



Prognostic biomarkers in HCV cirrhosis

Version 3.0 – date 18.06.2015

MAIN SPONSOR: Nottingham University Hospitals NHS Trust
FUNDERS: Medical Research Council (as part of a programme grant under their Stratified Medicine scheme)
STUDY COORDINATION CENTRE: Queen's Medical Centre, Nottingham

REC reference: 14/WM/1128

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Protocol incorporates the following amendments:

1. Addition of new NHS sites
2. Amendment to working regarding the collection of urine samples
3. Addition of Principle Investigators to signature list

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Funder

This study is part of a programme grant from the Medical Research Council under their Stratified Medicine scheme.

GRANT TITLE: Stratified Medicine to Optimise Treatment for Hepatitis C Virus Infection (STOP-HCV)

Grant Ref: MR/K01532X/1 is funding this study.

It falls within workstrand 5 of STOP-HCV: **Biomarkers**.

This protocol describes the 'Prognostic biomarkers in HCV cirrhosis' study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

APRI	AST to platelet ratio index
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
AUDIT questionnaire	Alcohol Use Disorders Identification Test Questionnaire
ELF	Enhanced liver fibrosis
FIB-4	Fibrosis 4 score
GGT	Gamma glutamyl transferase
HA	Hyaluronic acid
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
IL-10	Interleukin 10
INR	International normalized ratio
MELD	Model for End Stage Liver Disease
RNA	Ribonucleic acid
STOP-HCV	STratified medicine to OPTimise Treatment of patients with HCV
TIMP	Tissue inhibitor of metalloproteinases
UK	United Kingdom
UKELD	UK End Stage Liver Disease
YKL-40	Chondrex

KEYWORDS

Hepatitis C virus; chronic hepatitis; cirrhosis; decompensation; hepatocellular carcinoma; standard of care therapy; spontaneous clearance; liver fibrosis; disease progression

STUDY SUMMARY

TITLE Prognostic biomarkers in HCV cirrhosis

DESIGN Prospective Longitudinal study

AIMS Prediction of clinical outcomes in chronic hepatitis C infection using serial biomarkers

OUTCOME MEASURES **Compensated study:**

Primary : Hepatic decompensation (as defined by first presentation of ascites, encephalopathy, variceal haemorrhage, jaundice (bilirubin >50 µmol/L) or hepatocellular carcinoma (HCC))

Secondary:

- 1) Liver related death or liver transplantation
- 2) All cause mortality
- 3) Development of hepatocellular carcinoma
- 4) Increase in Child-Pugh score of ≥ 2 points from baseline.

Decompensated study:

Primary: Liver related death or liver transplantation

Secondary:

Progression of liver disease (defined by increase of Child-Pugh score of 2) or regression of liver disease (defined by improvement of Child-Pugh score of 2)

POPULATION Adults with evidence of infection with hepatitis C virus and cirrhosis will be recruited into the study.

ELIGIBILITY Patients must sign a consent/assent form, have cirrhosis and evidence of current or past infection with HCV and are already or are willing to be enrolled into HCV Research UK

DURATION 5 years

REFERENCE DIAGRAM

Pre-Study	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Year 0	Year 1	Year 2	Year 3	Year 4	Year 5
Consent to join HCV Research UK	Enrolment to cirrhosis study**	Follow Up 1 yr after enrolment	Follow Up 2 yrs after enrolment	Follow Up 3 yrs after enrolment	Follow Up 4 yrs after enrolment

** could be 0-2 yrs, average will be 1 yr.

1. INTRODUCTION

1.1 BACKGROUND

Cirrhosis is a distinct pathological entity resulting from chronic liver injury and specifically liver fibrosis. The progression of fibrosis to cirrhosis represents a change in morphology, haemodynamics and function of the liver. The natural history of cirrhosis is broadly divided into a compensated phase, which is largely asymptomatic, and a decompensated phase classically associated with the development of ascites, hepatic encephalopathy, variceal haemorrhage and jaundice. The transition from compensation to decompensation represents a landmark in the progression of disease and there are markedly different survival times after the development of liver cirrhosis.

