides "No Fault Compensation" for participants which provides an indemnity to participants for non-negligent harm.

19 DISSEMINATION OF RESEARCH FINDINGS:

All publications from the study will be published with joint authorship which will include all members of the study team. No member of the study team may publish any data from the study without the express consent of the management committee and any publications will be co-authored by all members of the study teams.

20 REFERENCES

1. Shiina, T., et al., *WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: basic principles and terminology.* Ultrasound Med Biol, 2015. **41**(5): p. 1126-47.

2. Sandrin, L., et al., *Shear elasticity probe for soft tissues with 1-D transient elastography.* IEEE Trans Ultrason Ferroelectr Freq Control, 2002. **49**(4): p. 436-46.

3. Sandrin, L., et al., *Transient elastography: a new noninvasive method for assessment of hepatic fibrosis.* Ultrasound Med Biol, 2003. **29**(12): p. 1705-13.

4. Oudry, J., et al., *Comparison of four different techniques to evaluate the elastic properties of phantom in elastography: is there a gold standard?* Phys Med Biol, 2014. **59**(19): p. 5775-93.

5. Bamber, J., et al., *EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology.* Ultraschall Med, 2013. **34**(2): p. 169-84.

6. Kamath, P.S., et al., *A model to predict survival in patients with end-stage liver disease.* Hepatology, 2001. **33**(2): p. 464-70.

7. Kamath, P.S. and W.R. Kim, *The model for end-stage liver disease (MELD).* Hepatology, 2007. **45**(3): p. 797-805.

8. Procopet, B., et al., *Real-time shear-wave elastography: applicability, reliability and accuracy for clinically significant portal hypertension.* J Hepatol, 2015. **62**(5): p. 1068-75.

9. Caesar, J., et al., *The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function.* Clin Sci, 1961. **21**: p. 43-57.

10. Grainger, S.L., et al., *Clearance and non-invasive determination of the hepatic extraction of indocyanine green in baboons and man.* Clin Sci (Lond), 1983. **64**(2): p. 207-12.

11. SE, P.M.S., *ICG-Pulsion - physicians prescribing information*, P.M.S. SE, Editor. 2015.

12. Faybik, P. and H. Hetz, *Plasma disappearance rate of indocyanine green in liver dysfunction.* Transplant Proc, 2006. **38**(3): p. 801-2.

13. de Graaf, W., et al., *Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green.* J Hepatol, 2011. **54**(4): p. 738-45.

14. Baker, K.J., *Binding of sulfobromophthalein (BSP) sodium and indocyanine green (ICG) by plasma alpha-1 lipoproteins.* Proc Soc Exp Biol Med, 1966. **122**(4): p. 957-63.

15. El-Desoky, A., et al., *Experimental study of liver dysfunction evaluated by direct indocyanine green clearance using near infrared spectroscopy.* Br J Surg, 1999. **86**(8): p. 1005-11.

16. Jiao, L.R., et al., *Effect of liver blood flow and function on hepatic indocyanine green clearance measured directly in a cirrhotic animal model.* Br J Surg, 2000. **87**(5): p. 568-74.

17. de Liguori Carino, N., et al., *Perioperative use of the LiMON method of indocyanine green elimination measurement for the prediction and early detection of post-hepatectomy liver failure.* Eur J Surg Oncol, 2009. **35**(9): p. 957-62.

18. Sheng, Q.S., et al., *Indocyanine green clearance test and model for end-stage liver disease score of patients with liver cirrhosis.* Hepatobiliary Pancreat Dis Int, 2009. **8**(1): p. 46-9.

19.   Corradi, F., et al., *Assessment of liver fibrosis in transplant recipients with recurrent HCV infection: usefulness of transient elastography.* Dig Liver Dis, 2009. **41**(3): p. 217-25.

20.   Castera, L., X. Forns, and A. Alberti, *Non-invasive evaluation of liver fibrosis using transient elastography.* J Hepatol, 2008. **48**(5): p. 835-47.

21.   Wilder, J. and K. Patel, *The clinical utility of FibroScan((R)) as a noninvasive diagnostic test for liver disease.* Med Devices (Auckl), 2014. **7**: p. 107-14.

22.   Gupta, S., et al., *Indocyanine green clearance test (using spectrophotometry) and its correlation with model for end stage liver disease (MELD) score in Indian patients with cirrhosis of liver.* Trop Gastroenterol, 2012. **33**(2): p. 129-34.

23.   Vos, J.J., et al., *Green light for liver function monitoring using indocyanine green? An overview of current clinical applications.* Anaesthesia, 2014. **69**(12): p. 1364-76.

24.   GR Foster, J.M., W. Irving, M. Cheung, B. Hudson, S. Verma, K. Agarwal, HCV Research UK EAP Group, *Treatment of decompensated HCV cirrhosis in patients with diverse genotypes: 12 weeks sofosbuvir and NS5A inhibitors with/without ribavirin is effective in HCV Genotypes 1 and 3.* EASL, 2015.

25.   Marcellin, P., et al., *Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study.* Lancet, 2013. **381**(9865): p. 468-75.

3.6 Patient information sheet

The Liver Unit   Main switchboard: 020 7377 7000
Directorate of General and Emergency Medicine   Secretary only: 020 7377 7442

The Royal London Hospital   www.bartsandthelondon.nhs.uk
Whitechapel
London E1 1BB

Professor G R Foster
Professor of Clinical Hepatology
PATIENT INFORMATION SHEET (V6 24.02.17)

Changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy.

Invitation

You are invited to take part in a clinical research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Feel free to ask any questions and, if you wish, to discuss this information with your doctor, family and friends before making your decision. Please ask us if there is anything that is not clear or if you would like more information. You will be given as much time as you need to make a decision. Your participation in this study is voluntary. If you decide not to participate this will not affect the way you are treated and you will continue to be cared for in exactly the same way.

What is the purpose of the study?

The liver is vital to survival as it carries out many functions from digesting food to breaking down toxins in our food. Many diseases can damage the liver but the liver can regenerate and regrow if the cause of the damage is removed. However, if the liver is very badly damaged it may not be able to recover. Patients with viral hepatitis who have liver damage can now be treated with drugs which often get rid of the virus. However, we do not know how much or how quickly the liver recovers when the virus has been eliminated. This study addresses this question and looks at how the liver is functioning before and after treatment.

These tests take maximum of four hours to perform, spread over four visits if you are about to start treatment or a single one hour appointment if you have completed treatment. Two tests will be performed – the first involves a special scan (a Fibroscan®) in which a probe is placed on the abdomen and an ultrasound beam used to measure the elasticity of the liver. The test involves no radiation and uses ultrasound waves that are believed to be harmless. The second test involves an injection of a dye that is broken down by the liver. A doctor or nurse will insert a small needle into a vein, usually on your arm, and inject the dye into your bloodstream. You will then be asked to place a small probe on your finger to measure the amount of the dye in your blood.

This study is being conducted as an academic research study as part of research degree under the supervision of Professor Graham Foster, head of hepatology at The Royal London hospital. If this

study is successful we hope to publish in a medical journal though the results will also be sent to you with all information provided being anonymised.

Why have I been invited to take part?

You are being asked to take part in this study because you have been diagnosed with either chronic hepatitis C or hepatitis B with liver scarring and are starting a new treatment or have completed a course of therapy.

Do I have to take part?

No – you do not have to take part and it is up to you to decide whether you want to take part or not. If you choose to take part, you will be given this leaflet to keep and you will be asked to sign a consent form. You are still free to back out of the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive. If you withdraw from the study, we will continue to use the data collected up to your withdrawal but we will collect no further data.

What will happen to me if I take part?

Please be aware every patient who agrees to take part in this study receives the same medical treatment and this is unaffected by entering this study. If you decide that you would like to take part, you will meet the research team at a time convenient to you for a 40-60 minutes appointment.

If you are starting treatment this will be within the three months prior to starting. You will then see them again 1 month after completing your treatment. You will be invited to return again 12 months and 5 years after starting your treatment. You will thus be asked to attend for a maximum of FOUR extra visits, for a maximum time of 1 hour per visit. In total, you will receive three extra Fibroscan® tests and four ICG tests in addition to your normal care.

If you have completed treatment 3 or 5 years ago, a single 1 hour appointment will be organised and you will receive one Fibroscan® test which sometimes is offered in normal care and one ICG test which is not normal care.

At your appointment, some information regarding the severity of your condition will be retrieved from your notes along with demographics. You will then have two extra tests performed during each appointment. We ask for you to not to have eaten or drunk anything for the 3 hours before your appointment.

A small amount of dye will be injected into a vein in your arm and a small non-invasive monitoring device attached to your finger. This device measures how quickly the dye is eliminated from your body by your liver. The second test would be similar to an ultrasound scan on your abdomen for which a probe is placed on a water based gel and measurements

are taken. If you choose not to participate, a Fibroscan may be required for your clinical care but if you chose to participate this will always be performed.

What are possible disadvantages and risks of taking part?

You will be required to be present for a maximum of four appointments lasting around one hour each. You will have a cannula placed into your arm during this and small injection given. This may be mildly painful and can cause bruising, discomfort, a small chance of infection to the skin (7.3%) or within the rest of the body (0.3%).

The dye injected is safe on the most part with a small proportion of people having mild reactions e.g. rash or headache, which usually settle. There is a small chance the dye may leak into the skin and if this occurs you may have some green colouring to this portion of skin for a few weeks. This would cause no other ill effects. Rarely, moderate or severe allergic reactions occur such as anaphylaxis though trained medical personnel will be on hand should this occur. These reactions occur in 1in 40,000 patients receiving this test.

Please let your doctor know if you have had this test before and are allergic to the ICG dye or are allergic to iodine as this study may not be appropriate for you.

What are the benefits of taking part?

Though this study will not benefit you directly, we hope the results of this study will lead to more effective treatments for patients in the future.

What happens if there is a problem?

Please contact us immediately if you have concerns about any aspect of this study or your health. Whilst we do not anticipate any major problems one cannot predict every eventuality. We would not expect you to suffer any harm or injury because of your participation in this study. Queen Mary University of London has agreed that if you are harmed as a result of your participation in the study, you will be compensated, provided that, on the balance of probabilities, an injury was caused as a direct result of the intervention or procedures you received during the course of the study. These special compensation arrangements apply where an injury is caused to you that would not have occurred if you were not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

Please contact Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care you have received, or as an initial point of contact if you have a complaint. Please telephone Tel: 020 3594 2040, e-mail: pals@bartshealth.nhs.uk or you can also visit PALS by asking at any hospital reception.

Contact details for further information:

For questions relating to this research study, to report a research related injury or information about study procedures you should contact:

Investigator: Prof Graham Foster
Daytime: 020 3594 6773 / 020 7377 7457
Out of hours: Tel: 020 7377 7000 (Trust Switchboard) & ask for the Hepatology Registrar "on-call"
Address:
Barts Health NHS Trust, Royal London Hospital, Grahame Hayton Unit, (GHU), Ambrose King Centre, Whitechapel Road, London E1 1BB.

If you have any questions about your rights as a research subject, again you can go through the NHS Complaints Procedure through PALS as listed above.

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available. Any significant new findings that are made during the course of the research, and that may relate to your willingness to continue participating in the study, will be provided to you in a timely manner.

If you decide not to continue in the study, your research doctor will ensure that your hepatologist will continue to provide care for your liver problems. If you decide to continue in the study your will be asked to sign an updated consent form.

What will happen if I don't want to carry on with the study?

You may withdraw from the study at any time with no effect to your ongoing clinical treatment.

Information collected may still be used; if you want your data to be removed from the study you must notify the study team.

If you become unwell during the study and cannot tell us whether or not you want to carry on with the study we will continue to keep and use the information about you that we have already collected.

Will my taking part be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. If you agree to take part in the study, a copy of your signed consent form, containing your full name, will be sent to the Trial Manager at Queen Mary, University of London.

If you consent to take part in this trial we may look at sections of your medical notes to collect data relevant to this study which will be used in our final analysis. Only researchers of this study at QMUL will have access to your personal information for purposes of this study. Your personal information will not be passed to other researchers or organisations. People may also look at them from regulatory authorities to check that the study is being carried out correctly.

We will inform your GP and/or any other medical practitioner who currently treats you about your participation in this study with your consent.

All data collected during this trial will be processed in accordance with the Data Protection Act, 1998. Personal details such as your initials, date of birth and data collected during the trial will be entered onto a computer and stored electronically at Queen Mary, University of London. Responsible individuals from the trial team will access this information.

The study may be liable to audit or inspection by the study sponsor (Queen Mary University of London) and/or the research ethics committee. In this circumstance your data may be viewed by authorised members of the team from these organisations but your confidentiality will be maintained. Anonymised data might be used in further studies and will be archived for 20 years in anonymised form and after will be disposed of securely.

What will happen to the results of the research study?

At the end of the study the results will be published but details of the patients who took part will not be made known.

Who is organizing and funding the research?

This study is being organized and Sponsored by Queen Mary University of London. The study is being funded by LAPresearch UK (Registered Charity No 1130523) a charity committed to eradicating diseases affecting the liver, pancreas, gallbladder and biliary tree.

Who has reviewed the study?

This study has been reviewed and was given a favourable opinion by an independent Research Ethics Committee.

Thank you for considering participating in this study

3.7   GP Information Sheet

The Liver Unit   Main switchboard: 020 7377 7000
Directorate of General and Emergency Medicine   Secretary only: 020 7377 7442
The Royal London Hospital   www.bartsandthelondon.nhs.uk
Whitechapel
London E1 1BB

Professor G R Foster
Professor of Clinical Hepatology

GP INFORMATION SHEET (V5 24.02.17)

Changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy.

Dear Doctor,

Your patient, [INSERT PATIENT NAME, DOB AND HOSP NUMBER] has agreed to take part in a research project examining the use of pre-treatment testing to predict likelihood of successful therapy.

As you know new treatments for viral hepatitis are now available on the NHS. We are looking at recovery of liver function and reversal of fibrosis in patients who have been treated with the new drugs. Your patient will undergo two additional tests per appointment, organised before and after therapy and repeated at one and 5 years post treatment, therefore four extra appointments in total. Alternatively, if your patient has successfully been treated previously or is on ongoing hepatitis B treatment they will have a single appointment with these two tests performed. The tests are the Fibroscan test and the indocyanine green dye excretion test.

Fibroscan® tests use technology similar to ultrasounds to give information on the degree of fibrosis in the liver. Indocyanine green involves giving a dye intravenously to ascertain its excretion by the liver into bile. This is achieved using a finger probe similar to an oximeter. This is generally safe though some patients complain of urticarial reactions and rarely anaphylaxis. As the dye contains iodine there is some interference with thyroid uptake tests and they shouldn't be performed within one week of each other.

The trial involves these tests being performed within three weeks prior to starting treatment and repeated within three weeks following completion of treatment.

Your patient will be kept under close review through the hospital treatment centre and further details on your patient's progress will be sent to you as required.

If you have any questions regarding the trial please do not hesitate to contact me.

Yours sincerely,

Prof. Graham FOSTER
BA, FRCP, PhD
Professor & Consultant Hepatologist

3.8   Patient consent form

The Liver Unit
Directorate of General and Emergency Medicine
The Royal London Hospital
Whitechapel
London E1 1BB

Main switchboard: 020 7377 7000
Secretary only: 020 7377 7442
www.bartsandthelondon.nhs.uk

Professor G R Foster
Professor of Clinical Hepatology

PATIENT CONSENT FORM (V6 24.02.17)

Changes in dynamic liver function tests in patients with chronic viral hepatitis undergoing antiviral therapy.

Participant Study ID:

	Please initial each box
1. I confirm that I have had a chance to read the patient information sheet (PIS: v6) about this study, and had the opportunity to consider the information, ask questions and have these answered satisfactorily.	
2. I understand that participation in this study is entirely voluntary, and may be withdrawn at any time, without providing a reason, without my medical treatment being affected in any way.	
3. I understand that relevant sections of my medical notes may be looked at as part of the study by the research team, including research fellows and nurses involved in the study and I agree to this process and give permission for them to access these records.	
4. I understand that if I am due to have thyroid tests within one week of appointments I should notify the research team to reschedule.	
5. I agree to the research team holding personally identifiable information about me. I understand that this information will not be shared outside of the research team or Queen Mary University of London.	
6. I understand my data and participation will be unidentifiable to anyone outside of the direct research team and may be used for reports for the study.	
7. I understand that you will inform my GP about my participation in this study.	
8. I understand that data collected during the study will be kept as computerised records for 20 years.	

354

9. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from regulatory authorities or from the Barts Health NHS trust/Queen Mary University of London, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records	
10. I understand that should I lose ability to give consent for any reason or withdraw my consent my participation within the study will be discontinued though any data already collected may continue to be used.	
11. I agree to take part in the study.	

Name of Participant (in capitals)   Signature   Date

Name of Person taking Consent   Signature   Date

One copy of this form should be kept by the researcher and one copy given to the participant for their own records.

3.9   Correlation of individual patient changes over 1 year

To better understand changes for individual patients, we have further described those with derangements. Unfortunately, several patients with the largest derangements did not attend their 1 year follow up which does hinder our ability to review this important sub-set, namely patients 17, 32 and 42, all of whom had transient elastographies of over 20kPa, all of whom showed some slight improvement in liver function following treatment but remained markedly deranged. Patient 57 received all her functional tests but unfortunately attended on a day with no TE equipment was present. She was a genotype 3 patient who was treatment-naive with a history of varices and severe alcoholism and was determined as a CTP grade B. She had marked cirrhosis with pre-treatment stiffness of 69kPa with a functional deficit, PDR - 2%/min, ICGR15 - 74.1%; this had shown some mild improvement post-treatment, however, her liver function, though still deranged, had improved noticeably by the end of follow up and leaves the question of whether she will return to acceptable function in the future and whether her liver stiffness would have reflected this. Patient 52 had a similar history, though is genotype 1, attended with an elastography of 40.7kPa and deranged liver function (PDR - 13.3%/min, ICGR15 - 13.6%), however, she reached normal function 4 weeks following treatment despite ongoing increased liver stiffness (37.8kPa) but again did not attend for her final appointment. The single patient that did achieve SVR, however, did have an improvement in both TE and liver function.

Patient 46 was genotype 4 with previous HCC and varices and was CTP grade B at enrolment and showed an unusual pattern with an initial worsening in TE at four months before improving to below baseline (53.7kPa to 63.3kPa to 38.7kPa) at their final reading. Conversely, his liver function shows the opposite pattern with an initial improvement from baseline but then had worsened values at the end of follow-up (PDR - 2.8%/min to 3.6%/min

to 2.2%/min, ICGR15 - 65.7% to 58.3% to 71.9%). Similarly, patient 50, a genotype 3, treatment-naive, previous IVDU, CTP grade A, treated with Sofosbuvir and Velpatasvir, had a cirrhotic liver stiffness at the beginning of treatment (35.8kPa), which had initially improved but was back to baseline by one year (35.1kPa). Their liver function showed an initial improvement, though this improvement had stagnated and continued to be deranged at 1 year (PDR - 6.2%/min to 11.6%/min, ICGR15 - 39.5% to 17.6%). These patients may indicate patients that have reached the point of no return for their liver function or that co-morbidities (such as alcohol misuse) had influenced their response.

Patient 8 had a jump in liver stiffness at the end of their treatment (12.1kPa to 20.8kPa). They were a genotype 3, CTP grade A, who was treatment-experienced with no previous decompensation and was treated with Peg/Sof/Riba and had normal liver function throughout. TE readings had proven difficult, with an IQR of above 2.3 at all of her readings. Of note, she had a change in her treatment for her breast cancer between her 4 weeks and 1 year appointment, changing from tamoxifen to anastrozole, which may have attributed to this aberration. However, patient 41, who was a treatment-experienced, genotype 3 CTP grade A, with no previous decompensation and treated with Sofosbuvir and Velpatasvir, shows a similar pattern with an overall decline in TE (12kpa to 9.8kPa to 15.2kPa), despite a normal liver function throughout. In contrast, we see patients 13, 35, 39 and 49, improve their TE despite declining PDR over the year though they had a normal ICGR15.

Patients 45 and 51 both had an improvement at 1 year after an initial worsening of their liver stiffness before improving at the end of treatment (patient 45 - 21.3kPa to 23.8kPa to 18.4kPa, Patient 51 - 18.3kPa to 20.7kPa to 13.7). Both showed a deranged function at baseline which steadily improved over the year to both having a normal ICGR15 with a slight

derangement in PDR at their final appointment (patient 45 - PDR - 8.7%/min to 15.5%/min to 16.6%/min, ICGR15 - 27.1% to 9.8% to 8.3%, patient 51 - PDR - 11.2%/min to 13.8%/min to 15.6%/min, ICGR15 - 18.6% to 12.6% to 9.6%). Contradicting this is patient 40, who began with a normal liver function (PDR - 19.3%/min, ICGR15 - 5.5%) and a liver stiffness of 14.8kPa, however, they showed a worsening stiffness (17.4kPa to 18kPa) in conjunction with a worsened liver function following treatment (PDR - 10.6%/min, ICGR15 - 20.4%), which started to improve again by one year, though this was still deranged (PDR - 12.2%/min, ICGR15 - 16%) despite achieving SVR12 and maintaining this at SVR48.

3.10 Analysis of variables post treatment

To investigate the impact of treatment for hepatitis C on other variables, we evaluated blood tests and relevant scoring systems by visual inspection both individually and as a median, as well as, Wilcoxon Signed-Rank test between baseline, 4 months and 12-month results. This may then highlight which factors would be important for univariate analysis.

