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# FibroTest, transient elastography method, and combined FibroTest and transient elastography method for diagnosis of severe hepatic fibrosis and cirrhosis in adults with chronic hepatitis C (Protocol)

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[Diagnostic Test Accuracy Protocol]

# FibroTest, transient elastography method, and combined FibroTest and transient elastography method for diagnosis of severe hepatic fibrosis and cirrhosis in adults with chronic hepatitis C

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## ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To determine the diagnostic accuracy of FibroTest, transient elastography method, combined FibroTest and transient elastography method, no matter the sequence, using liver biopsy as reference standard, for assessment of severe hepatic fibrosis and cirrhosis in adults with chronic hepatitis C without any co-infections such as hepatitis B, HIV, and alcoholic liver disease.

- To compare the accuracy of FibroTest, transient elastography method, combined FibroTest and transient elastography method, for assessment of hepatic fibrosis in adults with chronic hepatitis C.
- To explore heterogeneity analysing the following study factors:
    - different grade of inflammation according to the liver biopsy;
    - different lengths of liver biopsy sample;
    - different number of portal tracts included in a liver biopsy sample;
    - different serum levels of ALT activity.
- different grade of inflammation according to the liver biopsy;
- different lengths of liver biopsy sample;

- different number of portal tracts included in a liver biopsy sample;
- different serum levels of ALT activity.

## BACKGROUND

Hepatic fibrosis is the main consequence of necroinflammation in liver tissue, most often caused by chronic viral hepatitis B or C. When fibrosis advances, it causes bridging between the portal areas or between the portal area and the central vein, and causes the formation of pseudo-lobule (i.e., cirrhosis develops). Hepatitis C virus infection is a major cause of severe illness and death. The economic burden of the disease is high. Worldwide, 130 million to 150 million people are infected with hepatitis C virus (WHO 2014). Every year, another three to four million people acquire the infection, and 350,000 to 500,000 people die from hepatitis C-related liver diseases in a year (WHO 2014). About 15% to 45% of infected people may eliminate the virus without any treatment, but 55% to 85% of chronically infected people will develop a chronic liver disease. About 30% to 50% of people infected with hepatitis C virus will develop hepatic fibrosis without clinical or laboratory symptoms of significant liver disease (Ascione 2007). However, the progress of hepatic fibrosis to cirrhosis in people with chronic hepatitis C is slow (Kenny-Walsh 1999; Wiese 2005). About 15% to 30% of infected people will develop liver cirrhosis within 20 years, and 1% to 5% of those will die from liver cirrhosis decompensation or liver cancer (WHO 2014).

Severe fibrosis and cirrhosis should be regarded with highest priority in terms of treatment in an attempt to prevent the development of further complications from the end stage of chronic hepatitis C virus infection such as oesophageal bleeding, hepatocellular carcinoma, hepatic insufficiency, etc. So far, four classes of direct-acting antiviral agents have become available, claiming their ability to eradicate the hepatitis C virus, irrespective of the stage of fibrosis (Pockros 2015). The direct-acting antiviral agents have also led to new interpretations of the hepatitis C virus ribonucleic acid (RNA) concentration results. The longer the delay in diagnosing and staging correctly the hepatitis C virus, the higher the risk of developing advanced fibrosis and the poorer the survival prognosis (AASLD/IDSA/IAS-USA 2015; EASL 2015; EASL-ALEH 2015).

The natural course of chronic hepatitis C virus infection depends on age at time of infection; sex; degree of inflammation presented; co-infection with human immunodeficiency virus (HIV) or hepatitis B virus infection; and co-morbid conditions such as immunosuppression, insulin resistance, non-alcoholic steatohepatitis, haemochromatosis, and schistosomiasis (Chen 2006; Ascione 2007). Approximately 80% of people with hepatitis C infection do not exhibit symptoms, and the stage of liver disease remains unknown (Marcellin 1999; WHO 2014).

Liver biopsy is considered reference standard for diagnosing severe and advanced hepatic fibrosis in people with chronic hepatitis C, who are expected to have higher benefit from treatment (Castera 2011).