The development of significant complications of cirrhosis secondary to chronic HCV infection has been reported to be between 20-30% at 5 years in a number of observational studies⁽¹⁻⁴⁾. Studies have shown that if compensated cirrhosis is stratified by disease severity, for example using the presence or absence of varices, prognostic information is improved. Specifically in those patients with varices, 66% reached a negative clinical outcome (new onset of ascites, encephalopathy, jaundice, variceal haemorrhage or hepatocellular carcinoma (HCC)) at 6 years compared to 26% of those with no evidence of varices⁽³⁾.

The Model for End Stage Liver Disease (MELD) score was originally developed as a prognostic score for patients undergoing trans-jugular intrahepatic Porto systemic shunt (TIPSS) insertion but has subsequently been adopted for making decisions on organ allocation in liver transplantation. The MELD score has evolved to incorporate sodium and in the UK this has led to the UK End Stage Liver Disease (UKELD) scoring system. A number of studies have shown superior performance of MELD and its derivatives in predicting clinical outcomes in liver disease. Whilst it is generally accepted that current scoring systems are useful in stratifying severe disease (as exemplified by their use in selection of patients to be listed for liver transplantation) there may be potential to improve upon this by using non-invasive biomarkers, particularly in earlier compensated cirrhosis⁽⁵⁾. Demonstrating incremental prognostic benefit over validated systems such as MELD and Child-Pugh score, using robust clinical end-points, will have both novelty and clinical utility.

The availability of non-invasive biomarkers potentially allows greater stratification of prognostic information. A number of studies have shown that biomarkers⁽⁶⁻⁹⁾ at baseline predict liver related events 5-10 years later across a range of liver diseases. However liver disease is a dynamic process and thus the serial measurement of biomarkers, with subsequent refinement, may be more informative. The evidence this improves prognosis is limited and largely originates from the HALT-C study. Fontana et al⁽¹⁰⁾ found serum markers of liver fibrosis predicted clinical outcomes, dependent on baseline levels and changes on serial measurement. Importantly, both baseline values and then subsequent changes of the biomarker were significantly raised in those reaching a clinical outcome. Using different biomarkers, but the same study cohort, Everson et al⁽¹¹⁾ found that serial quantitative liver tests, between year 2 and year 4, differentiated patients into a high risk group of developing clinical outcomes (11 to 30%) or low risk (benign outcome) 2 years later. These studies show proof of principle that serial biomarkers may improve prognosis and further studies in unselected cohorts are now required to substantiate these findings.

1.2 RATIONALE FOR CURRENT STUDY

Hypothesis: The serial measurement of biomarkers can be used to predict which patients with HCV cirrhosis develop a clinical outcome within 5 years of follow up.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

1. To define optimal prognostic biomarkers, in isolation or as part of an algorithm, that predict clinical outcomes.
2. To create a resource for the future development and validation of emerging biomarkers

2.2 SECONDARY OBJECTIVE

1. To assess the incremental prognostic value of a panel of biomarkers against established prognostic tools (e.g. Child-Pugh score, MELD score and Baveno classification)
2. To compare **a)** baseline stratification using biomarkers versus **b)** baseline + serial biomarker measurement for the prediction of clinical outcomes
3. To assess biomarker performance in a prospective cohort of decompensated patients with chronic HCV infection for **a)** progression to death or liver transplantation and **b)** regression of liver disease severity.

3. STUDY DESIGN

This will be a prospective, longitudinal, prognostic study with two parts:

- a. Compensated cirrhosis study

And

- b. Decompensated cirrhosis study.