3.1.1 Haemoglobin

We first investigated haemoglobin as this is used for general patient health, as well as oxygen delivery to hepatocytes. For the most part, patients remained stable with regards to haemoglobin though it should be noted that patients 39 and 42 did not have any available blood tests. Patient 35 had the most marked drop in haemoglobin (see Figure 3.1). There are no changes over the follow-up period when observed together (Figure 3.2) and as a median (p-value 0.833) (Figure 3.3).
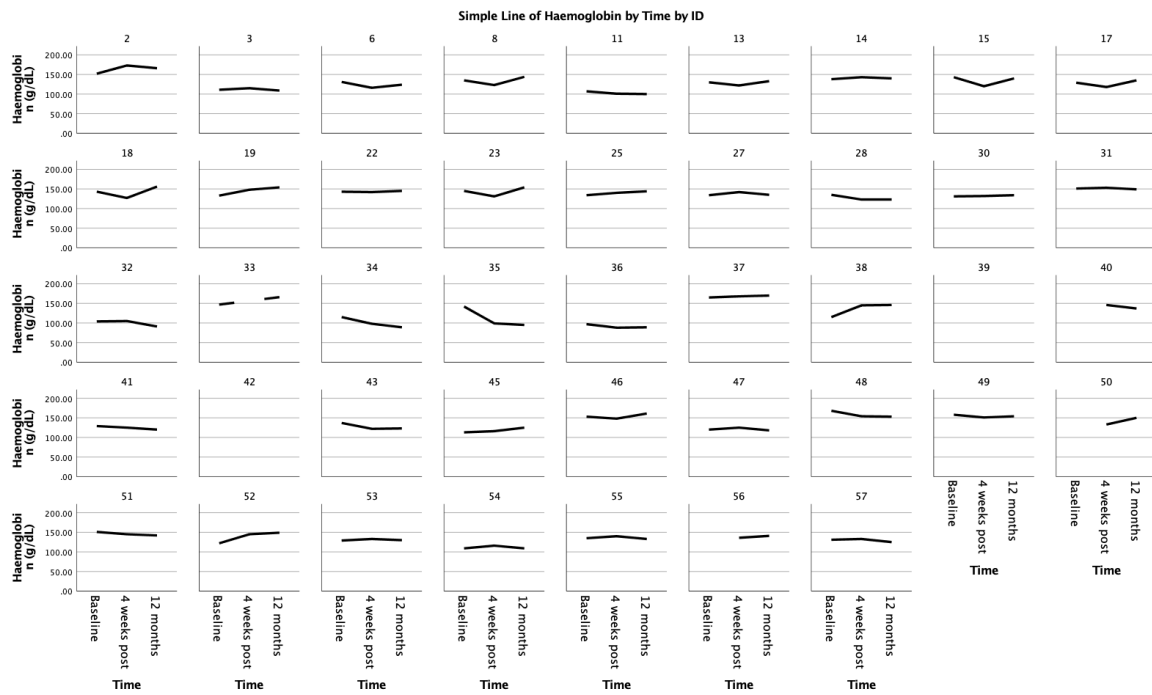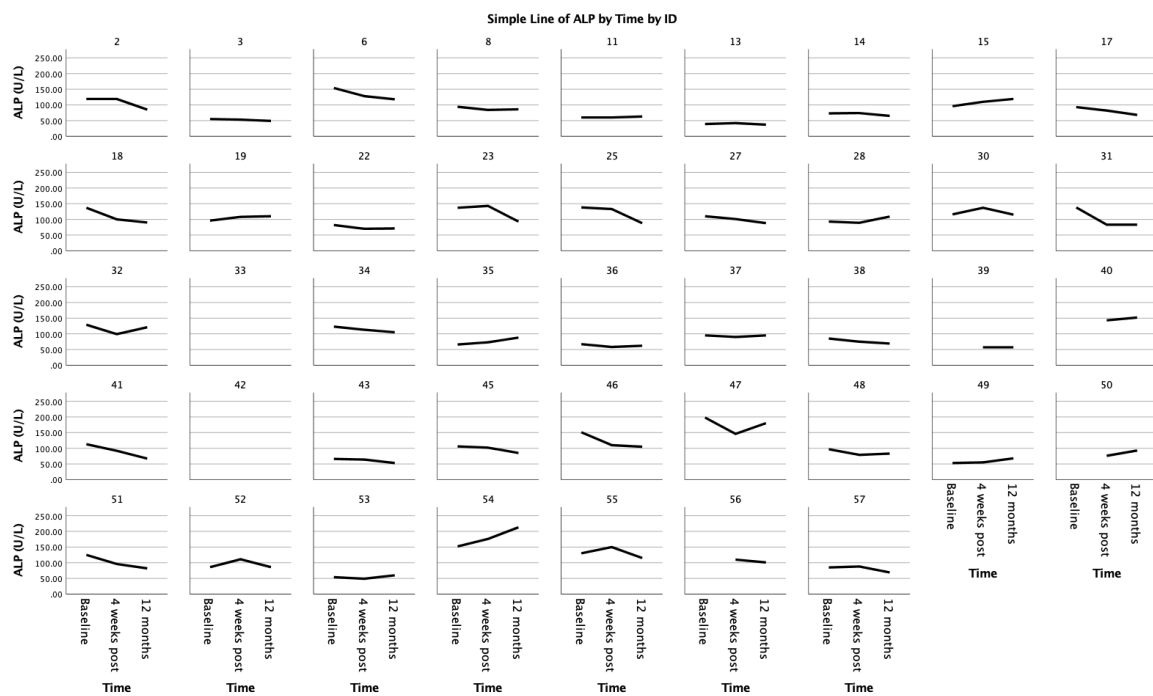
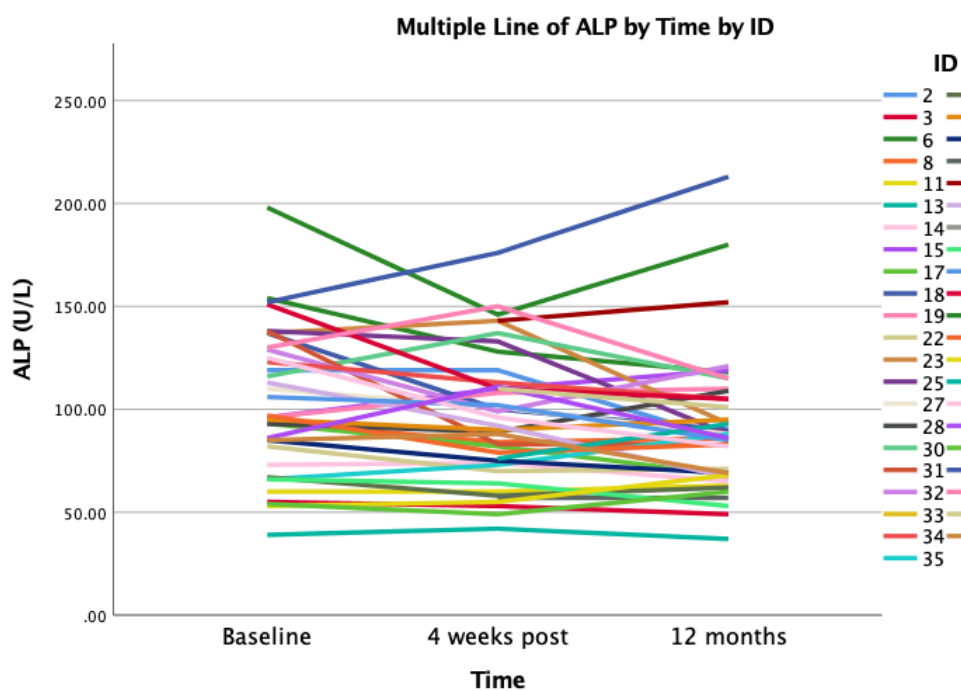Figure 3.1: Panel of individual changes in haemoglobin over one year following treatment initiation for hepatitis C
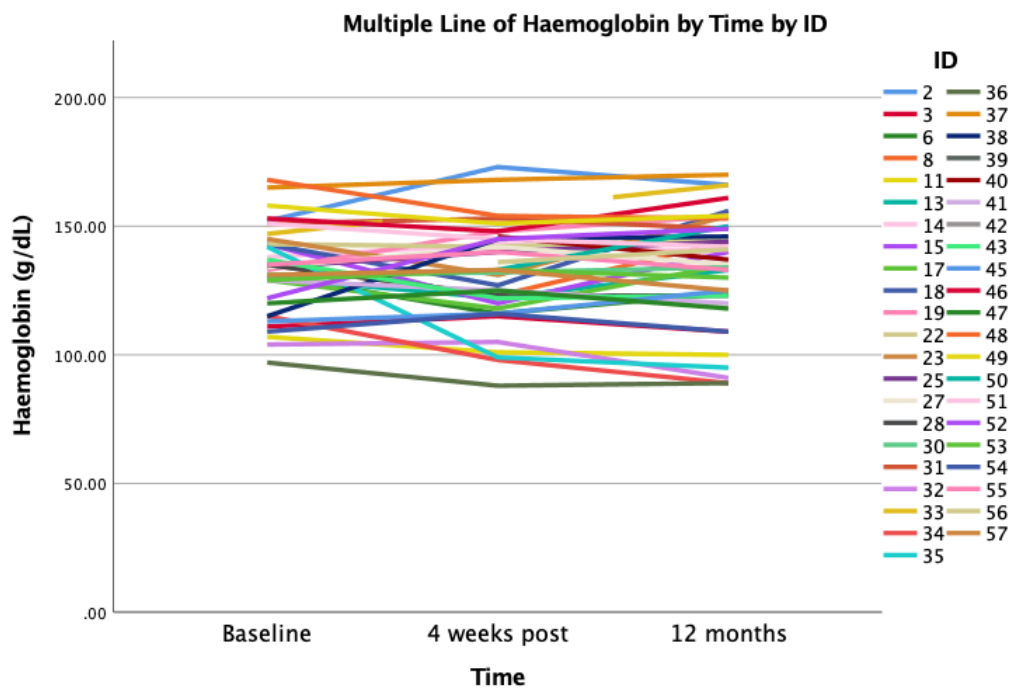
Figure 3.2: Multiple line graph showing all patients change for haemoglobin over one year following treatment initiation for hepatitis C

**Simple Line Median of Haemoglobin (g/dL) by Time**
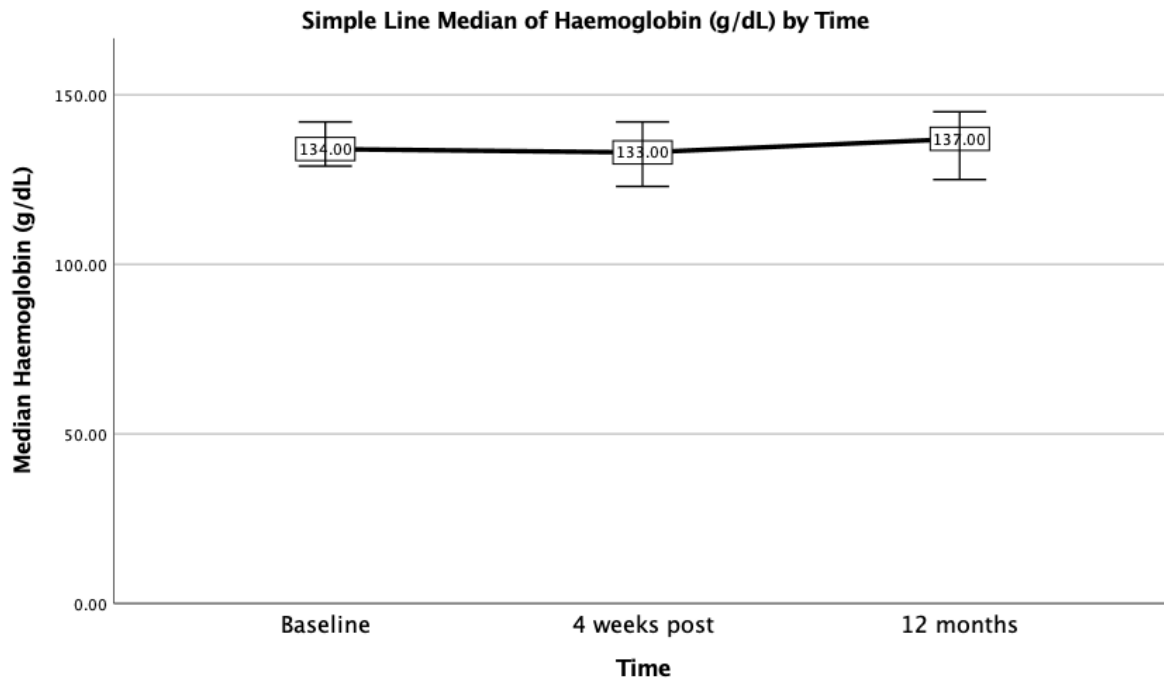
Figure 3.3: A line graph for the median plasma disappearance rate for ICG for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.1.2 **Platelets**

Platelet count is an important surrogate for the severity of cirrhosis and the development of portal hypertension. We have thus investigated them here. We found a slight improvement at one year following treatment, however, the values are within the normal range throughout and differences are not significant (p = 0.66) (Figure 3.4, Figure 3.5, Figure 3.6).
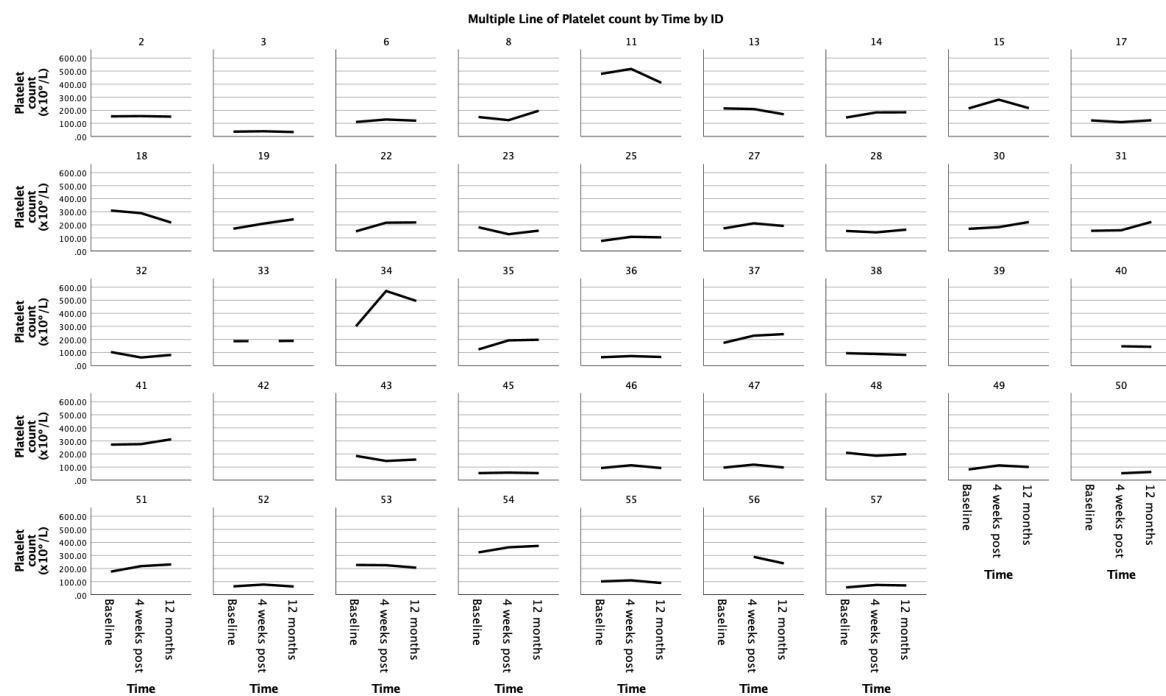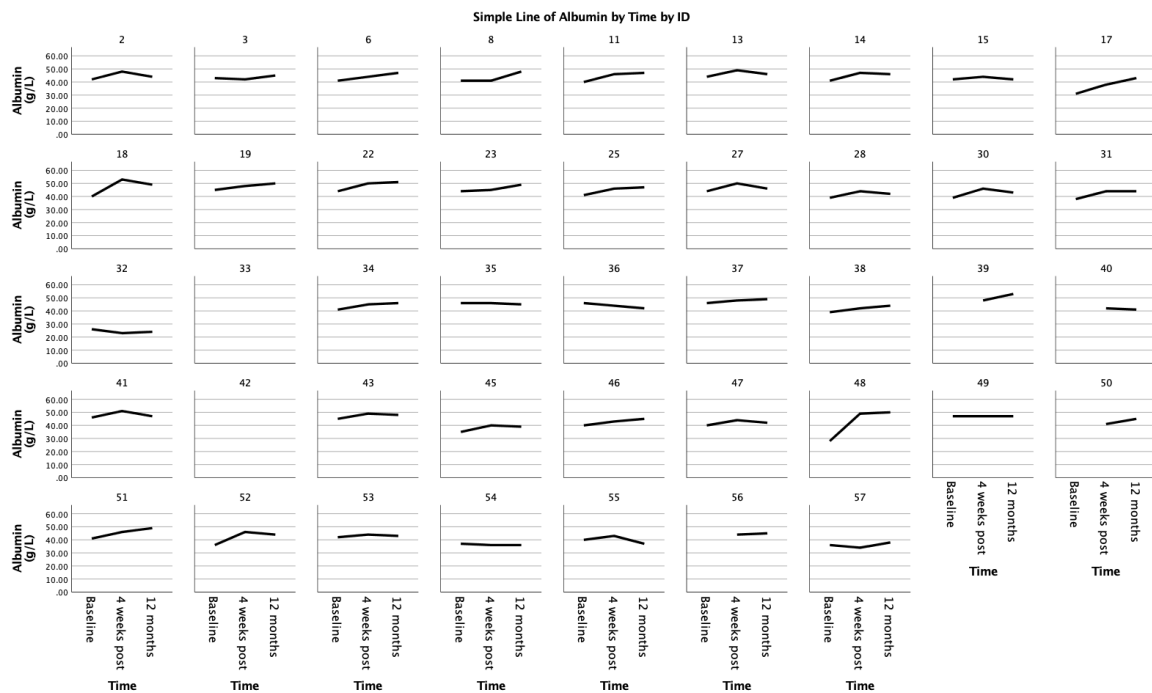
Figure 3.4: Panel of individual changes in platelet count over one year following treatment initiation for hepatitis C
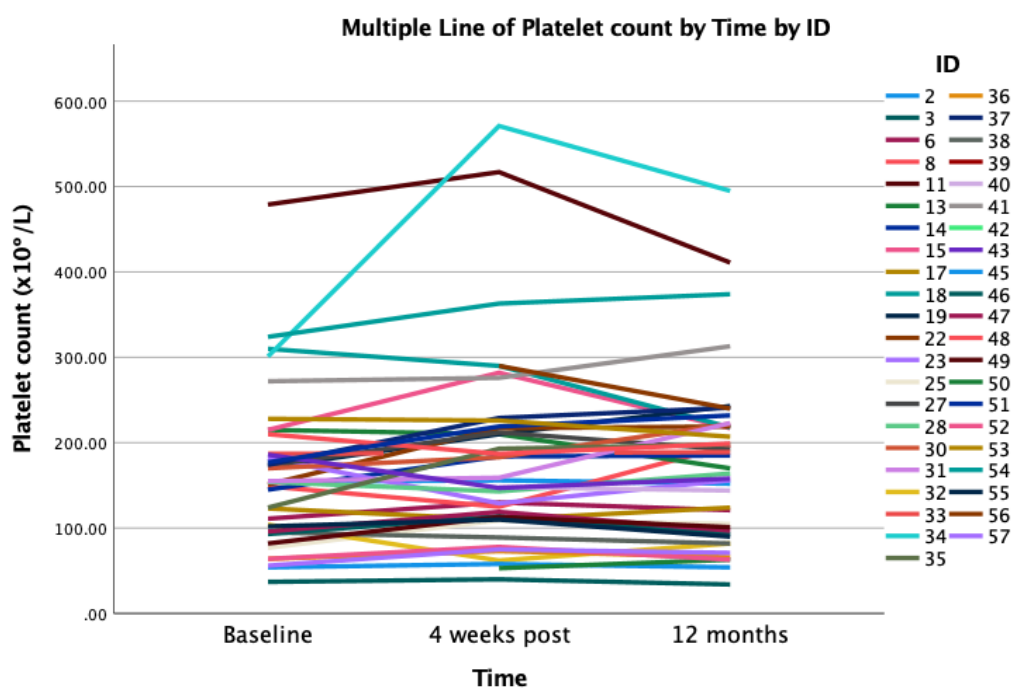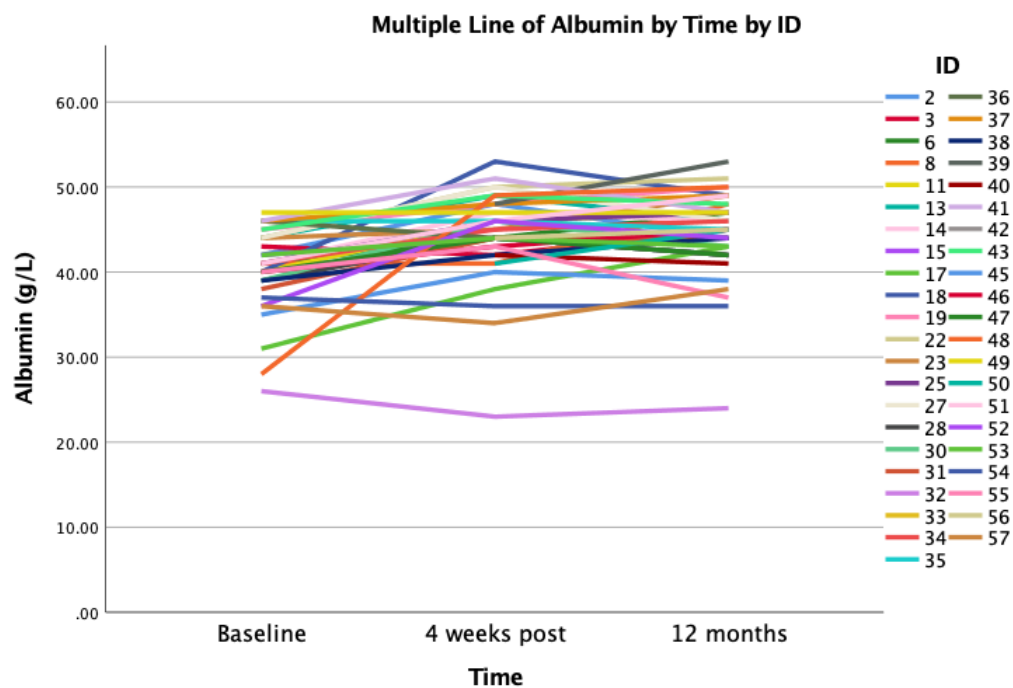