Liver biopsy provides information on the degree of inflammation and the amount of established fibrosis. Liver biopsy is obtained in three ways: percutaneous, transjugular or transfemoral, and laparoscopic (Kuntz 2008). Specimens are obtained either with a core aspiration needle (Menghini, Jamshidi, Klatskin style) or sheathed cutting needle (Tru-Cut style) that is at least 16-gauge in calibre. Specimens of liver tissue with a mean length of at least 15 mm and at least seven portal tracts are among the factors that can provide reliable morphological staging of hepatic fibrosis and grading of inflammation (Rockey 2009). As liver biopsy is painful, and in some cases it may lead to severe complications, people are not willing to undergo it (Castéra 2005). The accuracy of liver biopsy may also be affected by sampling errors and intra- and interobserver variability (Bedossa 2003). Various non-invasive methods have been suggested and used to detect or confirm the diagnosis of chronic hepatitis C infection (WHO 2014; EASL-ALEH 2015).

Non-invasive methods use two different approaches for diagnosing stages of liver fibrosis in chronic liver disease: one based on the quantification of biomarkers in serum blood samples (e.g., FibroTest®, Forns® Index, APRI (aspartate aminotransferase (AST) to platelet ratio index), etc.) and the other based on the measurement of liver stiffness (e.g., transient elastography, ARFI (acoustic radiation force impulse), magnetic resonance elastography (MRE), etc.) (EASL-ALEH 2015). Guidelines suggest that blood tests in combination with liver stiffness measurements may improve the diagnostic accuracy when stage of hepatic fibrosis is assessed, resulting in a significant reduction in the number of liver biopsies and in a better selection of patients to be investigated with the biopsy procedure (Castera 2011; EASL 2011).

### Target condition being diagnosed

Severe hepatic fibrosis and cirrhosis in adults with chronic hepatitis C. The diagnosis of chronic hepatitis C includes detection of both hepatitis C virus antibodies and hepatitis C virus RNA (lower limit of detection less than 15 IU/mL) in the presence of biological or histological signs of chronic hepatitis (either by elevated

aminotransferases or by histological changes of chronic hepatitis C) (WHO 2014; EASL 2015). We chose to study a homogeneous group of people as pathogenesis of liver injury may be influenced by different aetiological factors, co-infected patients may require different treatments, and the time for progression of fibrosis into cirrhosis.

There are a number of staging systems for evaluating hepatic fibrosis in people with chronic hepatitis C. METAVIR is the most widely used scoring system for interpretation of liver biopsy results based on the stage of fibrosis where F0 indicates no fibrosis, F1 indicates portal fibrous expansion, F2 indicates thin fibrous septa emanating from portal triads, F3 indicates fibrous septa bridging portal triads and central veins, and F4 indicates cirrhosis (Table 1). Hepatic fibrosis could be considered clinically significant if defined as F2 or worse, using METAVIR score (Franciscus 2007). Hepatic fibrosis could be considered clinically severe if defined as F3 or worse, using METAVIR score (F3 and F4), which is the subject of our review. In Table 1, we have also included other widely used systems for classification of hepatic fibrosis in people with chronic hepatitis C (Knodell 1981; Desmet 1994; Ishak 1995; Brunt 1999; Kleiner 2005), as liver pathologists have reached no universal consensus on the standardisation of scoring systems.

## Index test(s)

The non-invasive FibroTest and transient elastography are widely used tests for assessment of hepatic fibrosis in people with chronic hepatitis C (Sandrin 2003; Nahon 2008).

FibroTest (i.e., BioPredictive®, Paris, France and registered as FibroSure$^{TM}$ in the USA), is a test for determining the stage of hepatic fibrosis in people with chronic hepatitis C. The test uses six serum markers for identification of the existence of fibrosis in the liver tissue; alpha-2-macroglobulin, haptoglobin, gamma-glutamyl transpeptidase (GGT), total bilirubin, apolipoprotein A1, and alanine aminotransferase (ALT). In addition, it takes into account the age and sex of the people when defining the stage of hepatic fibrosis (Shaheen 2007; Gressner 2009). It can be performed in ambulatory conditions. However, there are also disadvantages. It is non-liver specific, is unable to discriminate between intermediate stages of fibrosis, has limited availability due to proprietary rights, and can be influenced by haemolysis, Gilbert's syndrome, or systematic inflammation (EASL-ALEH 2015). During its evaluation, the FibroTest was assessed on control liver biopsies using the METAVIR scoring system for substantial fibrosis of F2 or worse and activity score of the biopsy specimens (Imbert-Bismut 2001).