In the compensated study, 1000 patients who have already consented to or are willing to consent to the HCV Research UK national cohort will be enrolled. They will have laboratory evidence of HCV infection, either past or current, and a diagnosis of compensated liver cirrhosis. All will be attending specialist services for management and treatment of their disease.

In the decompensated study, 200 patients will be enrolled. They will have laboratory evidence of HCV infection, either past or current, and a diagnosis of liver cirrhosis. In addition they will have evidence of at least one feature of decompensation (any evidence of ascites, clinical evidence of encephalopathy, primary HCC, previous variceal haemorrhage or jaundice (bilirubin >50 µmol/L).

Enrolled patients will be asked to complete 5 visits; an Enrolment visit plus 4 further annual visits. At each visit patients will donate a blood (minimum 25ml) and if required urine (20ml) sample for analysis of biomarkers and subsequent storage in the HCV Research UK Biobank, and complete an alcohol intake questionnaire. Information from routine clinic care including laboratory blood results, fibroscan and ultrasound scans will also be obtained. For patients already enrolled in HCV Research UK, the HCV Research UK enrolment visit and bleed will be counted as the first visit for this cirrhosis study and therefore will need only a further 4 annual visits (urine will not have been collected at visit 1 for these patients).

Wherever possible, these study visits will be timed to coincide with routine clinic visits to minimise the burden for patients in terms of travel to the hospital, time spent in clinics, additional blood tests and other assessments.

3.1 STUDY OUTCOME MEASURES

a) Compensated study:

Primary:

- Hepatic decompensation (first presentation of ascites, encephalopathy, variceal haemorrhage, jaundice (bilirubin >50 µmol/L) or hepatocellular carcinoma)

Secondary:

- Liver related death or liver transplantation
- Development of hepatocellular carcinoma
- Increase in Child-Pugh score of ≥ 2 points from baseline

b) Decompensated study:

Primary:

- Liver related death or liver transplantation

Secondary:

- Progression of liver disease (defined by increase of Child-Pugh score of 2) or
- Regression of liver disease (defined by improvement of Child-Pugh score of 2)

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

All participants must have:

- Evidence of chronic HCV infection (RNA positive on a least 2 occasions 6 months apart) or have had previous HCV infection and a sustained virological response to therapy
- and
- Evidence of cirrhosis.

4.2 INCLUSION CRITERIA

a) Compensated study

- 1) Patients should have chronic HCV infection (RNA positive on a least 2 occasions 6 months apart) OR have had previous HCV infection and a sustained virological response to therapy
- 2) Patients are already enrolled or are willing to enrol in HCV Research UK
- 3) Patients have evidence of cirrhosis as defined by :
 - Histological assessment
 - Or at least 1 of the following criteria:
 - I. Validated non-invasive marker including:
 - Fibroscan with a reading greater than 13 kPA⁽¹²⁾
 - APRI score > 2⁽¹³⁾
 - ELF score greater than 10.48⁽¹⁴⁾
 - Fibrotest score >0.73⁽¹⁵⁾
 - II. Evidence of varices at endoscopy in the context of a patent portal vein
 - III. Definitive radiological evidence of cirrhosis (i.e. nodularity of liver and splenomegaly on Ultrasound/CT)

b) Decompensated study

- 1) Patients should have chronic HCV infection (RNA positive on a least 2 occasions 6 months apart) OR have had previous HCV infection and a sustained virological response to therapy
- 2) Patients are already enrolled or are willing to enrol in HCV Research UK
- 3) Patients have evidence of cirrhosis as defined by :
 - Histological assessment
 - Or at least 1 of the following criteria:
 - I. Validated non-invasive marker including:
 - Fibroscan with a reading greater than 13 kPA⁽¹²⁾
 - APRI score > 2⁽¹³⁾
 - ELF score greater than 10.48⁽¹⁴⁾
 - Fibrotest score >0.73⁽¹⁵⁾
 - II. Evidence of varices at endoscopy in the context of a patent portal vein
 - III. Definitive radiological evidence of cirrhosis (i.e. nodularity of liver and splenomegaly on Ultrasound/CT)
- 4) Patients have at least one of the following :
 - Clinical or radiological evidence of ascites
 - Clinical evidence of encephalopathy (West Haven classification ≥ grade 2)
 - Previous variceal haemorrhage
 - Jaundice (bilirubin >50 µmol/L)
 - HCC (as defined by EASL guidelines 2012)