Figure 3.5: Multiple line graph showing all patients change for platelet count over one year following treatment initiation for hepatitis C
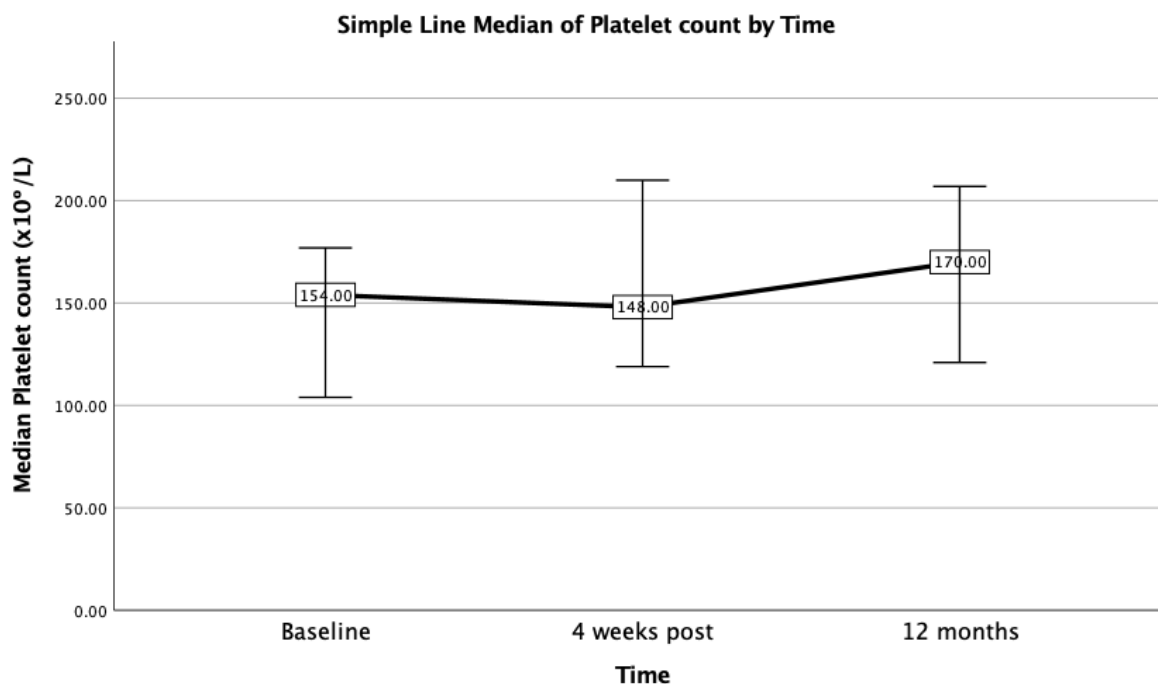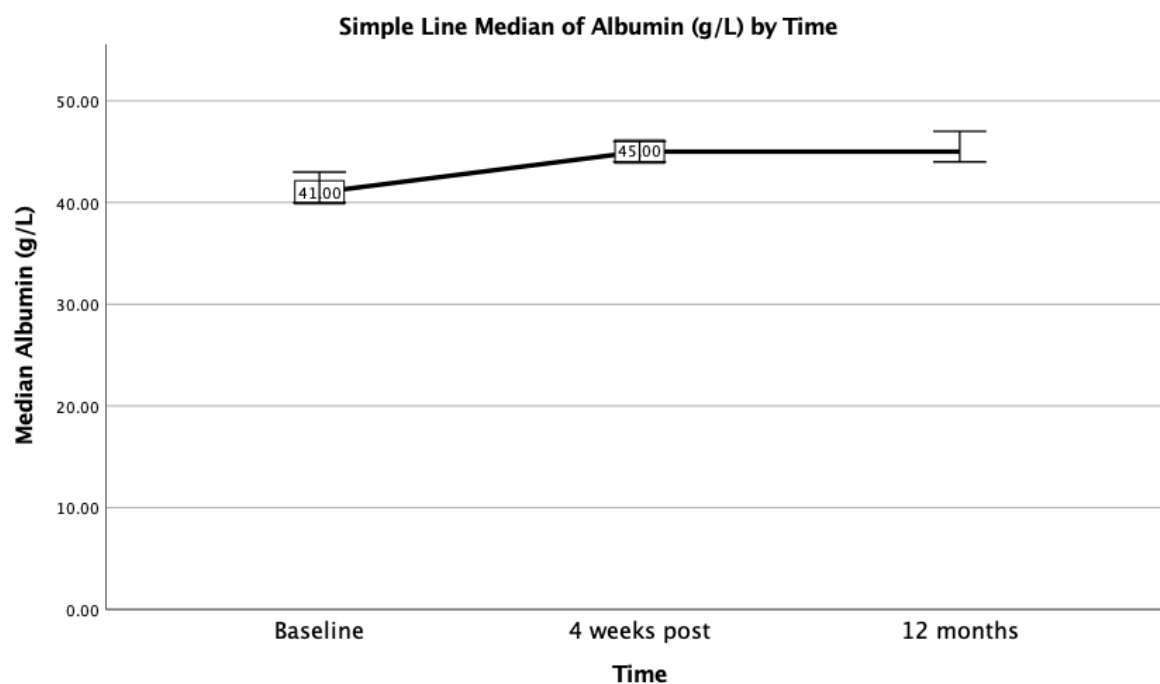
Figure 3.6: A line graph for the median platelet count for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.1.3 **Bilirubin**

The use of serial serum liver function tests gives a readily available and easily accessible means to monitor patients over their treatment and follow up. Therefore, the ability to delineate the effect of HCV treatment on these is helpful and may give added information on the extent of liver injury and may add to the ability to categorise patients prior to therapy (see Figure 3.7). We initially looked at bilirubin which did not change substantially in the majority of patients. Certain patients nevertheless, began the study with a raised value and this persisted, namely patients 11, 32, 34 and 36. Patient 52 started with a markedly increased value of 65 and was within normal limits at four weeks post-treatment. Patient 57 had a continually improving trend though they did not reach a normal value. When looking at the overall trends, there are no apparent trends observable and the medians are within normal limits (see fig Figure 3.8 and Figure 3.9) (p = 0.119).
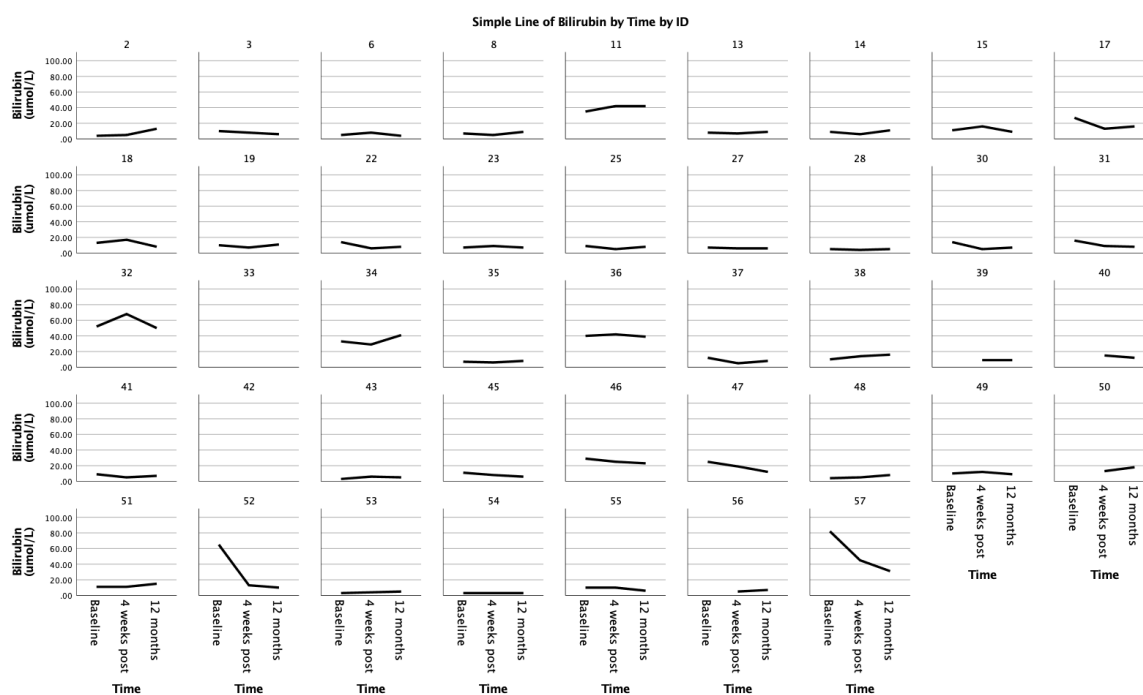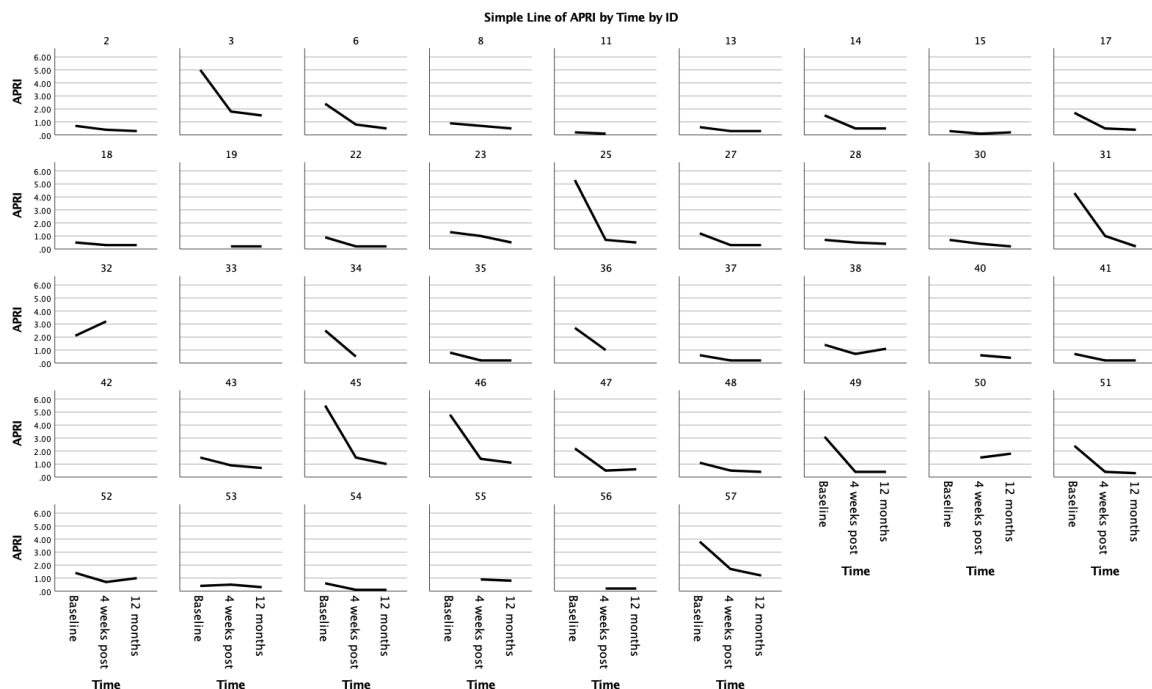
Figure 3.7: Panel of individual changes in bilirubin over one year following treatment initiation for hepatitis C
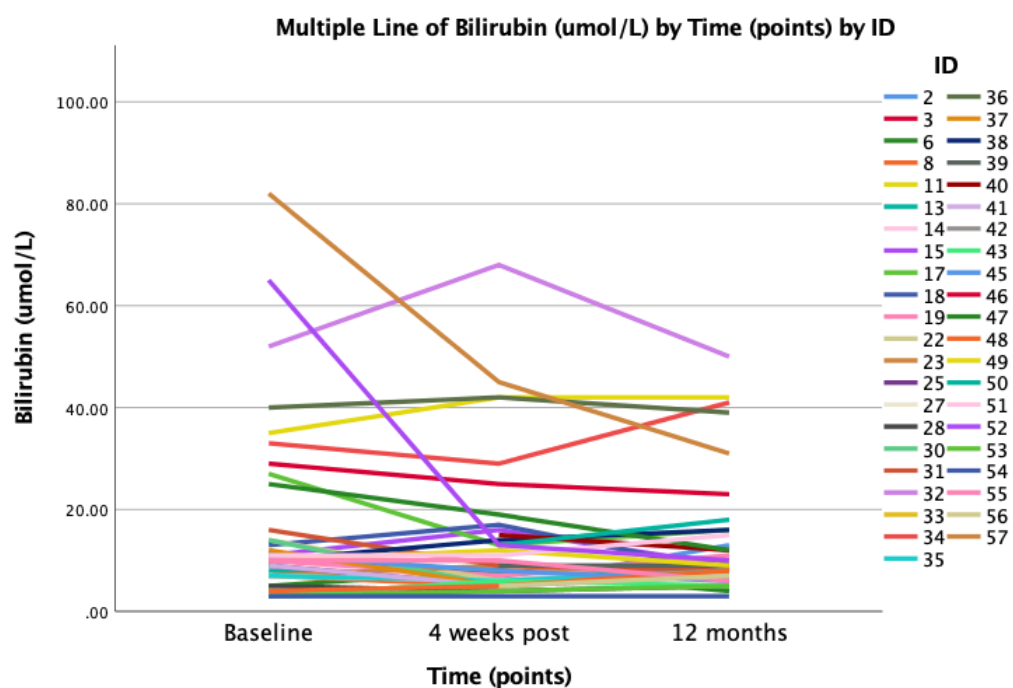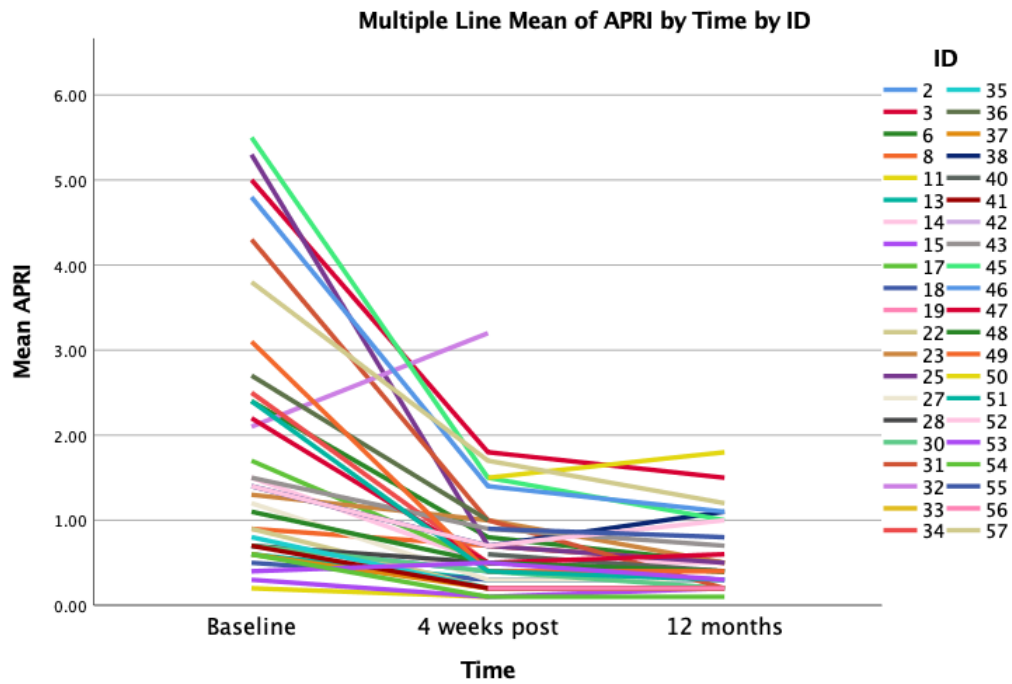

Figure 3.8: Multiple line graph showing all patients change for bilirubin over one year following treatment initiation for hepatitis C
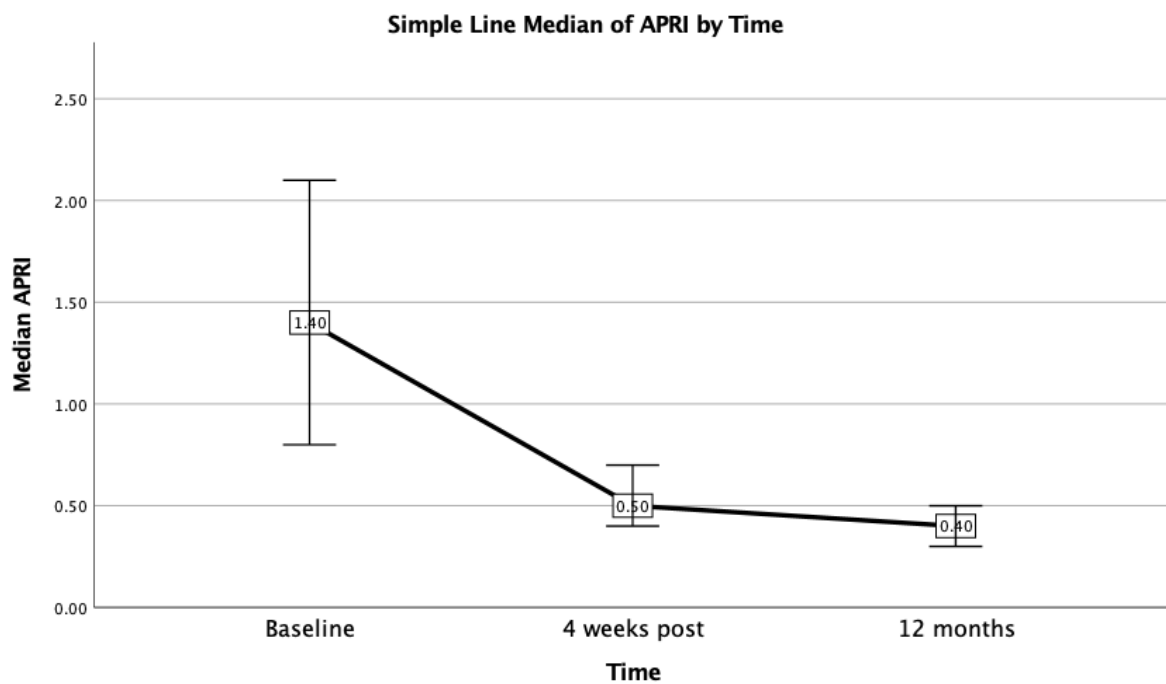
363
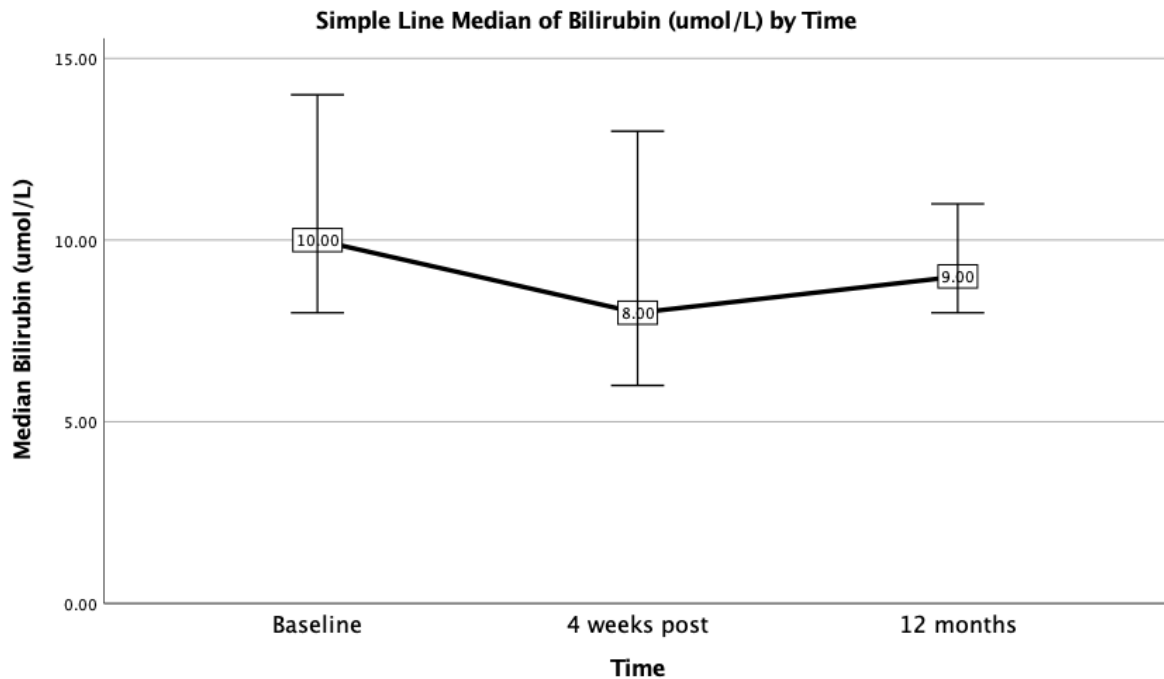
Figure 3.9: A line graph for the median bilirubin for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.1.4   **ALT**