Transient elastography (i.e., FibroScan® equipment, Echosens, Paris, France) is a mechanical test designed to measure liver stiffness in people with chronic hepatitis C virus. A probe is put on the skin surface overlying the liver. After pressing the button on the probe, a pulse wave is transmitted across the liver parenchyma. After a short interval, a second ultrasound wave is transmitted. The difference between the velocity of the two waves in the liver parenchyma is calculated using the Doppler technique (Sandrin 2003; Nahon 2008). As it is known from physical principles, the velocity of the pulse wave increases with the stiffness of the liver parenchyma.

Liver stiffness is expressed as a median value in kiloPascals (kPa). A pre-defined cut-off of 8.00 kPa is predictive of severe hepatic fibrosis in chronic hepatitis C that is F3 or worse by the METAVIR scoring system (Mueller 2010). The transient elastography method is simple, highly reproducible, and allows examination of at least 100 times larger volume of liver tissue than a biopsy sample ( de Lédinghen 2008). Extrahepatic cholestasis, food intake, and excessive alcohol use are among the factors influencing transient elastography measurements (EASL-ALEH 2015).

## Clinical pathway

The first-line diagnostic test for hepatitis C virus infection is measurement of hepatitis C viral antibodies. People with detectable hepatitis C viral antibodies, before undergoing anti-viral therapy, should have hepatitis C virus RNA levels (expressed in IU/mL) detected and quantified by molecular tests with a lower limit of detection of 15 IU/mL or less based on real-time reverse transcription polymerase chain reaction methods (EASL 2015). The hepatitis C virus genotype, levels of transaminases, and liver synthetic function should be assessed before start of treatment in order to determine the appropriate treatment regimens and duration. The severity of fibrosis should be assessed along with the presence of any co-morbid conditions possibly influencing the progression of the liver disease. Following EASL 2015, the stage of fibrosis using non-invasive methods should be assessed after the diagnosis of chronic hepatitis C and before start of its treatment. However, the stage of fibrosis using liver biopsy should be reserved for people with advanced stage fibrosis and with suspected additional aetiologies of liver injury or in case of discordance in the results obtained through any of the non-invasive methods (EASL 2015). Monitoring of hepatitis C virus viral load is performed to guide treatment duration, that is, to continue on therapy, or to determine whether to stop therapy.

Transient elastography, FibroTest, or their combination are recommended as non-invasive tests for diagnosis of severe fibrosis or cirrhosis in people with chronic hepatitis C (EASL-ALEH 2015).

## Prior test(s)

Hepatitis C virus antibody, hepatitis C virus RNA test, liver function tests (ALT, AST), FibroTest alone, transient elastography method alone, and combined FibroTest and transient elastography method could potentially be some of the first tests that people undergo after being diagnosed with chronic hepatitis C.

### Role of index test(s)

FibroTest, transient elastography method, and combined FibroTest and transient elastography method are non-invasive methods for the assessment of severe hepatic fibrosis and cirrhosis that could be used as triage or replacement tests of liver biopsy. We have not taken the cost-effectiveness into account when defining the role of the listed index tests, as this is not possible in this review.

### Alternative test(s)

There are different alternative non-invasive methods for measuring hepatic fibrosis. Based on their principle of defining fibrosis in people with hepatitis C virus, tests are grouped as follows:

- *Based on biochemical variables*: ALT and AST ratio, prothrombin time, hyaluronic acid, platelets (aspartate aminotransferase/platelet ratio index (APRI), Forn's index - combines age, GGT, cholesterol, and platelet count). The tests are based on recordings of liver biochemical variables. All the tests are used as surrogate markers for fibrosis, are inexpensive laboratory tests, performed routinely in people with chronic liver disease (Wai 2003; Degos 2010; EASL 2011).
- *Ultrasound-based modalities:* ARFI, and supersonic shear imaging (SSI) (Ersoz 1999; Liu 2007).
- *Magnetic resonance* is another imaging method which includes unenhanced magnetic resonance imaging (MRI), MRE, MRI with diffusion-weighted imaging (DWI) and magnetic resonance spectroscopy.
- *Breath tests:* methacetin breath test and C-aminopyrine breath test are markers of liver fibrosis in the setting of hepatitis C virus (Braden 2005; Lalazar 2008).
- *Algorithms:* several algorithms exist that combine blood tests or FibroScan in order to improve the accuracy of detecting liver fibrosis and cirrhosis (Sebastiani 2009; Castéra 2010; Sebastiani 2012).