4.3 EXCLUSION CRITERIA

Compensated study:

1. Patients will not be included if they are unable to give written informed consent.
2. Evidence of HCC
3. Active listing for liver transplantation
4. Portal vein thrombosis
5. Any evidence of ascites (radiological or clinical)
6. Any clinical evidence of encephalopathy (West Haven classification ≥ grade 2)
7. Jaundice with bilirubin >50 µmol/L
8. Previous variceal haemorrhage

Decompensated study:

1. Patients will not be included if they are unable to give written informed consent
2. Active listing for liver transplantation

4.4 WITHDRAWAL CRITERIA

Due to the observational nature of this study, there will be no criteria for stopping early. Patients can withdraw their consent at any time throughout the life of the study.

5. ADVERSE EVENTS

This section is not applicable as this is an observational study only and there are no protocolled treatment interventions.

6. ASSESSMENT AND FOLLOW-UP

End of Study:

- Compensated study: 1000 patients have completed their Year 5 visit or reached the primary end point
- Decompensated study: 200 patients have completed their Year 5 visit or reached the primary end point

6.1 CLINICAL DATA AT ENTRY

All patients willing to join the study will be asked to provide written consent.

On enrolment into HCV Research UK, each patient is assigned a unique study number. All data and biological samples are recorded against that number. On Enrolment to this study, each patient will retain their HCV Research UK number and that will be used.

The following information will be collected from all patients. Where possible, information will be obtained from the HCV Research UK Clinical Research database, but some may have to be identified specifically for this study (see Appendix 2):

- Demographics
- Risk factors for HCV infection
- Social history
- Physical Characteristics
- HCV data
- HCV Treatment data
- Co-morbidities
- Liver disease outcomes
- Laboratory data
- Alcohol history / current intake as documented in the validated AUDIT questionnaire

6.2 BIOLOGICAL SAMPLES

6.2.1 Handling and storage of biological samples

On enrolment, in addition to any samples taken for standard care, extra EDTA (10ml), clotted (10ml) and PAXgene (5ml) blood samples and if required 20ml urine samples will be collected from each patient.

Further blood (25ml) and if required urine (20ml) samples will be taken at yearly intervals for the subsequent 4 years after enrolment.

Samples will be shipped to the central HCV Research UK Biobank at the MRC-University of Glasgow Centre for Virus Research as soon as possible.

Patient consent for the analysis of biomarkers, and storage and future use for as yet unspecified research purposes of remaining blood and urine samples will be sought. Samples will be retained indefinitely.

6.2.2 Blood volumes

At each visit, a 25ml sample will be taken.

Over the life of the study, the total volume of blood taken will be 125ml.

6.2.3 Chain of custody

A full chain of custody will be maintained for all samples throughout their lifecycle. Each site will pass custodianship of the samples to the HCV Research UK biobank. Samples released to STOP-HCV then fall under the control of STOP-HCV. Any transfer of samples/data will be under the terms of a Material Transfer Agreement and there are explicit terms in the release document about what happens to any excess material not used for studies (either returned to HCV Research UK or destroyed).

All blood and/or urine-derived samples sent to the HCV Research UK Biobank will be anonymised with a unique barcode. The barcode number will link the sample to the patient study number and their clinical details. All future sub-divisions of the sample will be allocated a new unique barcode. Linking of clinical data to the samples will be possible by the use of unique patient study numbers.