Alanine aminotransferase is an intracellular enzyme released following damage to hepatocytes and is frequently used to monitor damage to the liver, particularly in an acute phase. Upon further investigation (see Figure 3.10), we found that almost all patients with values showed an improvement by four weeks post-treatment, with this being maintained or improved upon at 52 weeks post-treatment. This was not true for patient 41, who had an initial worsening before returning to baseline. When investigated as an amalgamation, this improvement is even starker with the most marked improvements for those with the highest values (median = 68units/L) (see Figure 3.11) and this is further confirmed when taken as an average with the median values returning the normal range (median = 21units/L)(see Figure 3.12) (p = <0.001).
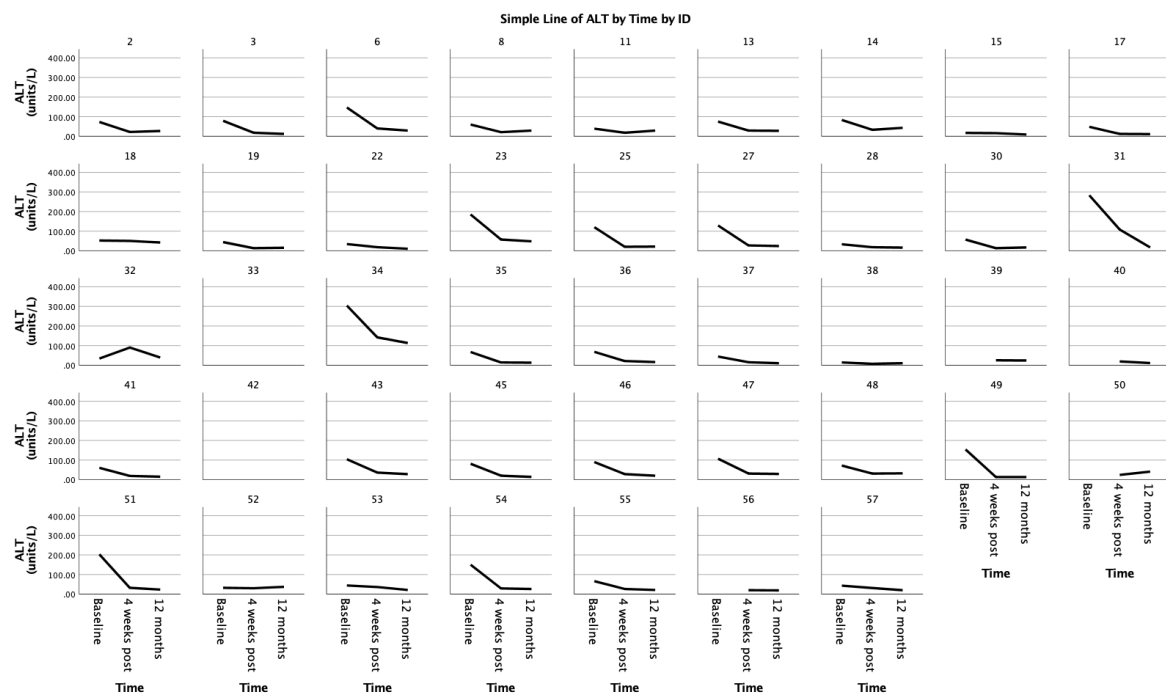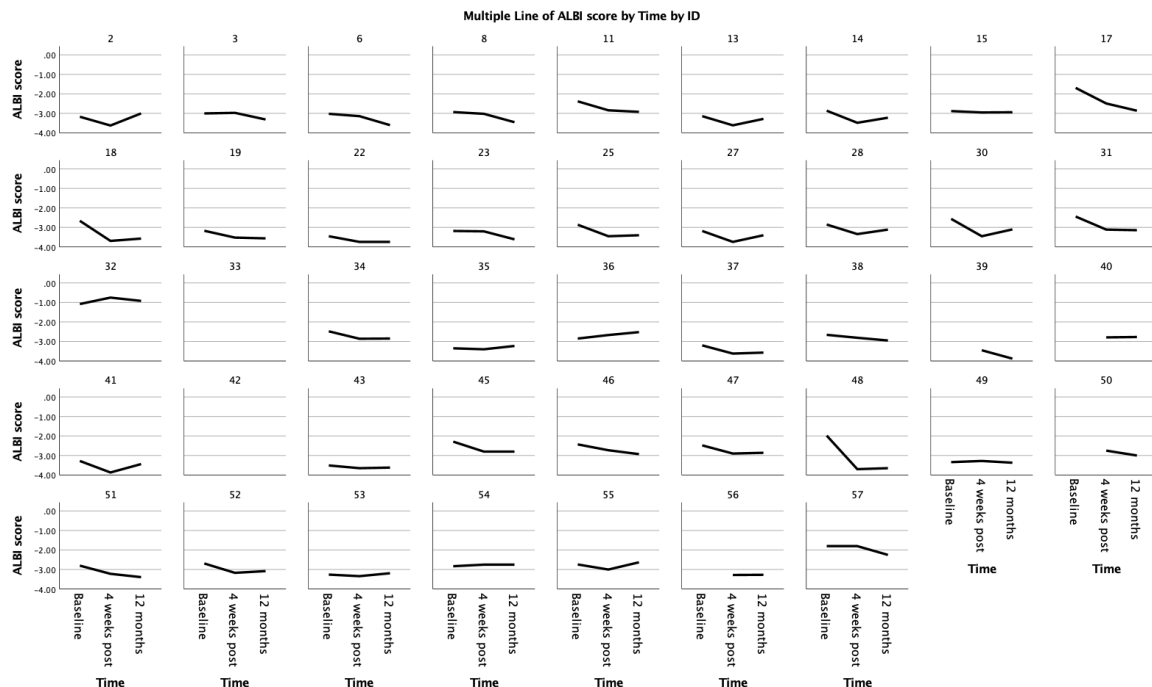
Figure 3.10: Panel of individual changes in alanine aminotransferase over one year following treatment initiation for hepatitis C
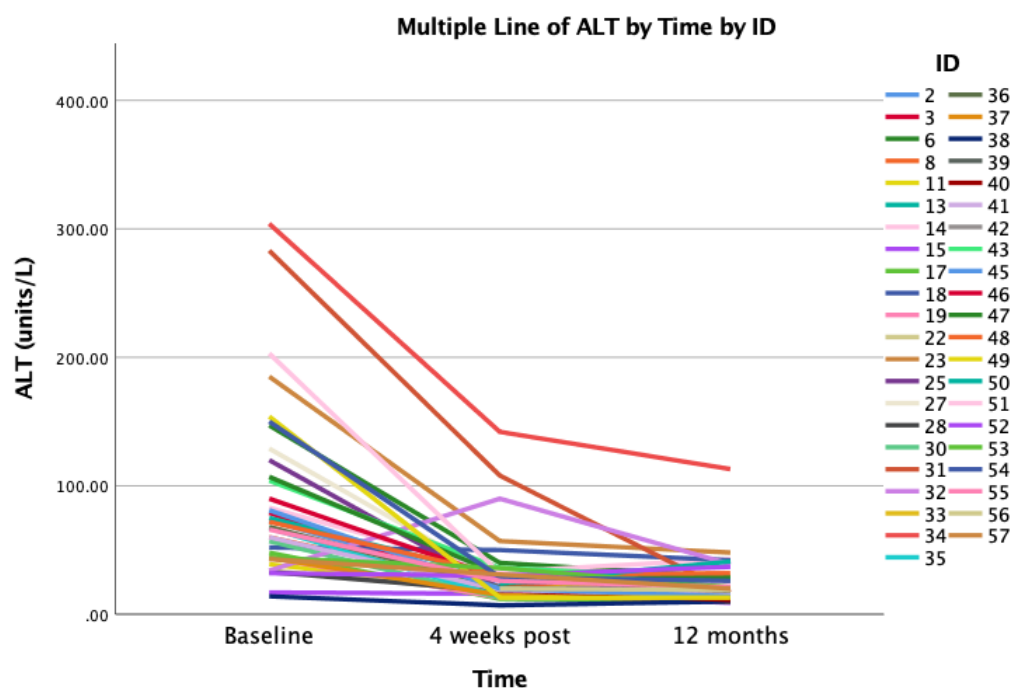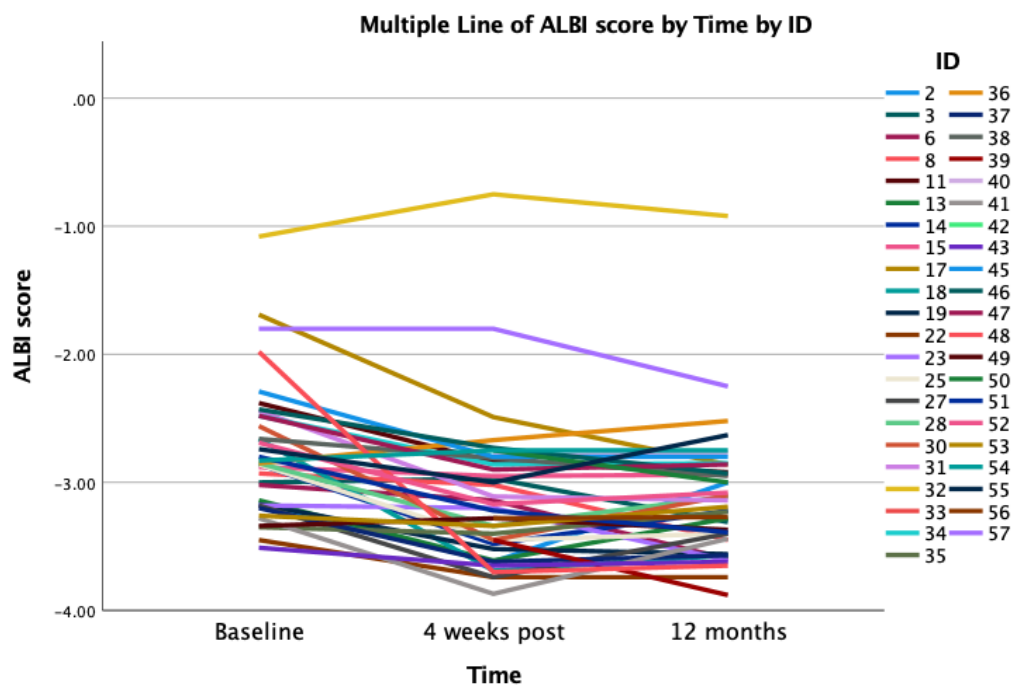

Figure 3.11: Multiple line graph showing all patients change for alanine aminotransferase over one year following treatment initiation for hepatitis C

365

Simple Line Median of ALT (units/L) by Time
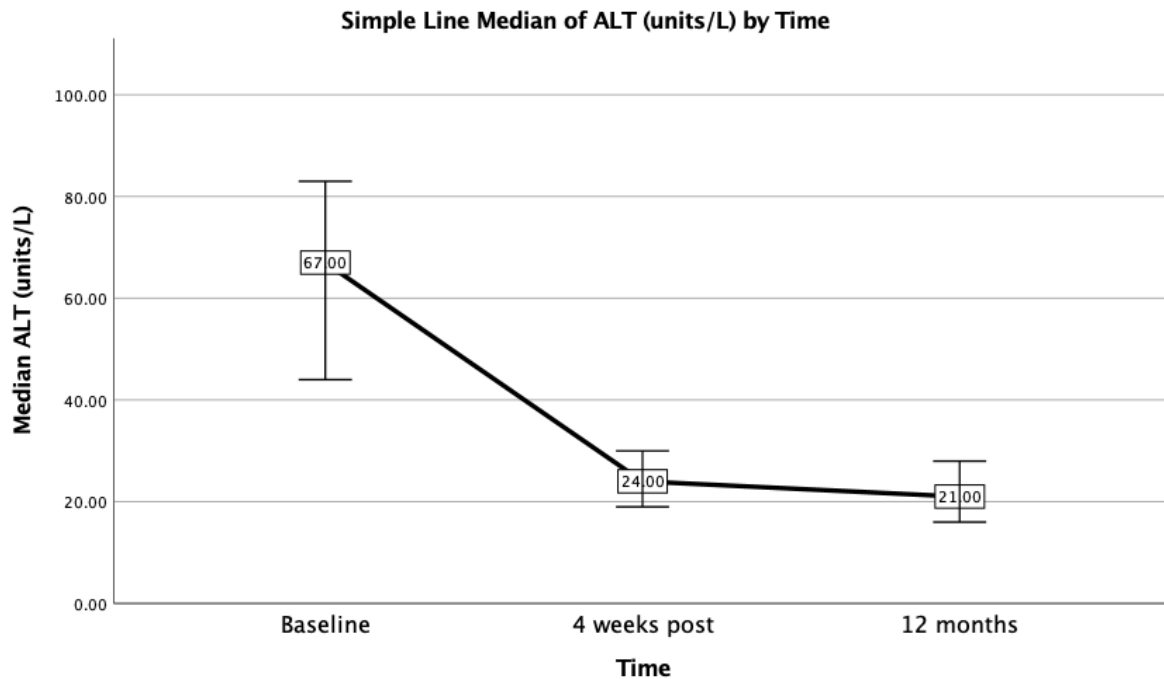
Figure 3.12: A line graph for the median alanine aminotransferase for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.1.5 **ALP and γGT**

Alkaline phosphatase and gamma-glutamyl transpeptidase are mainly found within the biliary tract. As ICG excretion is via this route, there is inherent importance, with cholestasis being a known cause of reduced excretion. Reviewing alkaline phosphatase, we found most patients showing an improvement or stagnation, with patient 54 having an increasing value consistently over the year (Figure 3.13). A reduction over the year is observed (p = 0.003), though it should be noted that these values are within the normal range (baseline ALP of 96U/L) (Figure 3.14 & Figure 3.15). This effect is viewed even more markedly for γGT (p = <0.001) (Figure 3.16, Figure 3.17 & Figure 3.18).

Figure 3.13: Panel of individual changes in alkaline phosphatase over one year following treatment initiation for hepatitis C

Figure 3.14: Multiple line graph showing all patients change for alkaline phosphatase over one year following treatment initiation for hepatitis C
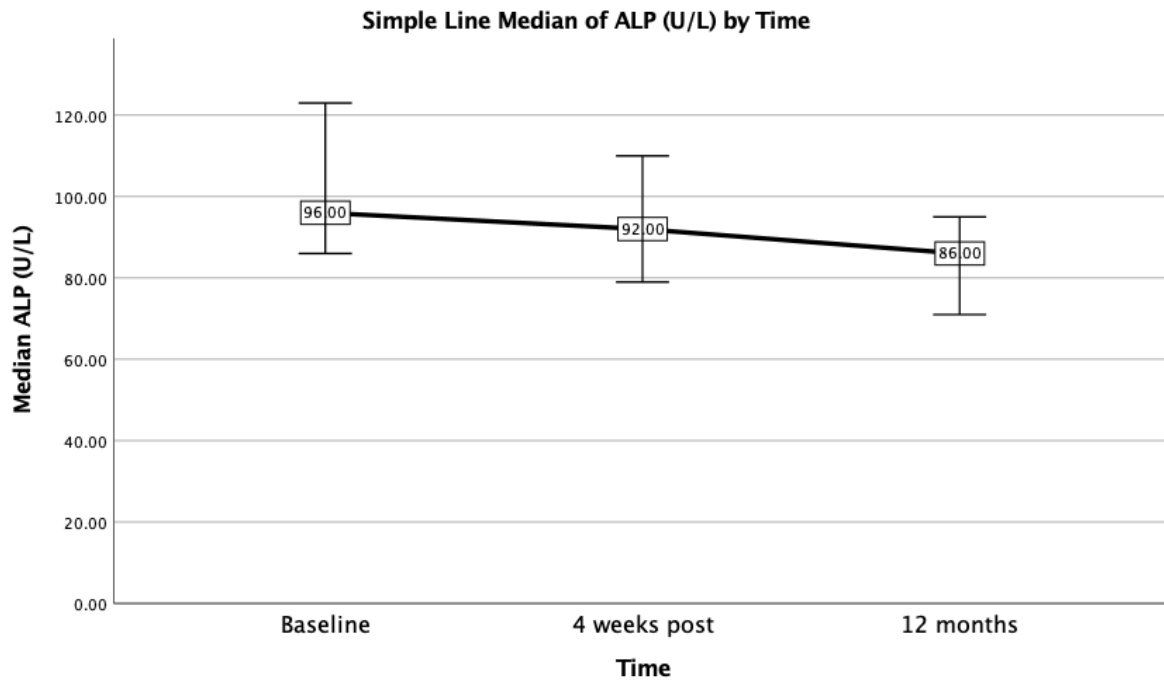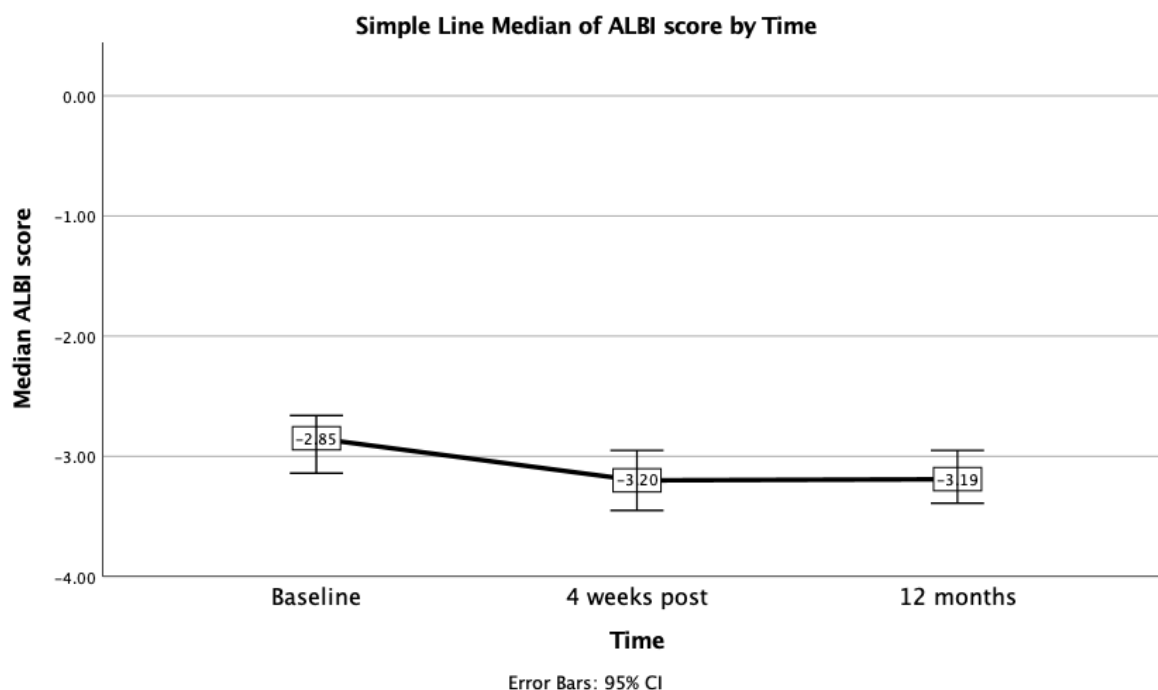
Figure 3.15: A line graph for the median alkaline phosphatase for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed
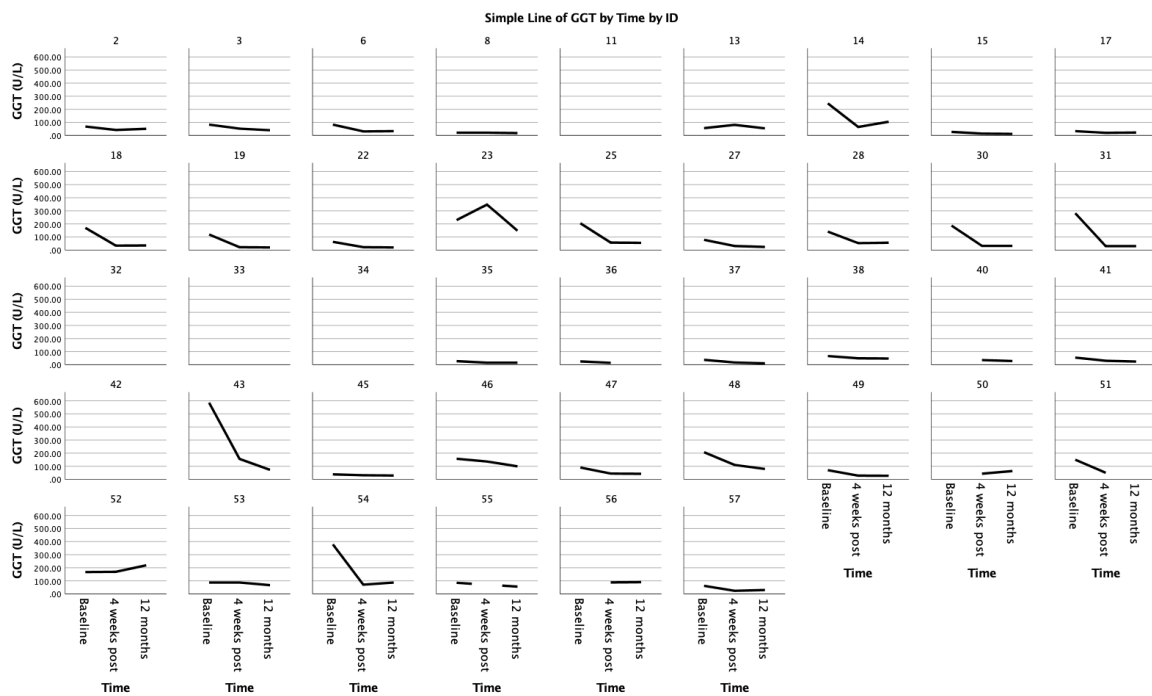
Figure 3.16: Panel of individual changes in gamma-glutamyl transpeptidase over one year following treatment initiation for hepatitis C

368
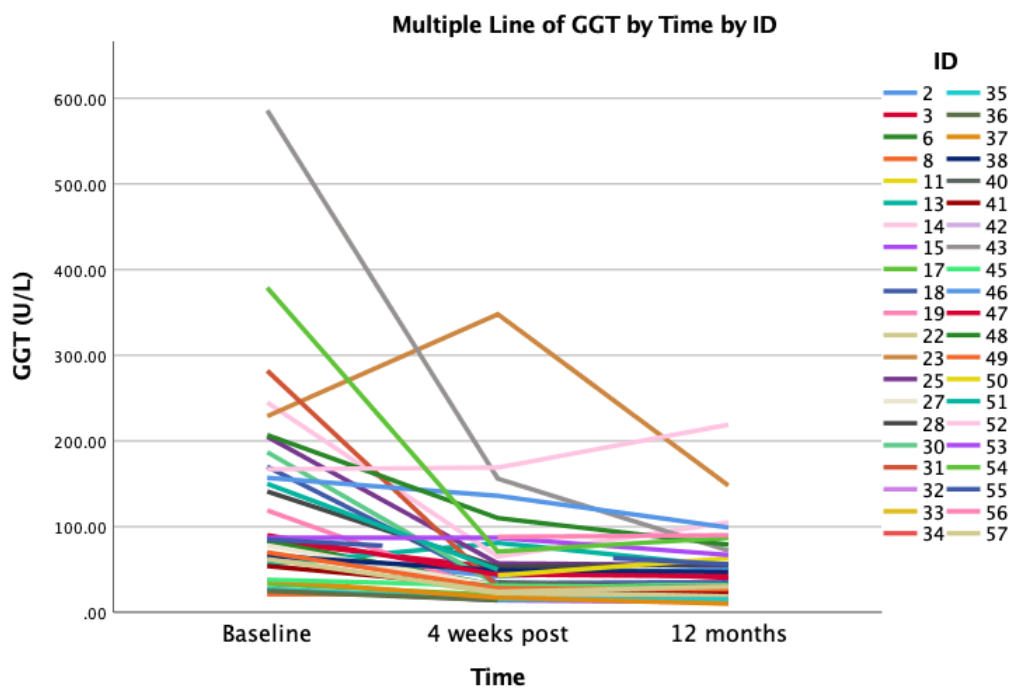
Figure 3.17: Multiple line graph showing all patients change for gamma-glutamyl transpeptidase over one year following treatment initiation for hepatitis C
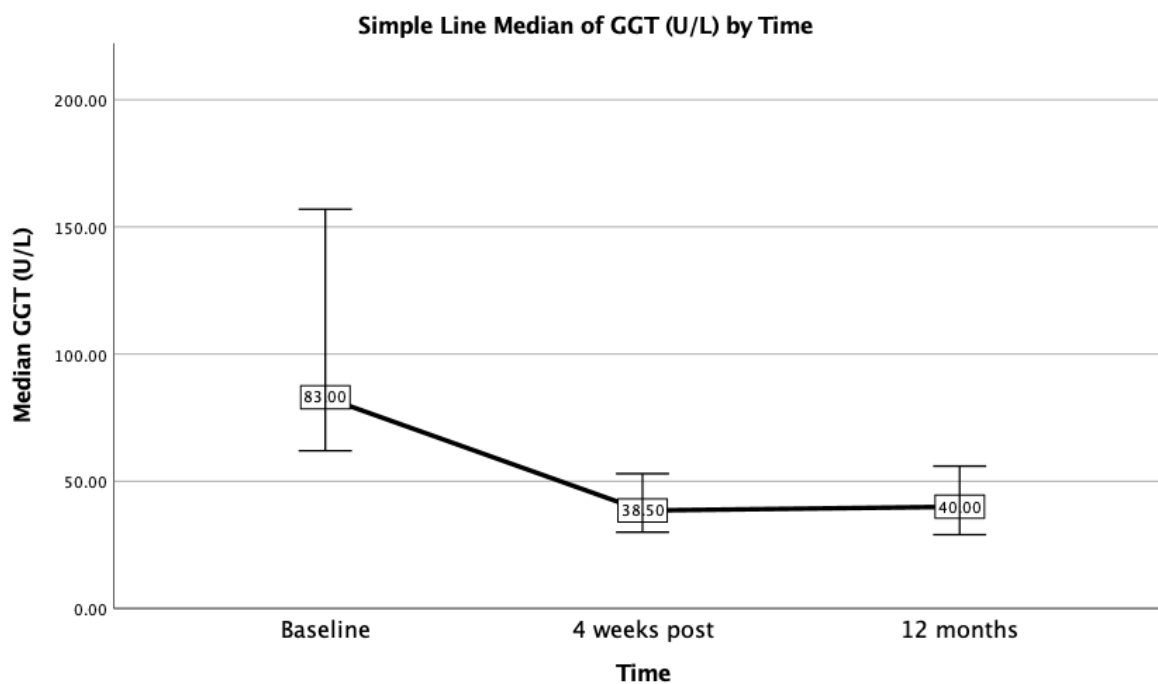
Figure 3.18: A line graph for the median gamma-glutamyl transpeptidase for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.1.6 **Albumin**

Albumin is produced by the liver and noted as a marker for the synthetic ability of the liver. Most readings, outside of patient 55, show an improvement in the reading of albumin (p<0.001) (Figure 3.19), though again, this is from a baseline within the normal range (median = 41g/L) (Figure 3.20 & Figure 3.21).