A few of the mentioned alternative tests are currently being studied in Cochrane diagnostic test accuracy reviews (Kalafateli 2015; Kalafateli 2016). However, their role and place in the clinical pathway in terms of diagnosing people with chronic hepatitis C still needs to be established.

### Rationale

Identifying people with cirrhosis or people with advanced fibrosis is of particular importance as their prognosis and their response to treatment differ (EASL 2011; EASL 2015). Liver biopsy is still regarded as the reference standard for assessing fibrosis in people with chronic hepatitis C. The advantage of liver biopsy for staging fibrosis in chronic hepatitis C is that this test not only fulfils its purpose, but it may also give diagnostic information for concurrent liver diseases such as alcoholic or non-alcoholic steatohepatitis, autoimmune liver disease, etc. (Poulsen 1979; Ismail 2011). Using liver biopsy for diagnosis of chronic hepatitis C is limited by sampling error, different levels of experience of the morphologists, invasiveness of procedure, and risk of both serious and non-serious complications (Seeff 2010; Castera 2011).
2015 clinical recommendations refer to the use of non-invasive serum markers (FibroTest, APRI, FIB4, etc.) and transient elastography for detection of hepatic fibrosis (EASL-ALEH 2015). It is suggested that the combined use of different non-invasive methods would possibly reduce the necessity of liver biopsy (EASL 2011). Non-invasive methods could also be used in the follow-up of people infected with chronic hepatitis C (Castera 2011). However, the optimal algorithm for use of non-invasive methods still needs to be established (Castera 2011).
We found no diagnostic test accuracy review prepared with Cochrane methodology to determine the diagnostic test accuracy of FibroTest, transient elastography method, combined FibroTest and transient elastography method, no matter the sequence, using liver biopsy as reference standard, for assessment of severe hepatic fibrosis and cirrhosis in adults with chronic hepatitis C.

## OBJECTIVES

To determine the diagnostic accuracy of FibroTest, transient elastography method, combined FibroTest and transient elastography method, no matter the sequence, using liver biopsy as reference standard, for assessment of severe hepatic fibrosis and cirrhosis in adults with chronic hepatitis C without any co-infections such as hepatitis B, HIV, and alcoholic liver disease.

### Secondary objectives

- To compare the accuracy of FibroTest, transient elastography method, combined FibroTest and transient elastography method, for assessment of hepatic fibrosis in adults with chronic hepatitis C.
- To explore heterogeneity analysing the following study factors:
    - different grade of inflammation according to the liver biopsy;
    - different lengths of liver biopsy sample;
    - different number of portal tracts included in a liver biopsy sample;
    - different serum levels of ALT activity.

## METHODS

### Criteria for considering studies for this review

### Types of studies

We will include cross-sectional cohort studies, case-control studies, and randomised comparisons of test accuracy that compare FibroTest, transient elastography method, and combined FibroTest and transient elastography method to the reference standard.
We will include studies that are published in any language as full paper articles or in the form of abstracts, published in conference proceedings, or presented as posters.

### Participants

We will include men or women aged above 18 years, with chronic hepatitis C virus infection, in any setting (i.e., hospitalised or ambulatory participants). The participants could have had any stage of fibrosis, including cirrhosis.
We will not consider studies with people with recurrent hepatitis C infection who have received a liver transplant or studies with participants with concomitant liver diseases.

### Index tests

FibroTest alone, transient elastography method alone, and a combination of the FibroTest and the transient elastography method.

### Target conditions

The presence of hepatic fibrosis in people with chronic hepatitis C. Based on the METAVIR histopathological score for interpreting liver biopsy, there are five stages of hepatic fibrosis: no fibrosis - F0; mild fibrosis - F1; significant fibrosis - F2 or worse; severe fibrosis - F3 or worse; cirrhosis - F4 (Table 1).
We will dichotomise the hepatic fibrosis estimated by the METAVIR score as follows:

- we will consider people with METAVIR score of F3 or worse 'diseased' and people with METAVIR score of F0 plus F1 plus F2 'non-diseased';
- we will consider people with METAVIR score of F4 'diseased' and people with METAVIR score of F0 plus F1 plus F2 plus F3 'non-diseased'.