6.3 FOLLOW UP

Further visits will be completed at 1, 2, 3, and 4 years after enrolment. It is expected that (on average) a large proportion of patients will have at least one year's data already captured through HCV Research UK, so this is a minimum 5 year study.

For a list of all data that will be collected in the database, refer to Appendix 2.

7. STATISTICS AND DATA ANALYSIS

Data and all appropriate documentation will be stored for a minimum of 5 years after the completion of the study, including the follow-up period.

Sample size consideration and candidate prognostic factors:

The sample size calculations are based on the main event of interest being hepatic decompensation in compensated cirrhosis (first presentation of ascites, encephalopathy, variceal haemorrhage, jaundice (bilirubin >50 µmol/L) or hepatocellular carcinoma).

The proportion of patients expected to have an event by 5 years is 30%.

Therefore if 1,000 patients are followed-up by at least 5 years each, then it is expected that 300 events will be seen. However, not all individuals will be followed-up for the 5 years, as some will be censored earlier due to drop-out or end of the study. If we conservatively suggest that 20% of patients are censored before 5 years and without an event, then we expect to see 240 events in our study.

A general rule of thumb for examining prognostic factors is to have at least 10 events per candidate predictor^(16,17). Therefore our study will allow consideration of 24 candidate prognostic factors.

The following candidate prognostic factors are of primary interest and additional markers that emerge during the study will be added :

- MELD

- UKELD
- Baveno classification
- AST/ALT ratio
- Platelet count
- Hylauronic acid
- TIMP
- YKL-40
- FIB-4
- APRI
- IL-10
- Vitamin D
- Viral Sequencing data
- Host genetic markers
- Transcriptomic biomarkers

In addition routinely available NHS information will be collected to allow determination of:

Child-Pugh Score (INR, bilirubin, albumin, severity of ascites and presence of encephalopathy),

Analysis (a): Independent prognostic value of biomarkers measured at baseline

The correlation at baseline between the candidate predictors will be examined, to identify those that are closely related to each other. Then to examine the independent prognostic effect of candidate predictors at baseline, a multivariable Cox regression model will be fitted containing all the predictors together. This will produce hazard ratios (HRs) for each candidate predictor. Proportional hazards will be investigated for each variable, and time-dependent effects modelled as necessary. A sensitivity analysis will be undertaken to examine the impact of collinearity of variables on HR results: the HRs for each variable will be re-estimated after excluding other variables that are highly correlated at baseline. Continuous variables will be analysed on their continuous scale, with non-linear trends investigated using a suitable method such as restricted cubic splines or fractional polynomials.

For variables (such as biomarkers) that are potentially measured with error and subsequently have repeated measurements over time, a joint longitudinal and survival model will be used to account for the measurement error in the baseline value⁽¹⁸⁾. How HRs after adjusting for measurement error have changed will then be examined in comparison to the earlier analysis, where measurement error was ignored.

Analysis (b): External validation of existing prognostic models

Two prognostic models have been proposed for this area, for predicting outcome risk in new individuals diagnosed with this condition: MELD and UKELD as noted in the Introduction. Using the cohort data, the performance of these models will be examined, in terms of both calibration (agreement between observed and predicted risk over time) and discrimination (separation of predicted risk for those who do and do not experience events over time). Calibration will be measured by such as the calibration slope, and examined visually by plotting the predicted risk from the models with the observed risk over time in the cohort (from a Kaplan-Meier curve), for each of a number of risk groups (e.g. low, medium, high) defined by the models. Discrimination will be summarised by Harrell's C-statistic and Royston's D statistic.

Analysis (c): Extension of existing prognostic models by incorporating baseline values of biomarkers

The prognostic biomarkers (and other factors not included in previous models) identified as important from part (a) will be added to the existing prognostic models (e.g UKELD), to investigate their added value. Added value will be measured by apparent improvement in discrimination, calibration and net reclassification as appropriate. Flexible parametric survival models will be used, using the Royston-

Parmar approach that models the baseline hazard using restricted cubic splines^(19,20). Modelling of the baseline hazard in this way will allow absolute risk predictions for individuals. Bootstrapping will be used to adjust for optimism in improvement from using biomarkers identified as important from the same data, and adjustment for measurement error made as appropriate.