Figure 3.19: Panel of individual changes in albumin over one year following treatment initiation for hepatitis C

Figure 3.20: Multiple line graph showing all patients change for albumin over one year following treatment initiation for hepatitis C

Figure 3.21: A line graph for the median albumin for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.1.7 __APRI__

APRI is an amalgamated score that non-invasively quantifies fibrosis. It does however involve liver function tests that may change with viral elimination and its value in assessing changes in fibrosis is questionable. As with transient elastography, there is an improvement which is most marked directly after treatment with all patients with a complete dataset improving in a much more drastic manner than on liver stiffness alone (p <0.001) (see figures Figure 3.22, Figure 3.23 & Figure 3.24).

Figure 3.22: Panel of individual changes in APRI over one year following treatment initiation for hepatitis C

Figure 3.23: Multiple line graph showing all patients change for APRI over one year following treatment initiation for hepatitis C

Figure 3.24: A line graph for the median APRI for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

3.1.8 **ALBI Score**

Another serum test for the liver function is the Albumin-bilirubin score which can also be expressed as a grade and was originally investigated as a prognostic score in HCC but has recently been investigated for liver function in HCV (Grade 1, best liver function (≤-2.6), grade 2 (-2.6 to -1.39) and grade 3 (worst liver function(>-1.39)). We see a significant improvement in the function of the median over the year though this improvement is all within grade 1 (p = <0.001) (Figure 3.25, Figure 3.26 & Figure 3.27).

Figure 3.25: Panel of individual changes in ALBI score over one year following treatment initiation for hepatitis C

Figure 3.26: Multiple line graph showing all patients change for ALBI score over one year following treatment initiation for hepatitis C

Figure 3.27: A line graph for the median ALBI score for all subjects over one year following treatment initiation for hepatitis C, with 95% confidence intervals displayed

4   What is the distribution of transient elastography results in patients deemed at risk of Sickle cell liver disease

3.11 Age

Advanced age is an individual risk factor for fibrosis. To determine whether fibrosis increased with age we compared age and elastography score.
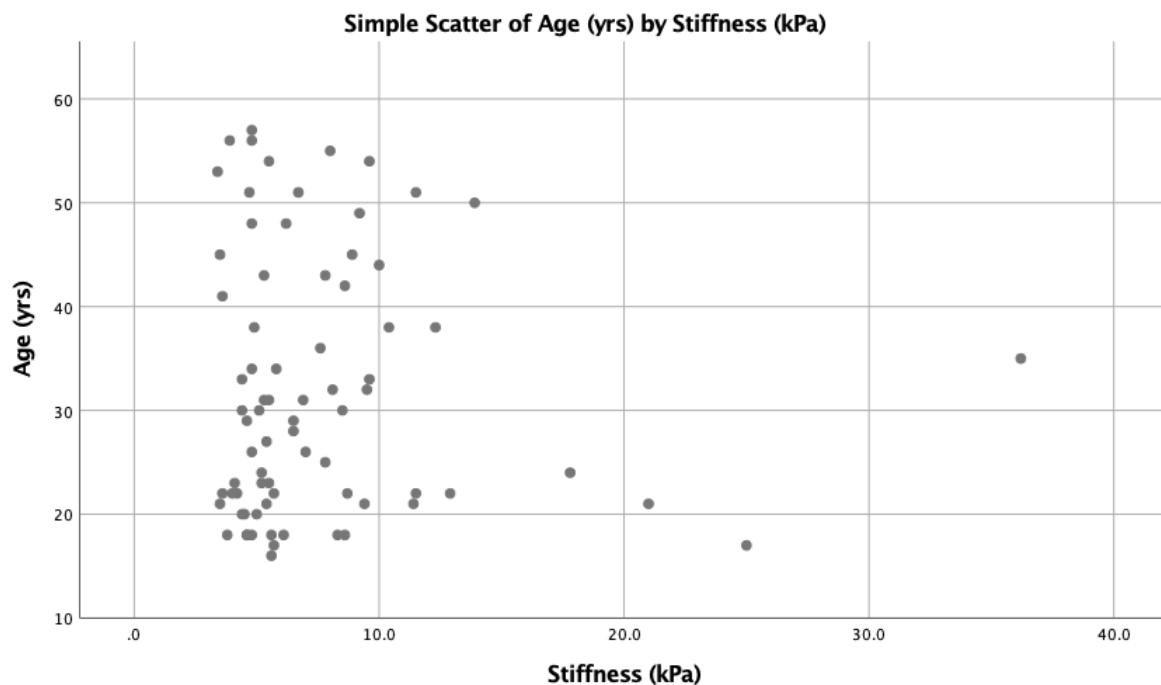
Figure 4.1: Scattergram showing age against liver stiffness. Spearman's rho test, r = 0.061, p = 0.598

Population Pyramid Frequency Age (yrs) by Severe fibrosis
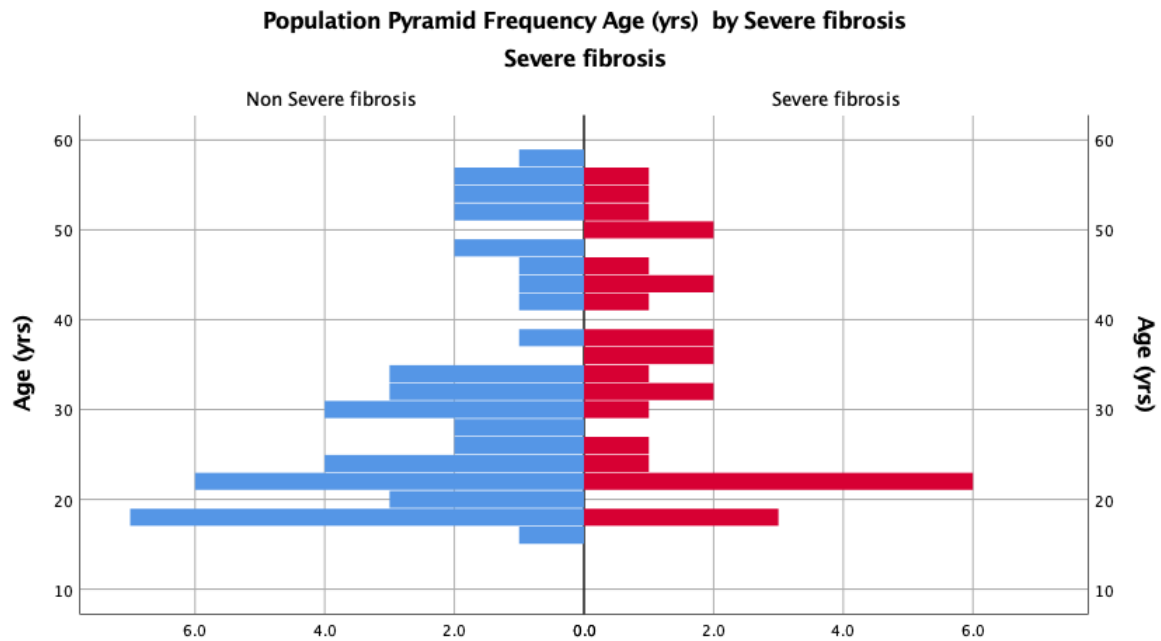
Figure 4.2: Histogram showing distribution of age when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There is no correlation between an increasing liver stiffness and age (Figure 4.1). Median age for patients without fibrosis did not differ between patients with non-severe fibrosis (27.5 yrs) and those with severe fibrosis (32.5 yrs), p = 0.321 (Figure 4.2).

3.12 <u>Haematological investigations</u>

To determine whether routinely measured haematological investigations could be used to identify patients at risk of fibrosis we examined platelet count, foetal and sickle haemoglobin percentages.
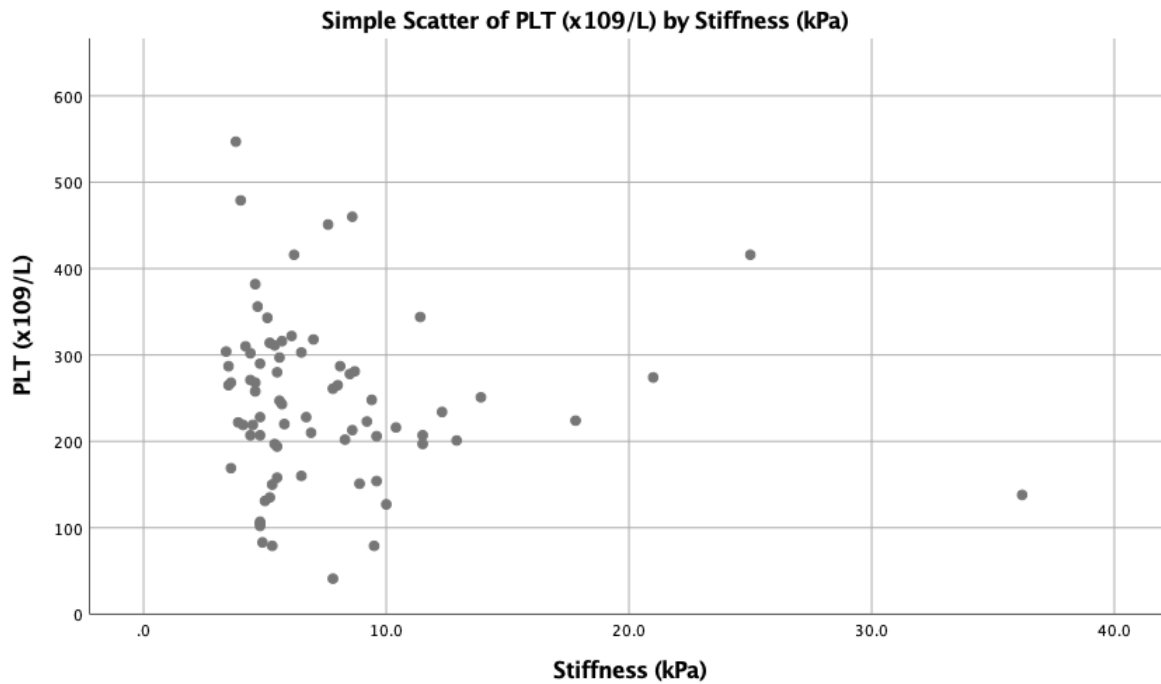
Figure 4.3: Scattergram showing platelet concentration against liver stiffness. Spearman's rho test, r = -0.141, p = 0.223
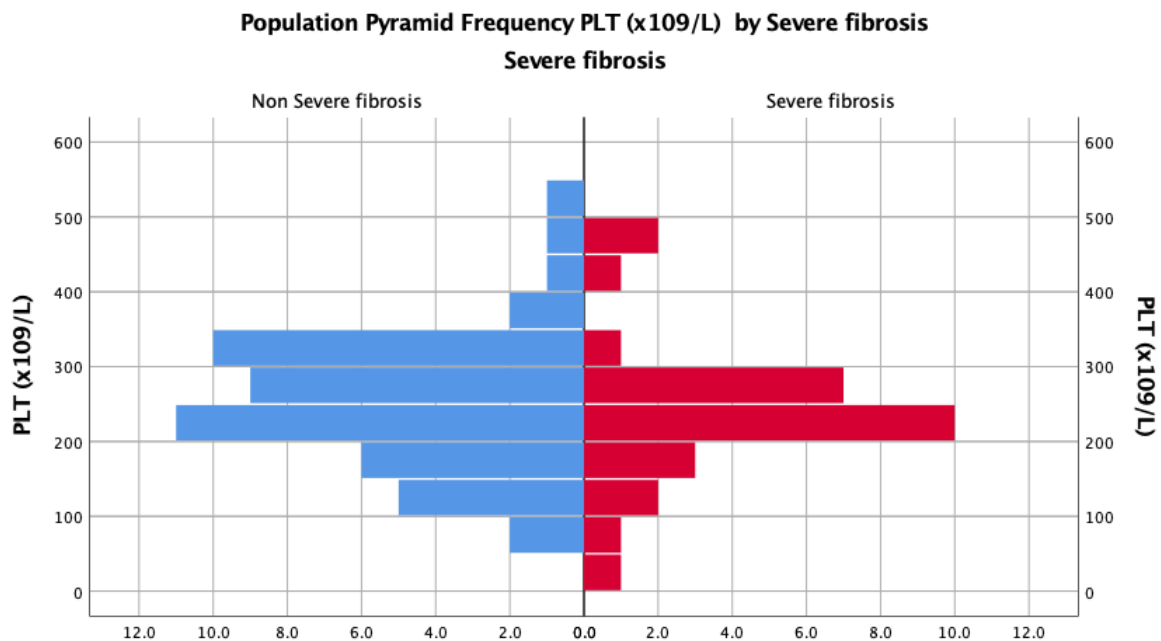
Figure 4.4: Histogram showing distribution of platelet concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

Two patients had splenectomies; both had normal platelet counts with one having a normal

Fibroscan® (5.7kPa) and one with some fibrosis, Fibroscan® score of 8.3kPa.

There was no correlation between an increasing liver stiffness and platelet concentration (Figure 4.3). Median platelet concentration was not different between patients with non-severe fibrosis (252.5×10^9/L) and those with severe fibrosis (223.5×10^9/L), p = 0.352 (Figure 4.4).

A higher foetal haemoglobin percentage decreases the intracellular polymerisation of HbS and therefore the denaturation of red blood cells. HbF is increased by hydroxyurea usage and thus can also be used as a marker for the effectiveness of treatment as well as adherence {Alapan, 2016 #2264}. We thus examined this to see whether a higher value indicated protection against fibrosis.
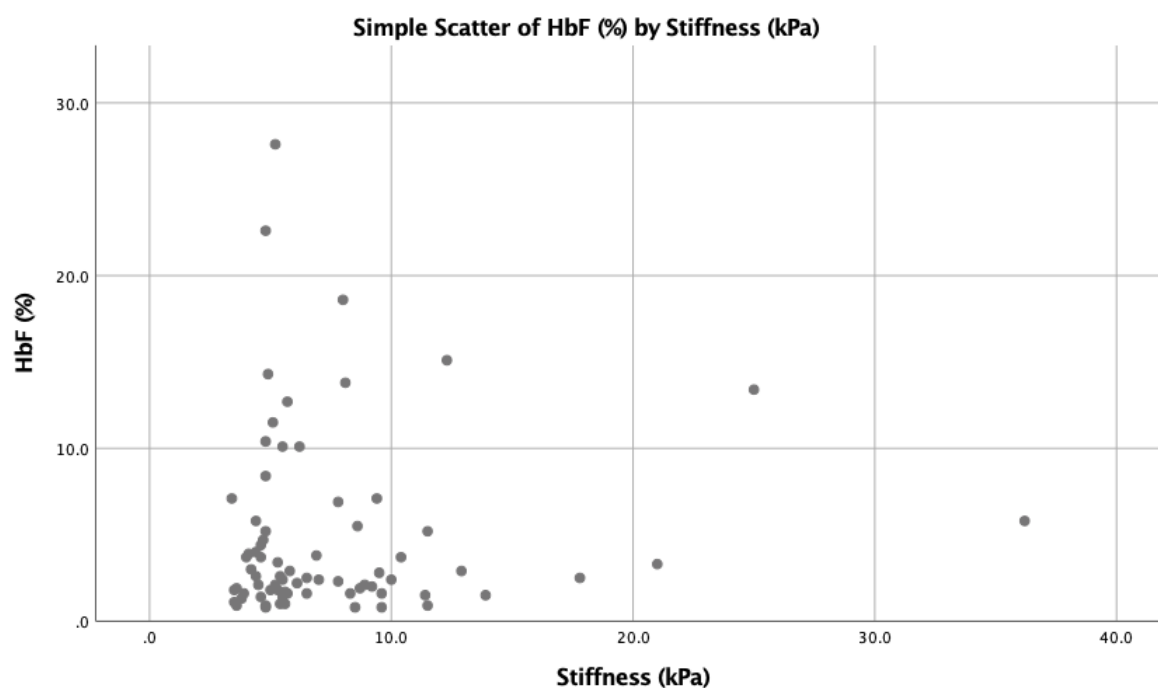
Figure 4.5: Scattergram showing foetal haemoglobin percentage against liver stiffness. Spearman's rho test, r = 0.041, p = 0.730
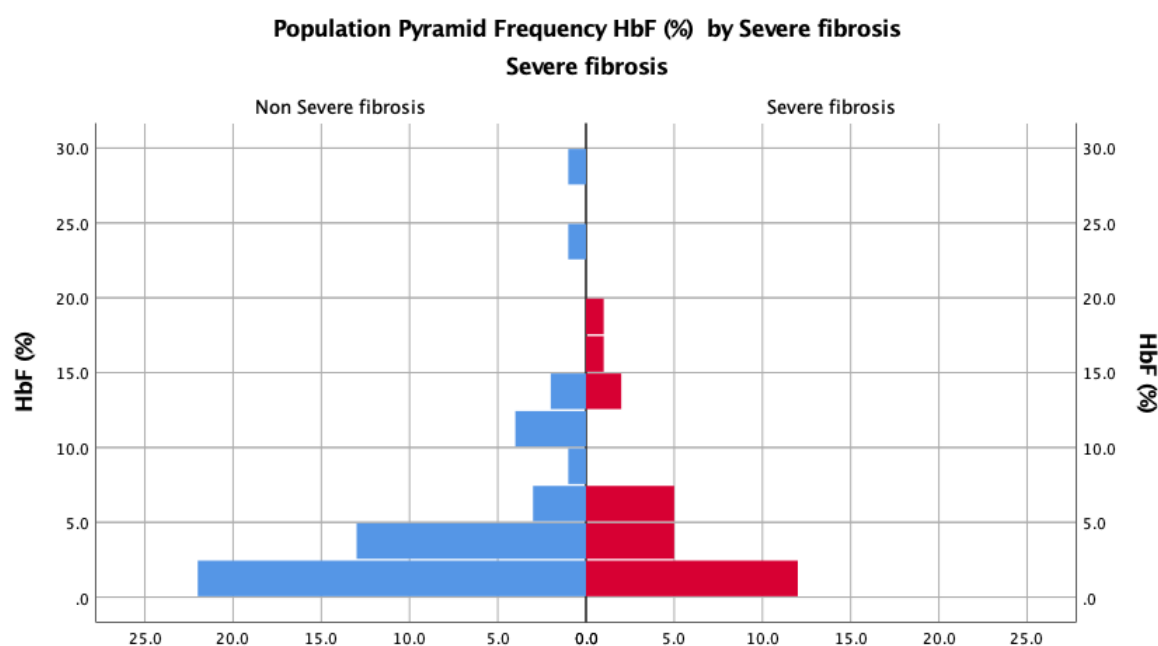
Figure 4.6: Histogram showing distribution of foetal haemoglobin percentage when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was no correlation between an increasing liver stiffness and foetal haemoglobin

percentage (Figure 4.5). Foetal haemoglobin percentage for patients with non-severe fibrosis

(mean rank = 36.72) were not significantly different than for those with severe fibrosis (mean rank = 37.5), p = 0.881 (Figure 4.6).

A higher percentage of HbS indicates an increased likelihood for patients to progress to sickled cells and thereby sickle crisis. One of the goals and thereby ways to monitor treatment, particularly transfusions, is to measure the HbS percentage {Alapan, 2016 #2264}. We thus correlated HbS percentage with TE to determine whether increased fibrosis occurs with an increased HbS.
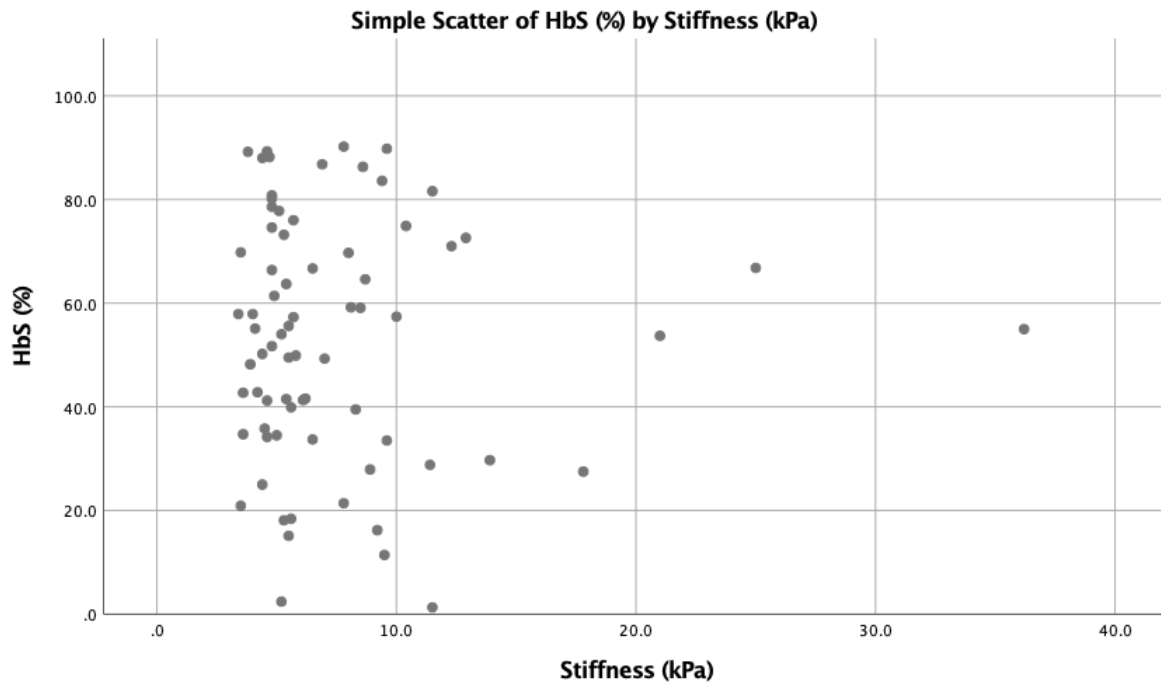
Figure 4.7: Scattergram showing sickle haemoglobin percentage against liver stiffness. Spearman's rho test, r = 0.043, p = 0.717
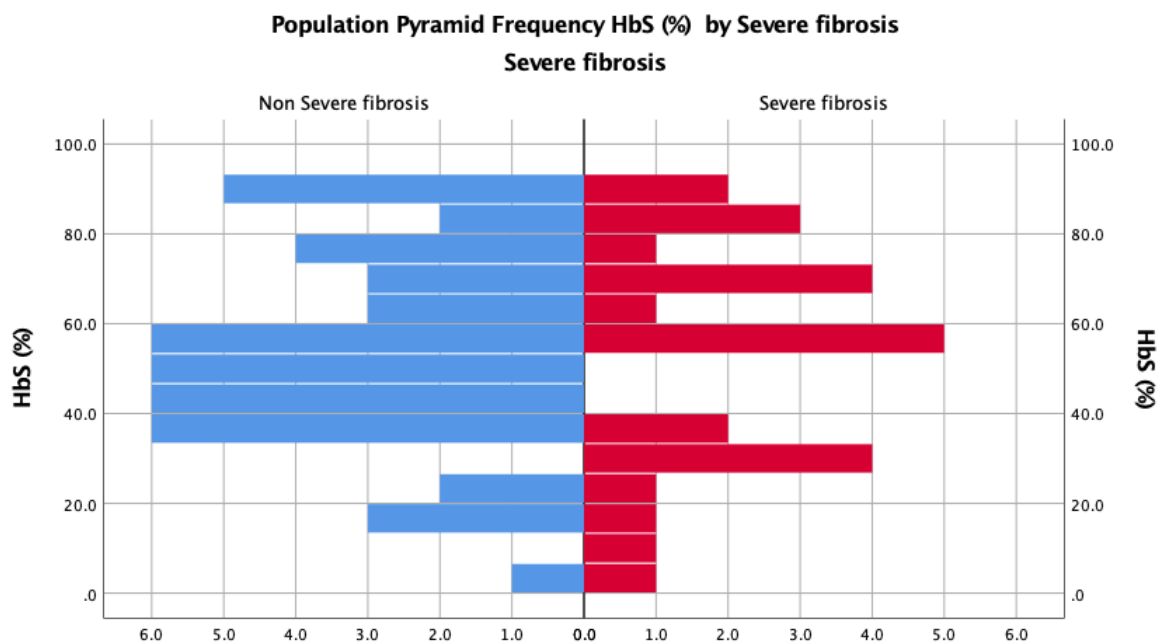
Figure 4.8: Histogram showing the distribution of sickle haemoglobin percentage when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was no correlation between an increasing liver stiffness and sickle haemoglobin

percentage (Figure 4.7). Sickle haemoglobin percentage for patients with non-severe fibrosis

(mean rank = 36.96) were not statistically significantly different than for those with severe

fibrosis (mean rank = 37.08), p = 0.982 (Figure 4.8).