### Reference standards

Liver biopsy.

## Search methods for identification of studies

We will combine electronic searches with reading references of identified studies of possible interest.

### Electronic searches

We will search The Cochrane Hepato-Biliary Group Controlled Trials Register (Gluud 2016), The Cochrane Hepato-Biliary Group Diagnostic Test Accuracy Studies Register (Gluud 2016), the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (OvidSP), EMBASE (OvidSP), and the Science Citation Index Expanded (Royle 2003; de Vet 2008).
We will also read references of the retrieved citations for additional studies of interest. We will include abstracts only if these are identified through the electronic or manual searches.
Appendix 1 shows the preliminary search strategies for the different databases with the expected time spans for the searches. The given search strategies may be improved at the review preparation phase.

## Data collection and analysis

### Selection of studies

Two review authors (CP and Ekaterina Liusina (EL) (to join at the review stage)) will independently identify studies for possible inclusion in the review by reading the abstracts of the search results. Authors will exclude references with a study design not fulfilling the inclusion criteria of the review protocol. We will retrieve publications in full for a second selection of relevant studies. We will identify multiple publications for inclusion and read through for extraction of data, not provided in the earliest publication.
The studies that we will include must evaluate FibroTest, transient elastography method, or combined FibroTest and transient elastography method in the diagnosis of hepatic fibrosis stage using liver biopsy as the reference standard. In order to provide data for our analyses, the studies must provide data that will enable us to calculate the true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) diagnostic values of transient elastography and FibroTest in diagnosing the stages of hepatic fibrosis, based on cut-off points for liver stiffness as described by the authors of the identified studies.
We plan to contact authors of studies in which data are missing, either by e-mail or letter. If we receive no reply, we will list the study under 'Excluded studies'.
We will put no maximum limit on the time interval of investigation with liver biopsy and transient elastography and FibroTest alone or in combination when we select the studies for inclusion in this review. However, the accepted time interval is advised to be no more than six months.

### Data extraction and management

Three review authors (CP, EL, GC) will independently extract data, using a data extraction sheet.

We will collect data on study origin, year of publication, participants' epidemiological and laboratory characteristics, technical failures in undertaking liver biopsy and transient elastography, cut-off values of the index tests, stage of hepatic fibrosis estimated by histological score, and information related to the QUADAS-2 items for evaluation of the methodological quality (Whiting 2011).

We will also extract all necessary data to calculate TP, RP, TN, and FN values, using the reference standard of liver biopsy. If information on any of the TP, FP, FN, and TN diagnostic test values or results are missing, we will attempt to derive them using other information that the study may provide. We will also attempt to obtain missing information from authors of the included studies.

If liver biopsy samples are reported with any of the five semi-quantitative scores (i.e., METAVIR (Franciscus 2007), Knodell (Franciscus 2007), Ishak (Franciscus 2007), Kleiner (Kleiner 2005), Scheuer (Regev 2002)), we will use a conversion grid for hepatic fibrosis staging adapted after Goodman 2007 (Table 1).

For grading steatosis, as zone 1 steatosis is a common distribution in chronic hepatitis C, we will use the Nonalcoholic Steatohepatitis Clinical Research Network scoring system (Kleiner 2005; Kleiner 2012) (Table 2).

## Assessment of methodological quality

Design flaws in test accuracy studies can produce biased results (Lijmer 1999; Whiting 2004; Rutjes 2006). In addition, evaluation of study results is quite often impossible due to incomplete reporting (Smidt 2005).

To limit the influence of different biases, three review authors (CP, GC, EL), in pairs or independently of one another, will assess the risk of bias of the included diagnostic test accuracy studies, using QUADAS-2 domains (Whiting 2011). A fourth review author (ET) will act as an arbitrator in case of disagreements between the authors assessing the risk of bias of the studies. We will contact study authors if information on methodology is lacking in order to assess correctly the risk of bias of the studies.

Appendix 2 shows the adopted items that will we will use to address the participant spectrum, index test, target condition, reference standard, and flow and timing, and which answers would also reflect the general quality of the included studies.

QUADAS-2 is not used to generate a summary 'quality score' because of the well-known problems associated with such scores (Jüni 1999; Whiting 2005). If a study is judged as 'low' on all domains relating to bias or applicability, then it is appropriate to have an overall judgement of 'low risk of bias' or 'low concern regarding applicability' for that study. If a study is judged as 'high' or 'unclear' on one or more domains, then it may be judged 'at risk of bias' or as having 'concerns regarding applicability'.