Analysis (d): Prognostic value of serial measurements of biomarkers

The prognostic value of serial (repeated) biomarkers measurements over time will be investigated, extending the analyses undertaken in parts (a) and (c). Joint longitudinal and survival models will be fitted, that allow the longitudinal trend in biomarker measurements to be modelled jointly alongside a survival model that examines the prognostic value of that trend. This will allow the estimation of whether a patient's biomarker profile provides prognostic value at a particular time, conditional on the patient being event-free at that time. The independent prognostic value of each serial biomarker profile will be examined by adjusting for baseline prognostic factors identified by part (a), to reveal whether the profile adds value over and above information collected at baseline. A joint model will be fitted within the flexible parametric modelling framework discussed above^(21,22).

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Chief Investigator has obtained approval from the NRES Committee West Midlands – The Black Country Research Ethics Committee. Where applicable the study must be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the SSA approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 CONSENT

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. All participants are free to withdraw at any time from the protocol without giving reasons and without prejudicing further treatment.

8.2.1 Withdrawal of consent

If a patient withdraws consent, samples and clinical data already collected will continue to be used unless the patient specifically requests that they are destroyed in which case they will be asked if their consent withdrawal relates to samples, data or both. A record of withdrawal of consent will be retained and an audit trail will be maintained of all actions.

As all samples in the tissue bank are barcoded and traceable, samples can be traced and recalled. Issued samples where possible will be disposed of if requested by the patient or their family.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

On Enrolment to HCV Research UK, each patient is assigned a unique study number and all data and biological samples are recorded against that number. On Enrolment to this study, each patient will retain their HCV Research UK number and that will be used. The code for study numbers is held securely, locally in each clinic. Data will be entered into the HCV Research UK Clinical Research Database and access to the clinical data held in the database will be restricted. Data entered into the HCV Research UK Clinical Database will be transferred to a Biobank Management System that holds

all information on the samples collected from each patient. Before transfer of the data to the Biobank System, any possible patient identifiers will be removed. Data released to STOP-HCV researchers would originate from the Biobank System.

Site Investigators and their delegates will have password-protected access to the database for the purpose of recording and maintaining data. They will only be able to access the data from patients at their own centre. Access will only be granted once adequate training has been completed.

All blood and urine-derived samples sent to the HCV Research UK Biobank will be anonymised with a unique barcode number. The barcode number will link the sample to the patient study number and their clinical details. All future sub-divisions of the sample will be allocated a new unique barcode. Linking of clinical data to the blood and urine samples will be possible by the use of patient study numbers.

8.4 INDEMNITY

Standard NHS Indemnity applies.

8.5 SPONSOR

Nottingham University Hospitals NHS Trust will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING

This study is part of a programme grant from the Medical Research Council under their Stratified Medicine scheme

GRANT TITLE: Stratified Medicine to Optimise Treatment for Hepatitis C Virus Infection

Grant Ref: MR/K01532X/1 is funding this study.

It falls within workstrand 5 of STOP-HCV – **Biomarkers**

8.7 AUDITS

The study may be subject to inspection and audit by Nottingham University Hospitals under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

The HCV Research UK Biobank may also be subject to inspection and audit by Greater Glasgow & Clyde Health Board. Scotland does not fall within the Human Tissue Act but does comply with its principles.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through the project co-ordinator of STOP-HCV and the project co-ordinator of HCV Research UK, both externally funded. Regular contacts will be made with the sites to ensure ongoing adherence to the protocol, that data are being accurately recorded and provide information and support to the investigator and site team.