3.13 <u>Static liver function tests</u>

Standard serum liver function tests are readily available with serial measurements for liver

injury being assessed in most patients. Alanine aminotransferase (ALT) is an enzyme which

is present in hepatocytes and following cell damage can be detected at higher concentrations

within the serum. It should be noted that this rise is secondary to ongoing cell injury and thus,

the actual level may not reflect the level of fibrosis though may indicate ongoing damage. We

investigated the relationship between ALT and stiffness.
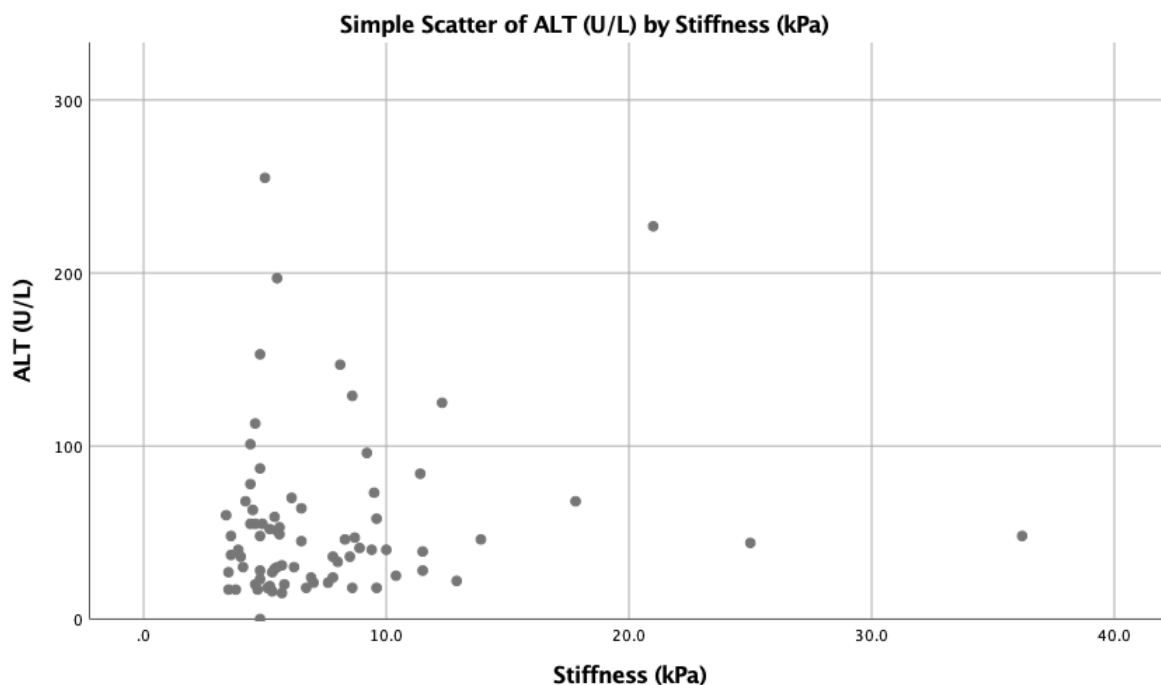
Figure 4.9: Scattergram showing serum alanine aminotransferase concentration against liver stiffness. Spearman's rho test, r = 0.076, p = 0.513

Population Pyramid Frequency ALT (U/L) by Severe fibrosis
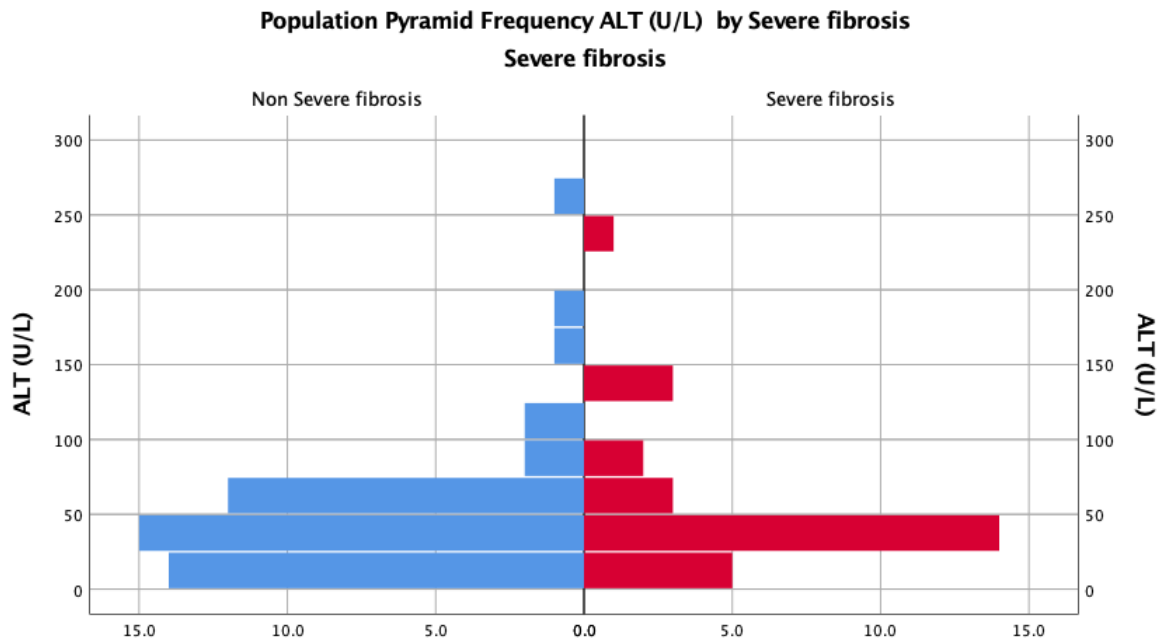
Figure 4.10: Histogram showing the distribution of serum alanine aminotransferase concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was no correlation between an increasing liver stiffness and serum alanine aminotransferase concentration (Figure 4.9) and median serum alanine aminotransferase concentration was not statistically significantly different between people with non-severe (38.5U/L) and severe fibrosis (42.5U/L), $p = 0.349$ (Figure 4.10).

Unconjugated bilirubin is conjugated by the liver, following which the majority is excreted into the biliary system. If this process is hindered, serum bilirubin rises and thus can be used as a marker of liver disease. It should also be noted, however, that unconjugated bilirubin is produced from the breakdown of red blood cells and this cell turnover is likely to be higher in sickle cell patients due to the inherent pathology. A way to overcome this would be to use a bilirubin assessment for conjugated and unconjugated, unfortunately, not enough patients had this measured consistently to be an adequate marker for analysis within this cohort. Within the inclusion criteria, an increased concentration of bilirubin was deemed pathological in accordance with this.
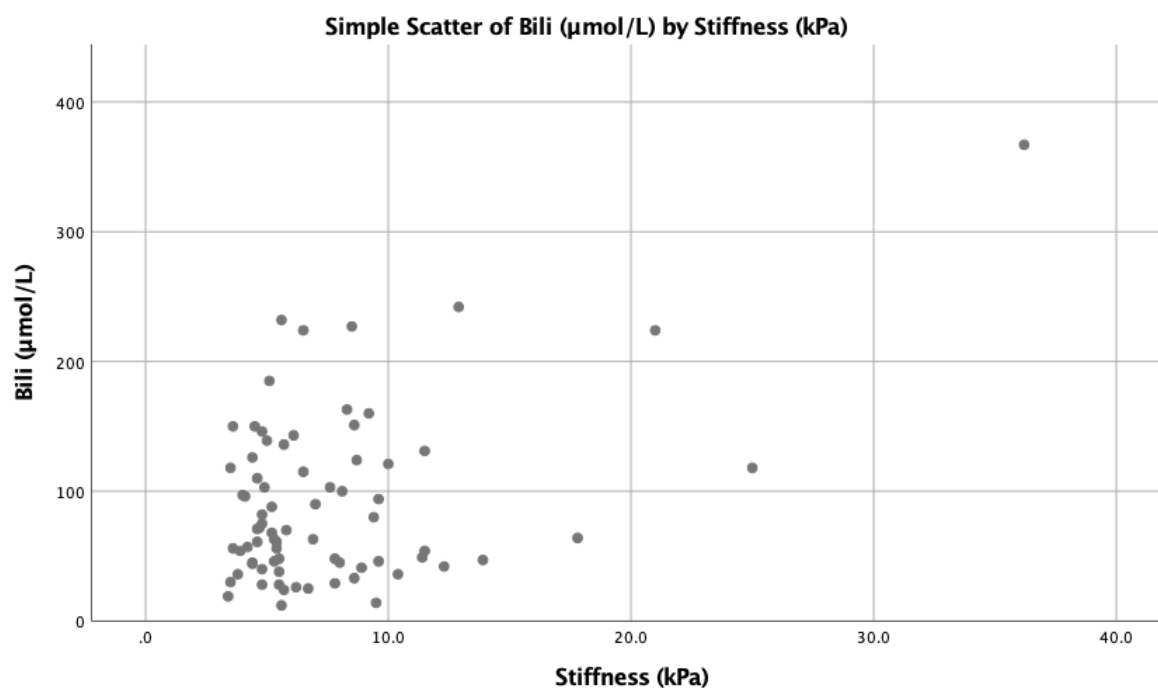
Figure 4.11: Scattergram showing serum bilirubin concentration against liver stiffness. Spearman's rho test, r = 0.125, p = 0.285.
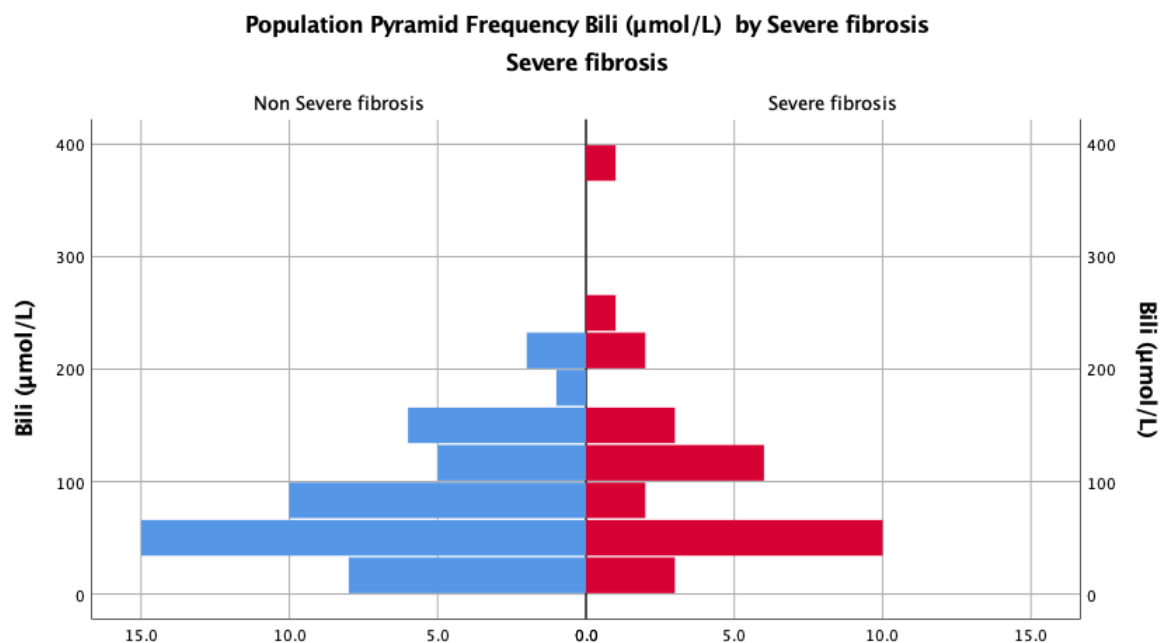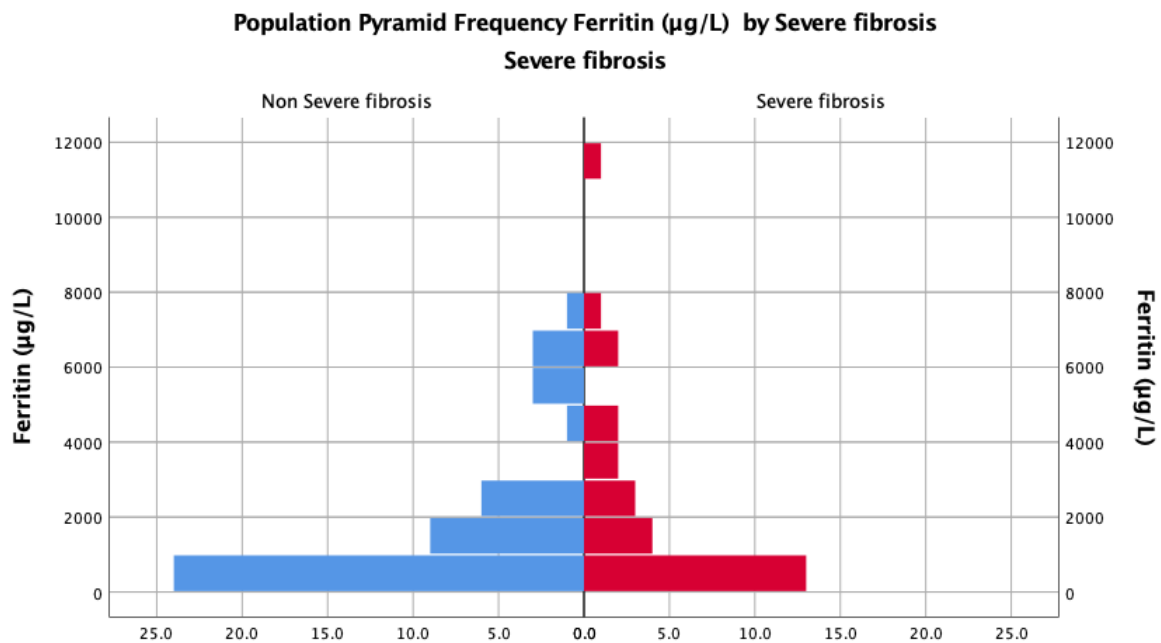

Figure 4.12: Histogram showing the distribution of serum bilirubin concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was no correlation between an increasing liver stiffness and serum bilirubin concentration (Figure 4.11) and median serum bilirubin concentration was not statistically significantly different between people with non-severe (68μmol/L) and severe fibrosis (87μmol/L), p = 0.335 (Figure 4.12).

Secondary to the increased bilirubin production, there is an increased frequency of gallstones, which then cause issues such as biliary colic and cholecystitis which may require a cholecystectomy. We thus investigated whether there was a relationship between the frequency of cholecystectomies and degree of fibrosis and found no difference (p = 0.940, OR: 0.96 95% CI: 0.376-2.47) (see Figure 4.13).
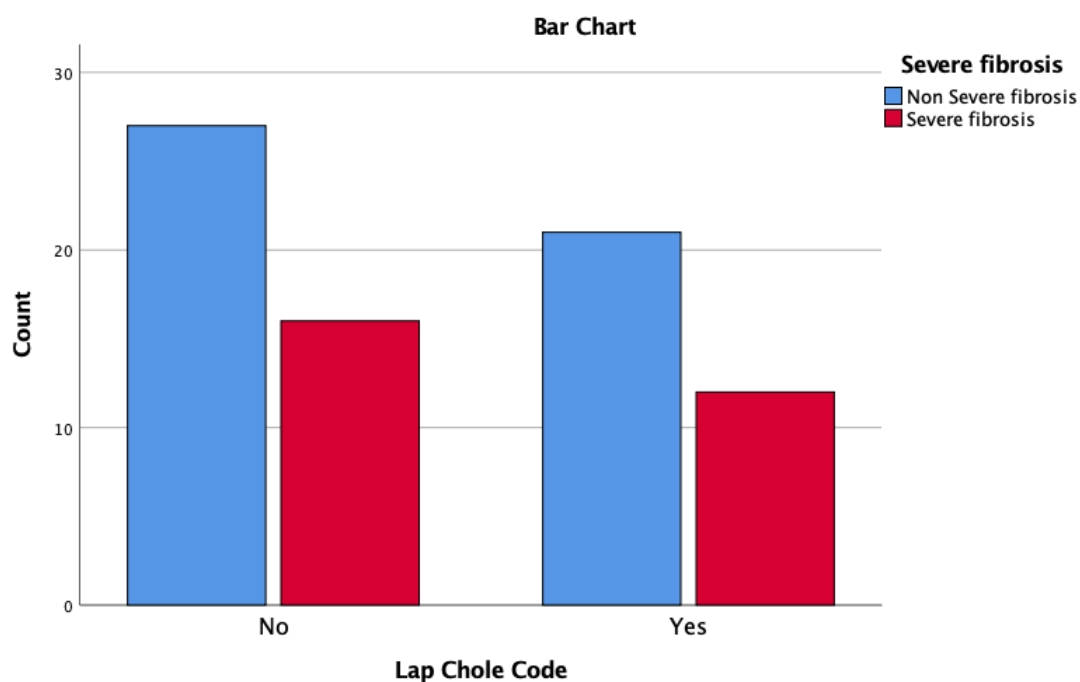
Figure 4.13: A clustered bar chart displaying the number of patients receiving a cholecystectomy according to degree of fibrosis. Chi sq test, p = 0.940, OR: 0.96 95% CI: 0.376-2.47

3.14 <u>Iron studies</u>

Serum ferritin has a controversial interaction with actual liver iron concentration which may contribute to fibrosis. Serum ferritin is easier and cheaper to measure than LIC and therefore a correlation would be clinically useful.
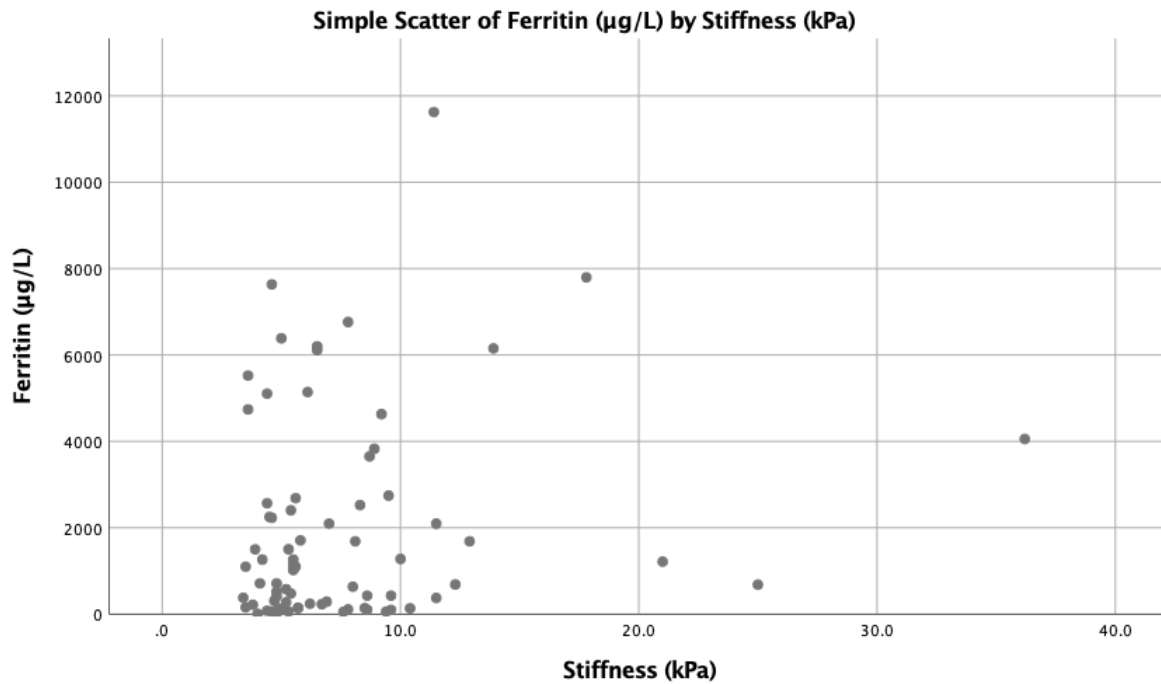
Figure 4.14: Scattergram showing serum ferritin concentration against liver stiffness, r = 0.151, p = 0.196

Figure 4.15: Histogram showing distribution of serum ferritin concentration when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was no correlation between an increasing liver stiffness and ferritin concentration (Figure 4.14) and median ferritin was not statistically significantly different between people with non-severe (712µg/L) and severe fibrosis (1246µg/L), p = 0.399 (Figure 4.15).