We will use tabular and graphical displays to summarise QUADAS-2 assessments.

## Statistical analysis and data synthesis

We will carry out the analyses following Chapter 10 (Analysing and Presenting Results), as recommended in the *Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy* (Macaskill 2010). The analyses will be performed using Review Manager 5 (RevMan 2014).

### Index tests

We will build 2 × 2 tables of the FibroTest and transient elastography performance, alone or in combination (TP, TN, FP, FN), for each primary study and for all of the pre-defined target conditions (mild hepatic fibrosis, significant hepatic fibrosis, severe hepatic fibrosis, and cirrhosis). We will estimate sensitivity, specificity, positive and negative likelihood ratios (LR+ and LR-) with their 95% confidence intervals (CI). First, we will perform a graphical descriptive analysis of the included studies: we will report forest plots (sensitivity and specificity separately, with their 95% CIs) and we will provide a graphical representation of the studies in the receiver operating characteristic (ROC) space (sensitivity against 1 - specificity). Second, where appropriate, we will perform meta-analyses. If the primary studies report accuracy estimates of transient elastography or FibroTest using different cut-off values, we will use the hierarchical summary ROC model (HSROC) to estimate a summary ROC (SROC) curve (Macaskill 2010). If studies have reported a common cut-off value, we will use the bivariate model (Macaskill 2010) to estimate summary sensitivity and specificity.

For the studies reporting the results of both the index tests (FibroTest plus transient elastography) on the same participants, we will build the 2 × 2 tables for the combination of the two index tests. We will consider as test negative all the participants negative to both the FibroTest and transient elastography; we will consider as test positive all the participants positive to at least one of the two tests. We will use the positivity cut-off values for the FibroTest and transient elastography used in the primary studies. If those cut-off values are the same across studies, we will use the bivariate model and we will provide the estimated summary sensitivity and specificity. In presence of heterogeneous cut-off values, we will perform the meta-analysis using the HSROC model (Macaskill 2010).

For primary studies that report accuracy results for more than one cut-off point, we will report sensitivities and specificities for all of the cut-off points, but we will use a single cut-off point for each study in HSROC (or bivariate model) analysis. We plan to base the choice of the cut-off value on the most commonly reported cut-off value for each stage of hepatic fibrosis depending on the availability of data.

### Comparison of the index tests

As descriptive preliminary analyses, we will plot studies in the ROC space, differentiating the three index tests as suitable, using different colours or symbols. We plan to perform the formal

comparative meta-analysis in an indirect way by adding the index tests as co-variates to the bivariate or HSROC model. For direct comparisons, we will use the methods as described in the *Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy* (Macaskill 2010)

One review author (GC) will perform all statistical analyses using SAS statistical software, release 9.4 (SAS Institute Inc., Cary, NC, USA).

### Investigations of heterogeneity

To investigate sources of heterogeneity, we plan to add co-variates (co-factors) to the bivariate or HSROC model as follows:

- liver biopsy as the reference standard: different grade of inflammation according to the liver biopsy (below two grades compared to two or greater grades of activity);
- different lengths of liver biopsy sample (less than 15 mm compared to 15 mm or more);
- different number of portal tracts included in a liver biopsy sample (fewer than seven compared to seven or more);
- different body mass indices (below 25 kg/m$^2$ compared to 25 kg/m$^2$ or more) (WHO 2014) (only relevant for the analyses of studies in which transient elastography method was used);
- serum levels of ALT activity (up to the upper limit of normal 40 IU/L compared to more than 40 IU/L) (only relevant for the analyses of studies in which transient elastography method was used).

### Sensitivity analyses

We will perform sensitivity analyses by considering only cross-sectional design studies (i.e., excluding case-control studies), and studies assessed as low risk of bias (Appendix 2).

### Assessment of reporting bias

We will not assess reporting bias as there is lack of sensitive tests, suitable for investigation of reporting bias.