Within HCV Research UK, there is a management group (MG) along with a larger inclusive Steering Committee (SC). STOP-HCV has a separate Project Steering Committee (PSC), which will have overall responsibility for overseeing the study. The STOP-HCV PSC consists of representation from each of the project work strands, patient groups (Hepatitis C Trust) and two of its industrial project partners. STOP-HCV has an external advisory board (EAB) to advise the PSC.

10. PUBLICATION POLICY

It is intended that all data generated by STOP-HCV will be published in peer-reviewed journals.

The dissemination/publication of results will be undertaken, without delay, in accordance with the RCUK and MRC's policies on open access and data sharing.

All publications will be submitted to the STOP-HCV PSC for review at least 45 days prior to submission for publication. Any requests for modification, or delay in submission, will be made to the publishing party within this period. Steps will be made to ensure that any delays in submission, for example to allow time for the consideration and preparation of patent applications, will be kept to a minimum and as a general rule shall not exceed 90 days from receipt of the publication by the PSC.

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APPENDIX 1. Signature of investigators

Investigator	Signature	Date of Signature
Prof Graham Foster		
Dr Graeme Alexander		
Dr Stephen Ryder		
Dr Ellie Barnes		
Prof Peter Mills		
Dr David Mutimer		
Dr Ben Stone		
Dr Matthew Cramp		
Dr Stuart McPherson		
Dr Kosh Agarwal		
Dr Mark Thursz		
Dr Fiona Gordon		
Dr Paul Richardson		
Dr Martin Wiselka		
Dr William Rosenberg		
Dr William Rosenberg		
Dr Mark Aldersley		
Dr David Gorard		
Dr Christopher Shorrock		
Dr Andrew Ustianowski		
Dr Richard Aspinall		
Dr Chin Lye Ch'ng		
Dr Daniel Forton		

Dr Aftab Ala		
Dr Jeffrey Butterworth		
Dr Sumita Verma		
Dr Matthew Foxton		
Dr Lynsey Corless		
Dr Moreea Sullerman		
Dr Nick Kennedy		
Dr Andrew Fraser		
Dr Peter Hayes		
Dr Clifford Lean		
Dr Stephen Barclay		
Dr John Dillon		

APPENDIX 2. List of data points to be collected

This list reflects the potential list of data points that are currently planned to be collected. It is not intended to be an exhaustive list and may require altering as new information comes to light. Where possible, laboratory data will be obtained from routine clinical tests at each participating centre, but will need to be specifically requested by the Investigator if not.

- **Clinical data** (see table below) – collected where available as part of patient’s routine care. As patients will be enrolled in the HCV Research UK National Cohort, all clinical data available within the Clinical Research Database (accessed through the HCV Research UK Biobank database), will also be available for use in this current study
- **Biomarker assays** – will include serum markers of fibrosis (e.g. HA and YKL-40) and validated panel marker algorithms (e.g. APRI, FIB-4, ELF etc). Experimental biomarkers as agreed by the STOP-HCV Steering Committee (e.g. Vitamin D, IL-10).
- **Host genetic markers** (baseline only) - including the cirrhosis risk score
- **Viral sequencing** (baseline & annually) - where viraemic samples are available

Date of Enrolment / Consent (first visit only)	
AUDIT Questionnaire	
Haematology	Haemoglobin (Hb) White Cell Count (WBC) Platelets INR
Clinical Chemistry	ALT GGT AST Serum Creatinine Creatinine Clearance Bilirubin (total) Clotting (INR) Serum sodium Serum bilirubin Serum albumin Alphafetoprotein
Virology	HCV PCR Anti-HCV Anti-HIV Anti-HBe

	HBV DNA Hepatitis B e antigen Hepatitis B surface antigen
Imaging (when available as part of routine NHS care)	Transient Elastography (Liver Fibroscan) Liver Ultrasound CT abdomen MRI abdomen Endoscopy
Clinical Findings	Severity of ascites Severity of encephalopathy