The frequency of transfusions may be used as a surrogate for the severity of sickle cell disease with those requiring more regular transfusions having a worse disease. Also, they will inherently be at higher risk of developing iron overload symptoms due to the increased exposure.
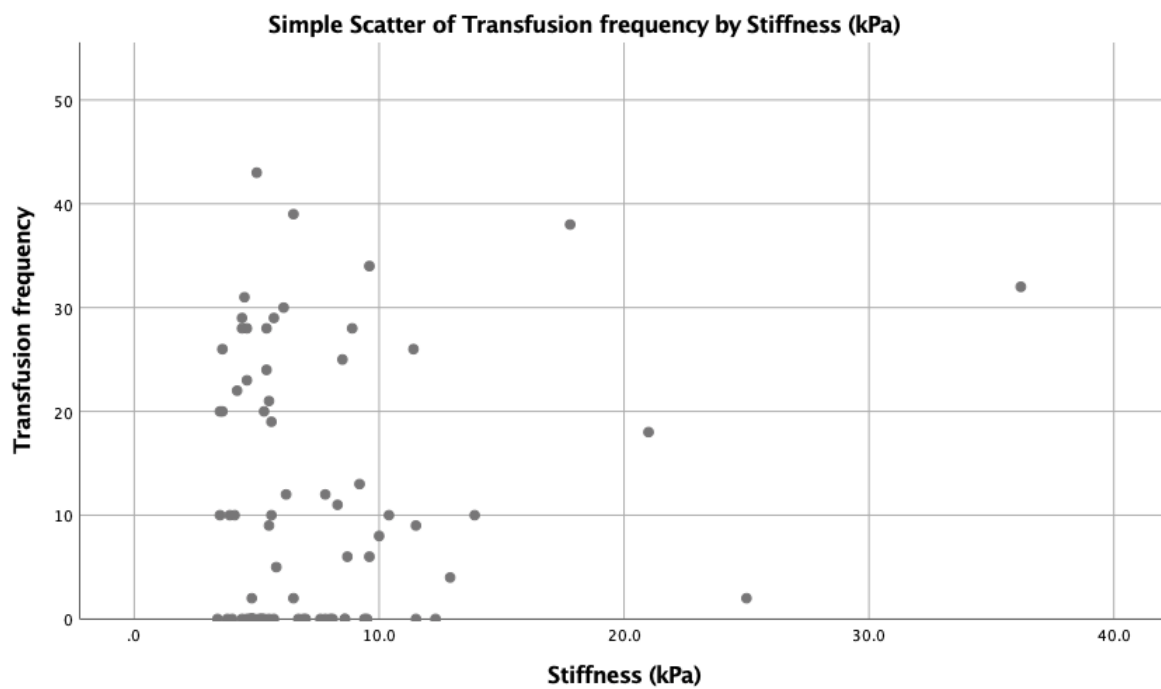
Figure 4.16: Scattergram showing transfusion frequency against liver stiffness. Spearman's rho test, r = 0.031, p = 0.789
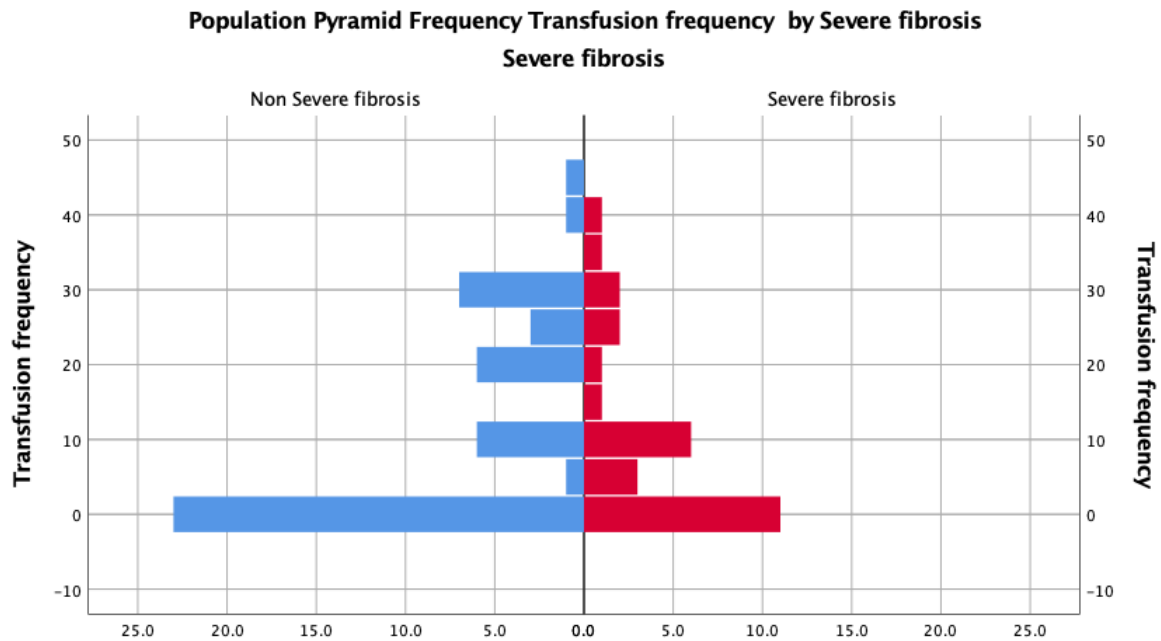
Figure 4.17: Histogram showing the distribution of transfusion frequency when divided by absence (TE <7.2kPa) or presence of severe fibrosis (TE>7.3kPa)

There was no correlation between an increasing liver stiffness and transfusion frequency (Figure 4.16) and median transfusion frequency was not statistically significantly different between patients with non-severe (7 transfusions) and severe fibrosis (7 transfusions), p = 0.982 (Figure 4.17).

Patients that are known to have iron overload, usually secondary to transfusions are either progressed to exchange transfusions, chelation therapy or a mixture of both. We thus interrogated whether there was a relationship between patients with severe fibrosis and chelation therapy and found that this was not the case (p = 0.259, OR: 0.449, 95% CI: 0.130-1.54) (see Figure 4.18).
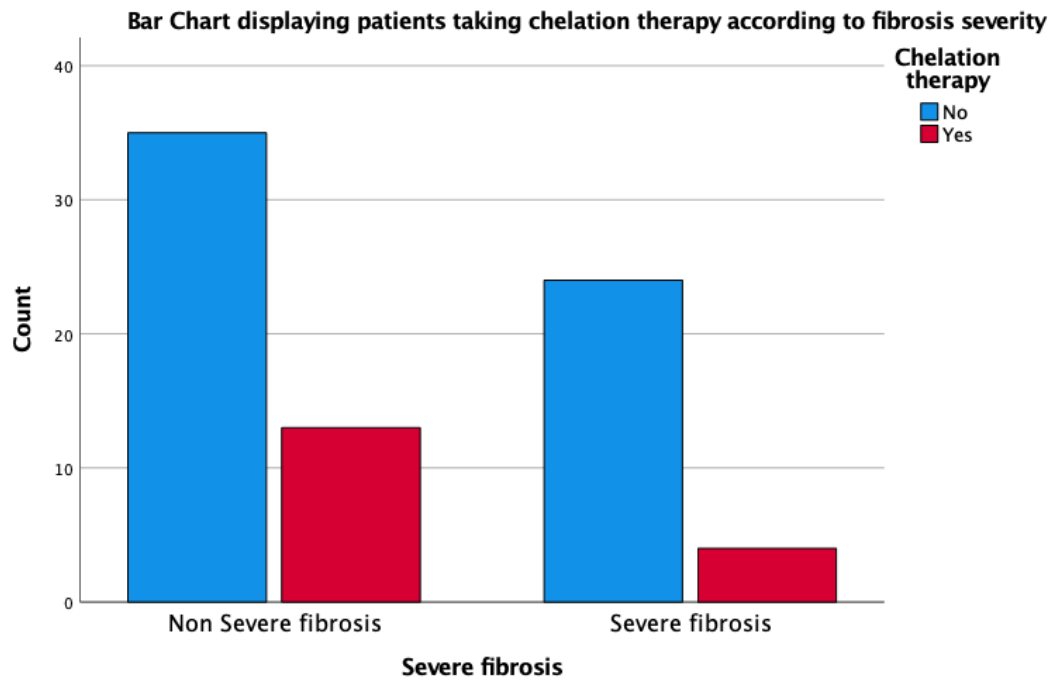
Figure 4.18: a clustered bar chart displaying the number of patients receiving chelation therapy according to patient with non-severe fibrosis and severe fibrosis. Fisher's Exact test, p = 0.259, OR: 0.449, 95% CI: 0.130-1

5   What is the distribution of transient elastography results of adult patients with cystic fibrosis

3.15 Age

Advanced age is an individual risk factor for fibrosis. We compared age and elastography scores to determine whether fibrosis increased with age.
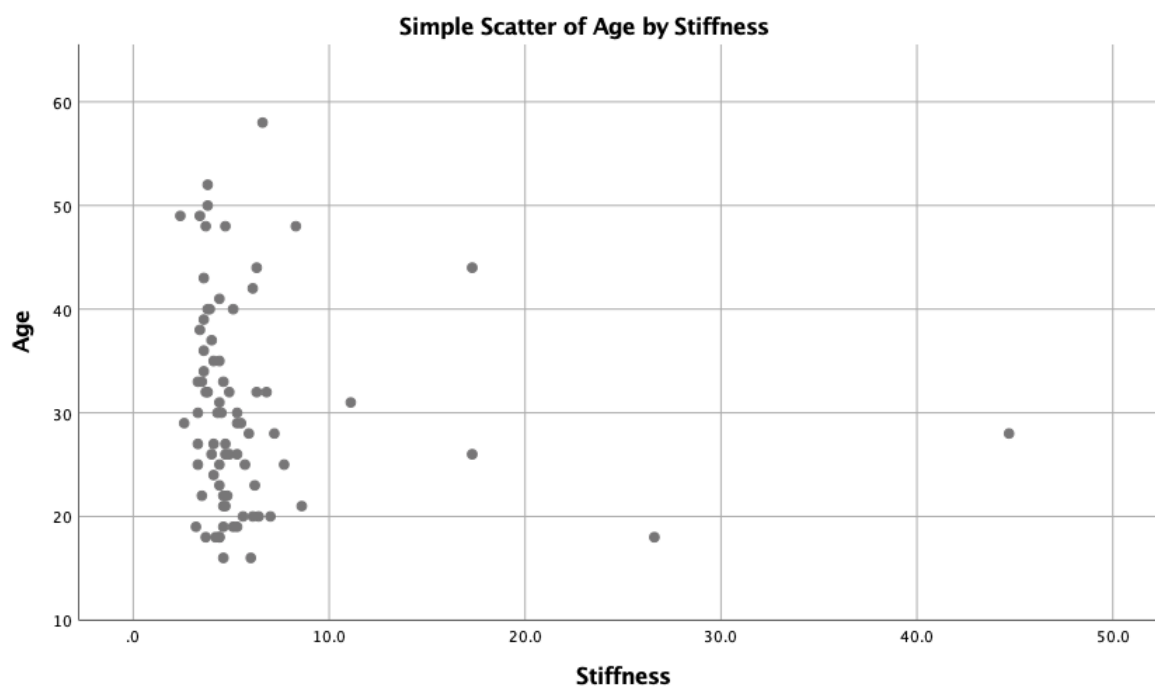
Figure 5.1: Scattergram showing age against liver stiffness. Spearman's rho test, r = -0.22, p = 0.051

Population Pyramid Frequency Age by Fibrosis
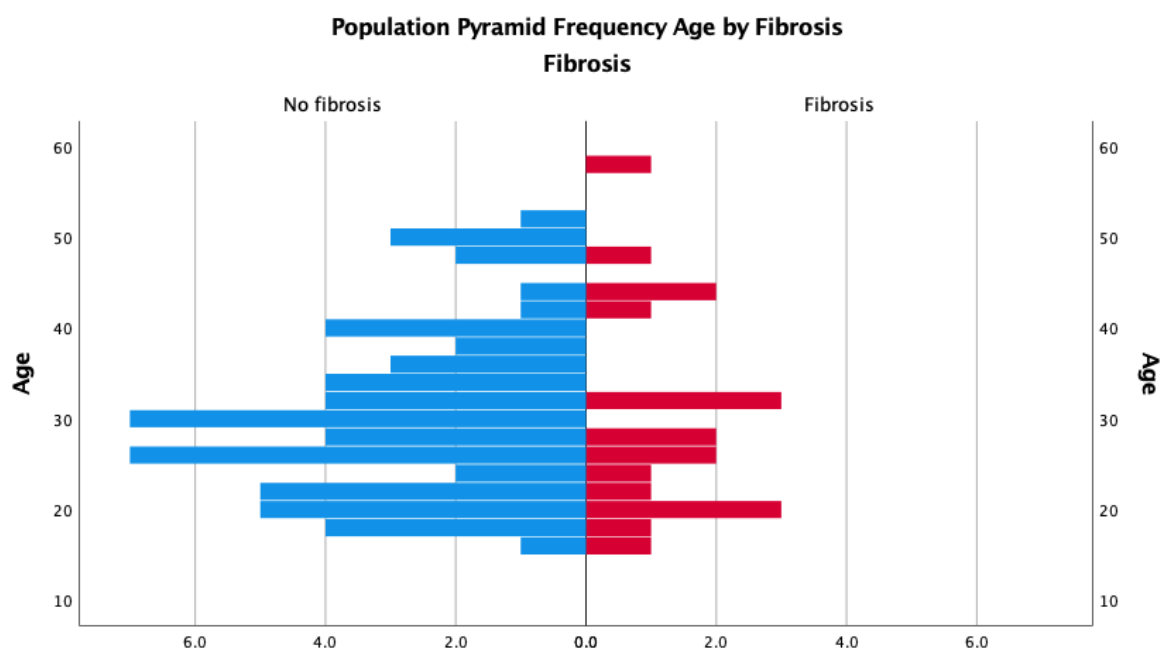
Figure 5.2: Histogram shows the age distribution when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

A weak negative correlation between increasing liver stiffness and age did not reach statistical significance (p = 0.051) (Figure 5.1). The median age for patients without fibrosis was not different between patients with no fibrosis (28 yrs.) and those with fibrosis (29 yrs.), p = 0.735 (Figure 5.2).

3.16 **CF markers**

CF is a genetic disease with many associated genotypes, F508 deletion is the most common genotype and therefore, this was investigated for the likelihood of fibrosis with no association found (p = 0.744). The diagnosis of CF in children can be associated with meconium ileus though this shows no relationship with CFLD (p = 0.579). Previous exposure to the hepatitis A virus was not significant (p = 0.148).

3.17 **Pancreatic insufficiency**

Pancreatic insufficiency is noted in patients with cystic fibrosis due to an increase in the viscosity of pancreatic secretions, leading to their build-up within the pancreas, thereby causing damage to pancreatic cells. We found that most patients within our cohort were identified with pancreatic insufficiency (79.7%) to some degree and pancreatic insufficiency was not found to be significant for CFLD (p = 0.332).

3.18 **Static liver tests**

We looked at bilirubin which did not show an association (p = 0.374) or difference (p = 0.822) with liver stiffness. We investigated alkaline phosphatase and similarly found no difference (p = 0.105) (Figure 5.3) though a significant association was found (r = 0.241, p = 0.032) (Figure 5.4).

Simple Scatter of ALP by Stiffness
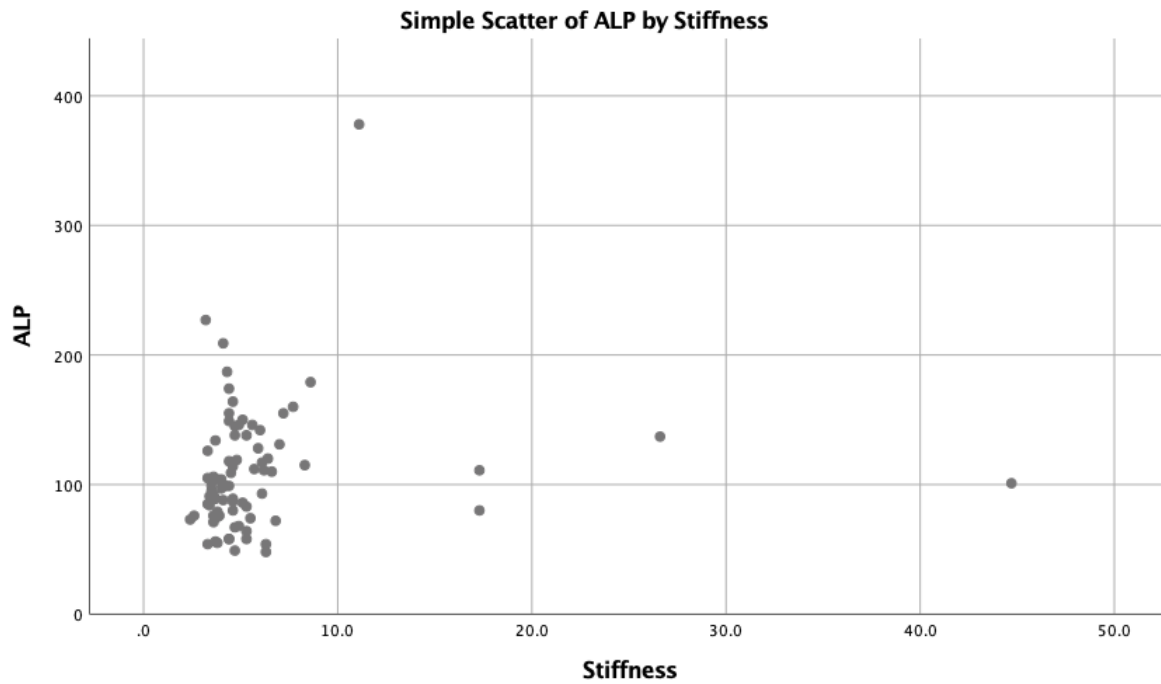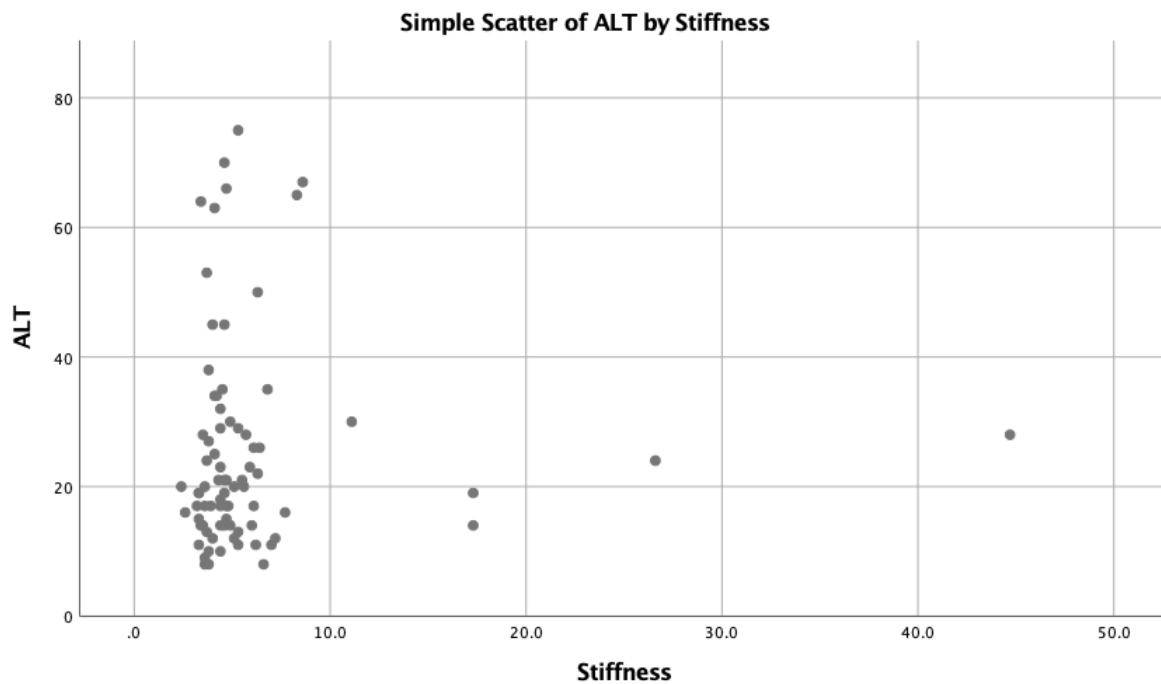

Figure 5.3: Scattergram showing serum alkaline phosphatase concentration against liver stiffness. Spearman's rho test, r = 0.241, p = 0.032
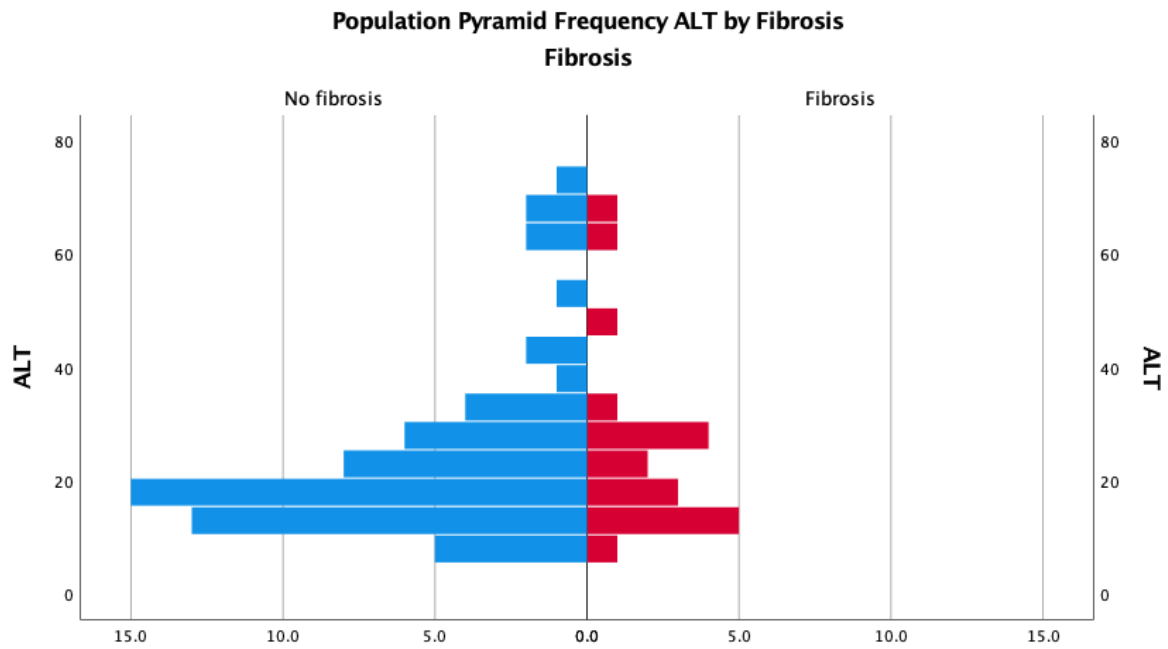
Population Pyramid Frequency ALP by Fibrosis
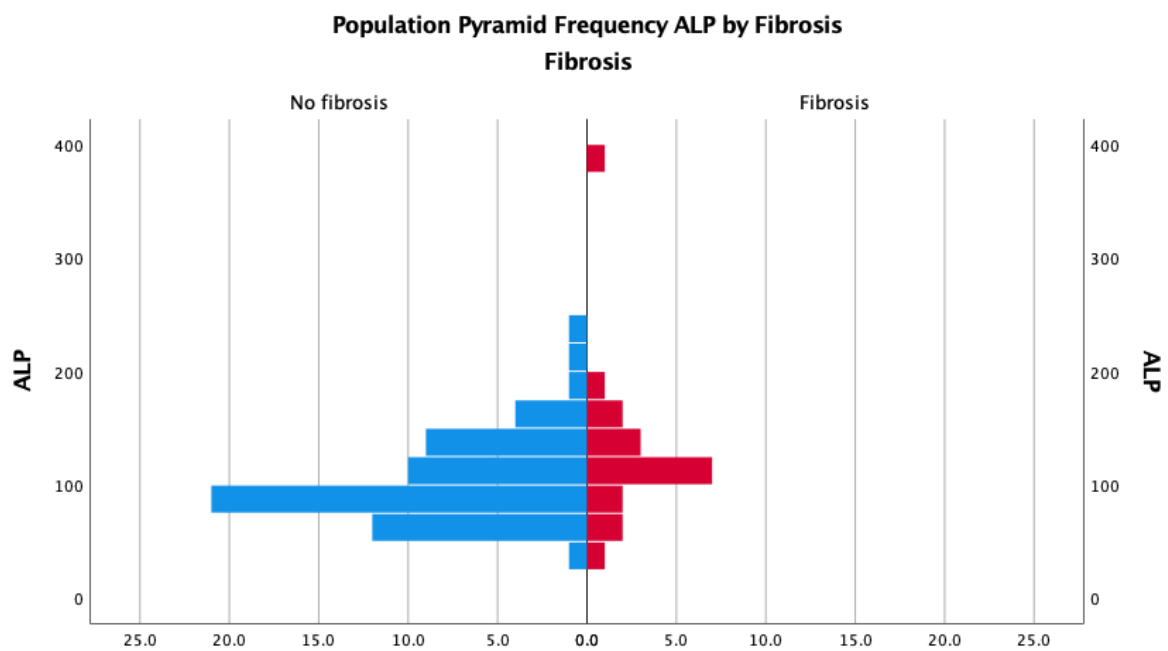
Figure 5.4: Histogram showing the distribution of serum alkaline phosphatase concentration when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

3.19

Standard serum liver function tests are readily available, with serial measurements for liver injury being obtainable for most patients. Alanine aminotransferase (ALT) is an enzyme

present in hepatocytes and following cell damage, can be detected at higher concentrations within the serum. It should be noted that this rise is secondary to ongoing cell injury and thus, the actual level may not reflect the level of fibrosis, though it may indicate ongoing damage. We investigated the relationship between ALT and stiffness.

Figure 5.5: Scattergram showing serum alanine aminotransferase concentration against liver stiffness. Spearman's rho test, r = 0.127, p = 0.264

Population Pyramid Frequency ALT by Fibrosis

Figure 5.6: Histogram showing the distribution of serum alanine aminotransferase concentration when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

There was no correlation between increasing liver stiffness and serum alanine aminotransferase concentration (Figure 5.5) and median serum alanine aminotransferase concentration was not statistically significantly different between those with no fibrosis (20U/L) and fibrosis being present (22U/L), $p = 0.877$ (Figure 5.6).

3.20 <u>Lung function</u>

CF is a disease that mainly affects the respiratory tract and is monitored by the percentage of predicted forced expiratory volume in 1 second (FEV1) on spirometry, we thus, investigated its relationship to liver disease.

Scatter Plot of Predicted FEV1 by Stiffness
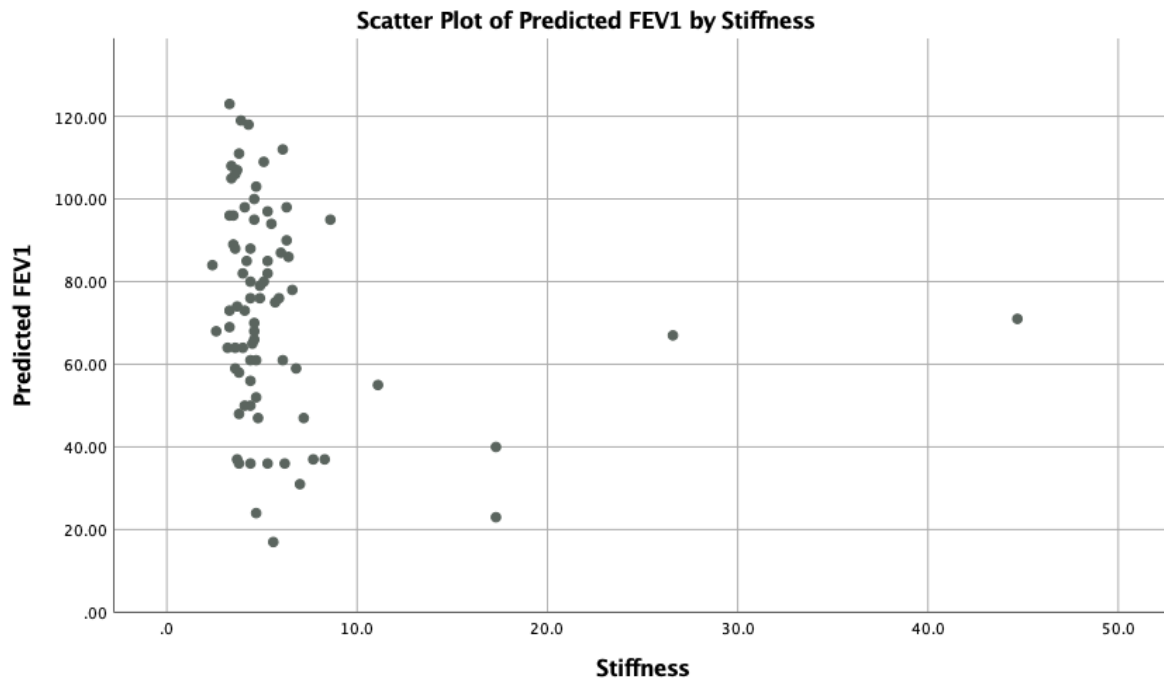
Figure 5.7: Scattergram showing percentage of the predicted forced expiratory volume in 1 second (predicted FEV1) against liver stiffness. Spearman's rho test, r = -0.160, p = 0.086
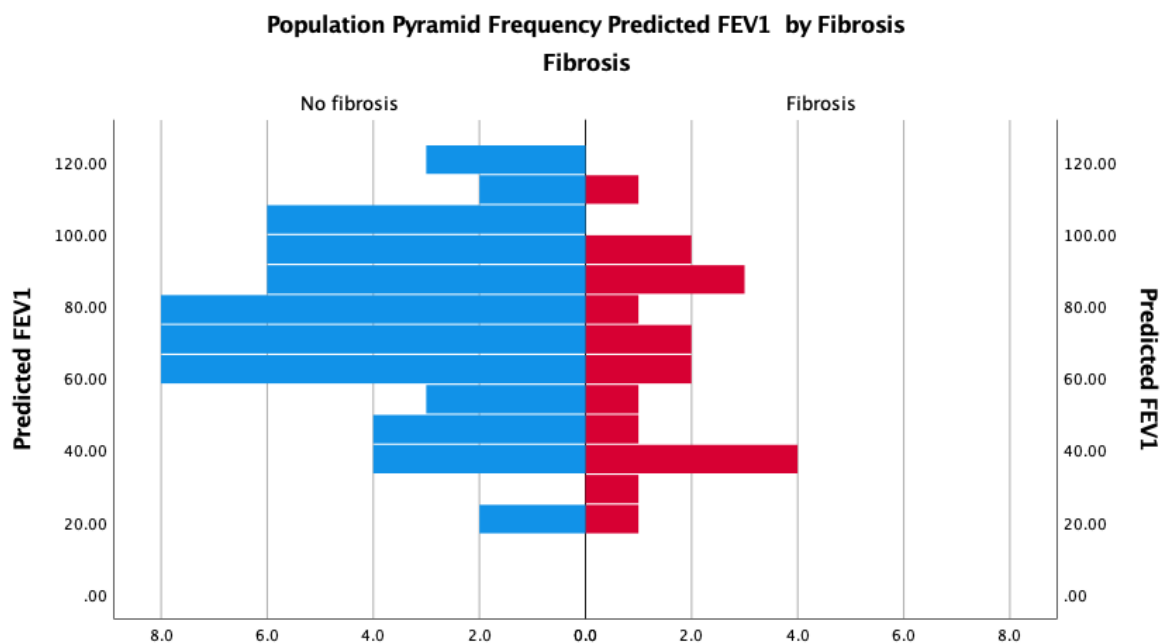
Population Pyramid Frequency Predicted FEV1 by Fibrosis

Figure 5.8: Histogram showing the distribution of predicted forced expiratory volume in 1 second (predicted FEV1) when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

There was no correlation between an increasing liver stiffness and predicted forced

expiratory volume in 1 second (pred FEV1) (Figure 5.7) and median pred FEV1 was not

398

statistically significantly different between no fibrosis (76%) and the presence of fibrosis (61%), p = 0.086 (Figure 5.8).

3.21

3.22 **Organisms in sputum**

CF patients have difficulty in clearing respiratory secretions and therefore suffer from several persistent infections, the presence of which may indicate damage to the liver, we thus investigated this with the most prevalent, pseudomonas aeruginosa infection, which showed a near statistical significance (p = 0.052) Figure 5.9. Burkholderia cepacia has been shown to indicate patients at risk of CF fibrosis, we found two patients colonised with this, both of whom had normal liver stiffness.
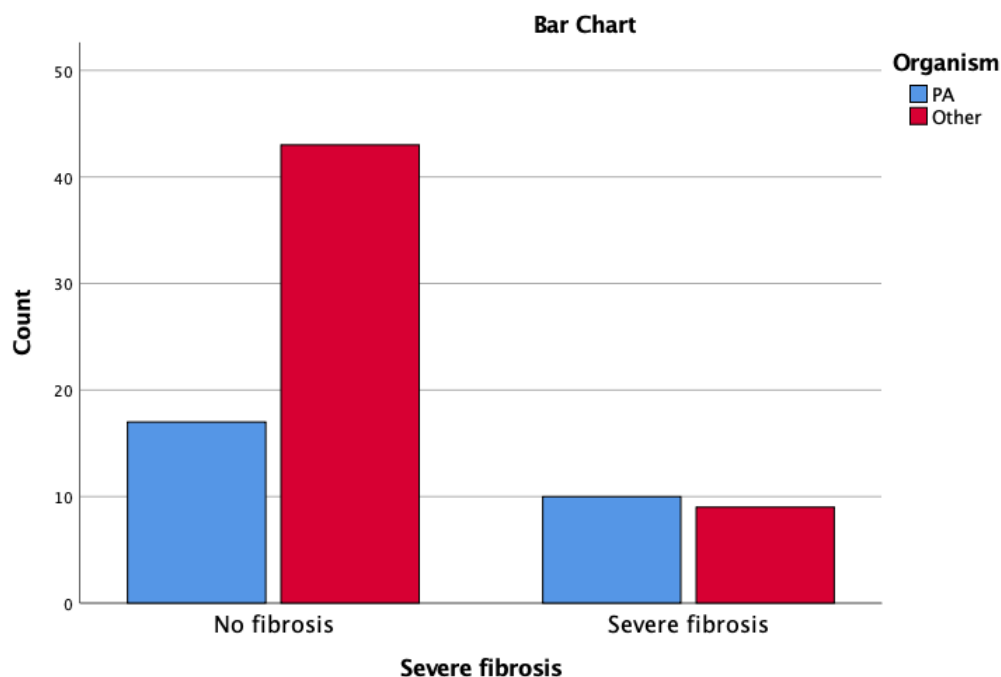

Figure 5.9: a clustered bar chart displaying the number of patients with pseudomonas aeruginosa infection when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa). Chi sq test, p = 0.052

3.23 <u>Haemoglobin concentration</u>

Haemoglobin carries oxygen around the body, however, most CF patients either have a
normal haemoglobin concentration or anaemia. We compared haemoglobin with
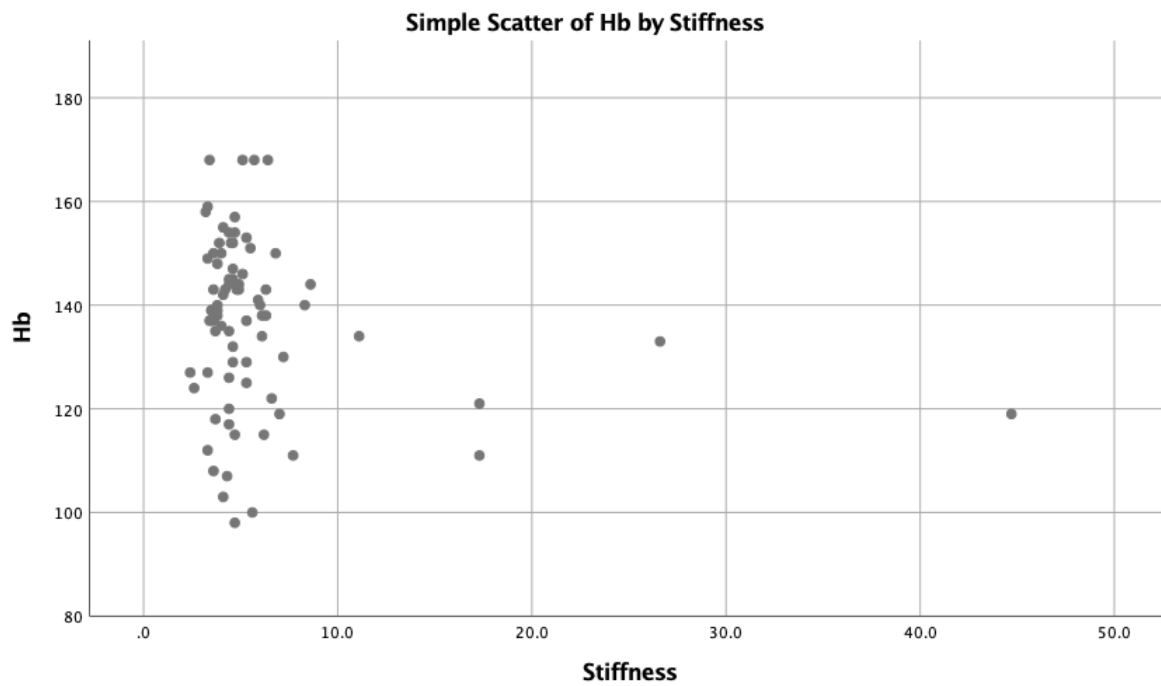elastography to determine whether haemoglobin levels are associated with increased fibrosis.

Figure 5.10: Scattergram showing haemoglobin concentration against liver stiffness. Spearman's rho test, r = -0.117, p =
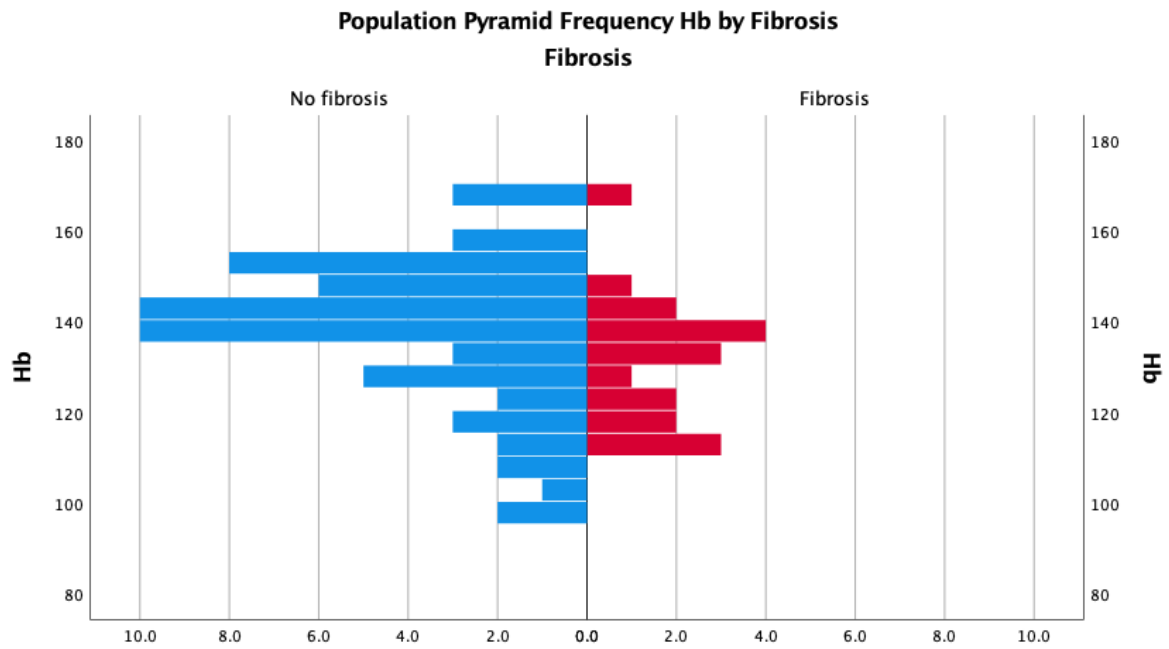0.303

Figure 5.11: Histogram showing the distribution of haemoglobin concentration when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

There was no correlation between increasing liver stiffness and haemoglobin (Figure 5.10).

Median haemoglobin concentration for patients without fibrosis (141g/dl) was not different from those with fibrosis (134g/dl), p = 0.065 (Figure 5.11).
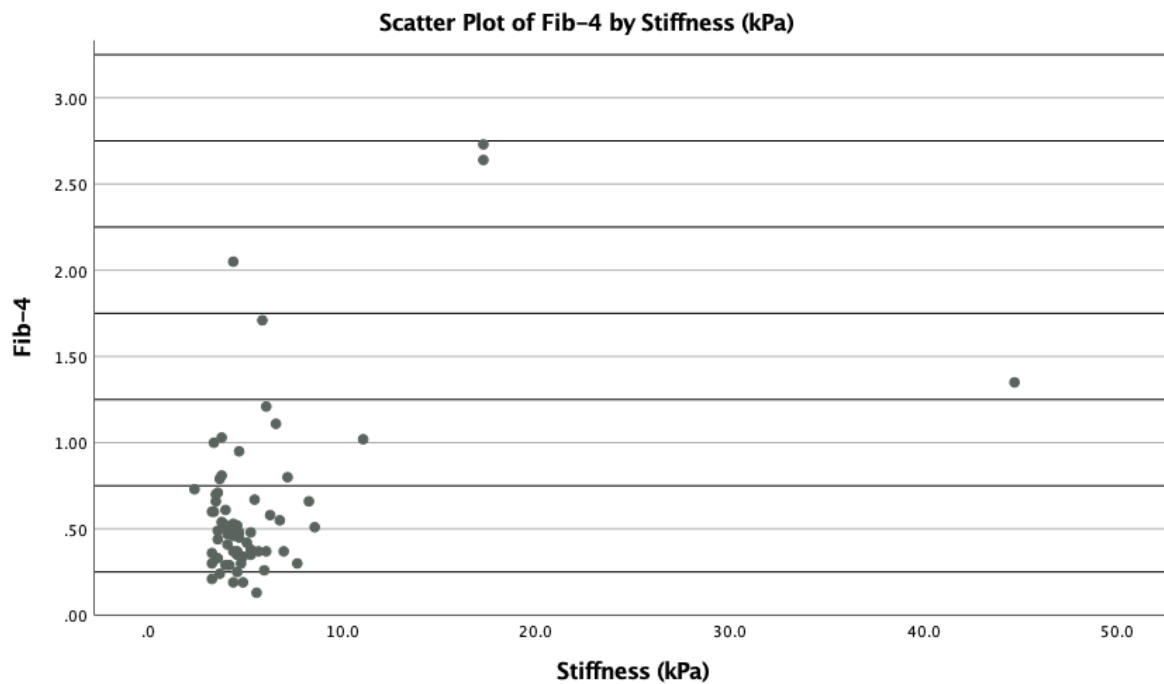
3.24 <u>Scoring systems</u>

Figure 5.12: Scattergram showing Fibrosis 4 (Fib 4) score against liver stiffness. Spearman's rho test, r = 0.121, p = 0.338
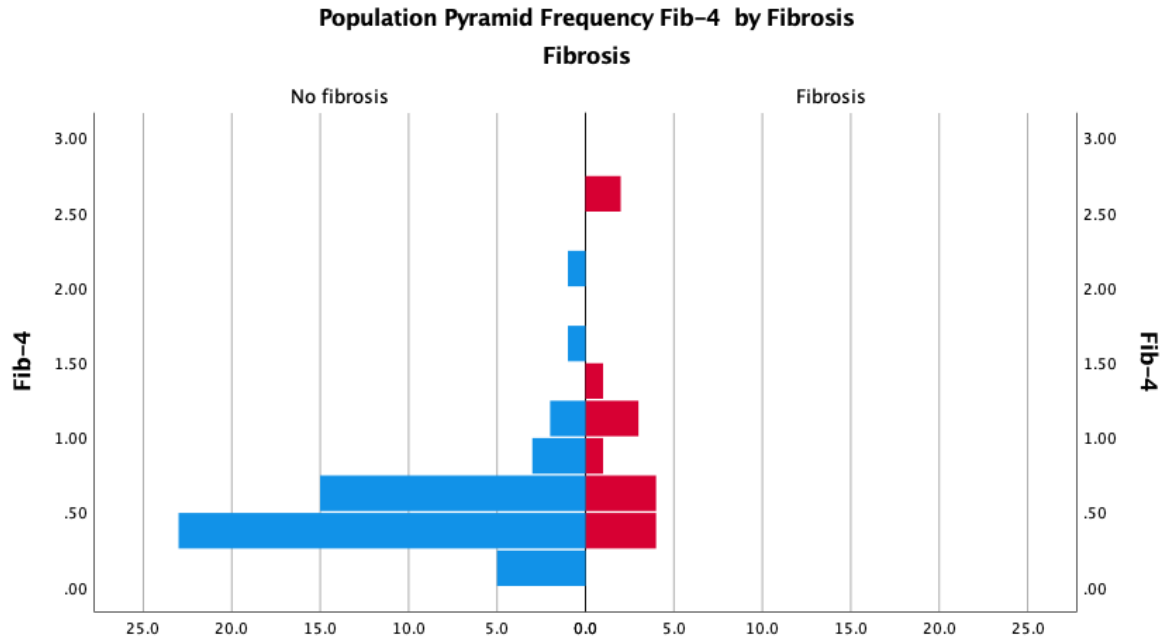

Figure 5.13: Histogram showing the distribution of fibrosis 4 (Fib 4) when divided by absence (TE <5.9kPa) or presence of fibrosis (TE>6kPa)

There was no correlation between increasing liver stiffness and fibrosis 4 (Fib 4) (Figure 5.12) and no difference for TE between patients categorised as having fibrosis (≥1.45) compared to those without fibrosis according to Fib 4 (p = 0.226) (Figure 5.13).

3.25 **Comparison of CFLD scoring systems**

There were eight patients which Debray criteria did not diagnose as CFLD, which the others did. They all had normal liver stiffness except for the one stated above. Otherwise, all patients which Debray did diagnose agreed with the other criteria. There were no patients missed by Koh criteria that were agreed upon by the other scoring systems, although there were eight that have CFLD according to Koh, which were not picked up by either of the other systems. These all had a raised ALP and AAR score, both exclusive to Koh, with all but one (6kPa) having a normal liver stiffness. Flass identified 19 patients as CFLD in disagreement with the other systems, three of which had transient elastographies showing early fibrosis (6.3-6.4kPa), which did not reach the threshold for Koh criteria.

All patients diagnosed as CFLD by Debray criteria were agreed upon by both other scoring criteria. However, Koh criteria identified 16 further patients (correlation = 0.618, p <0.001), while Flass identified 27 (correlation = 0.597, p <0.001). Koh and Flass criteria agreed on 24 patients diagnosed with CFLD and 28 not having CFLD (correlation = 0.445, p <0.001). This meant 27 patients were deemed to have CFLD by one criterion and not the other, eight by Koh and 19 by Flass.