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* *Indicates the major publication for the study*

## ADDITIONAL TABLES

**Table 1. Conversion grid for the stages* of hepatic fibrosis (adapted after Goodman 2007).**

| **METAVIR** *Stage of estimated fibrosis* | **Knodell** *Stage of estimated fibrosis* | **Ishak** *Stage of estimated fibrosis* | **Kleiner** *Stage of estimated fibrosis* | **Scheuer** *Stage of estimated fibrosis* |
|---|---|---|---|---|
| F0 | F0 | F0 | F0 | F0 |
| F1 | F1 | F1 | F1 | F1 |
| F1 | F1 | F2 | F1 | F1 |
| F2 | F3 | F3 | F2 | F2 |
| F3 | F3 | F4 | F2 | F3 |
| F4 | F4 | F5 | F3 | F4 |
| F4 | F4 | F6 | F4 | F4 |

F: stage of hepatic fibrosis.
Stage* is an assessment of fibrosis location (i.e., scar). It is potentially irreversible. Stage describes only parenchymal location of collagen and matrix deposition, and vascular/architectural alterations, but not absolute quantity (Kleiner 2012).

**Table 2. Nonalcoholic Steatohepatitis Clinical Research Network scoring system for grade* of hepatic steatosis**

| *Evaluation of parenchymal involvement by steatosis* | *Steatosis grade* |
|---|---|
| < 5% | 0 |
| 5% to 33% | 1 |

**Table 2. Nonalcoholic Steatohepatitis Clinical Research Network scoring system for grade* of hepatic steatosis** (*Continued*)

| | |
|---|---|
| 34% to 66% | 2 |
| > 66% | 3 |

*Grade is a global measure of hepatocellular and necroinflammatory injury; it describes amount and reflects features that are potentially reversible (Kleiner 2012).

# APPENDICES

## Appendix 1. Search strategies

**FibroScan, ultrasound impedance, magnetic resonance imaging (MRI) impedance**

| Database | Time span | Search strategy |
|---|---|---|
| The Cochrane Hepato-Biliary Group Controlled Trials Register | Date will be given at review stage. | ((transient elastograph* or fibroscan*) OR (fibrotest* or fibrosure*)) AND (hepatic or liver) and (fibrosis or cirrhosis) AND liver biops* |
| The Cochrane Hepato-Biliary Group Diagnostic Test Accuracy Studies Register | Date will be given at review stage. | ((transient elastograph* or fibroscan*) OR (fibrotest* or fibrosure*)) AND (hepatic or liver) and (fibrosis or cirrhosis) AND liver biops* |
| Cochrane Central Register of Controlled Trials (CENTRAL) | Latest issue. | #1 MeSH descriptor: [Elasticity Imaging Techniques] explode all trees<br>#2 (transient elastograph* or fibroscan*)<br>#3 (fibrotest* or fibrosure*)<br>#4 #1 or #2 or #3<br>#5 MeSH descriptor: [Liver Cirrhosis] explode all trees<br>#6 (hepatic or liver) and (fibrosis or cirrhosis)<br>#7 #5 or #6<br>#8 MeSH descriptor: [Biopsy, Needle] explode all trees<br>#9 liver biops*<br>#10 #8 or #9<br>#11 #4 and #7 and #10 |
| MEDLINE (OvidSP) | 1950 to the date of search. | 1. exp Elasticity Imaging Techniques/<br>2. (transient elastograph* or fibroscan*).mp. [mp=title, abstract, original title, name of substance |

(*Continued*)

| | | |
|---|---|---|
| | | word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]<br>3. (fibrotest* or fibrosure*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]<br>4. 1 or 2 or 3<br>5. exp Liver Cirrhosis/<br>6. ((hepatic or liver) and (fibrosis or cirrhosis)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]<br>7. 5 or 6<br>8. exp Biopsy, Needle/<br>9. liver biops*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]<br>10. 8 or 9<br>11. exp Hepatitis C, Chronic/<br>12. (chronic hepatitis c or hep C).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]<br>13. 11 or 12<br>14. 4 and 7 and 10 and 13 |
| EMBASE (OvidSP) | 1980 to the date of search. | 1. exp elastography/<br>2. (transient elastograph* or fibroscan*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]<br>3. (fibrotest* or fibrosure*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]<br>4. 1 or 2 or 3<br>5. exp liver cirrhosis/<br>6. ((hepatic or liver) and (fibrosis or cirrhosis)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, |

(*Continued*)

| | | |
|---|---|---|
| | | keyword]<br>7. 5 or 6<br>8. exp liver biopsy/<br>9. liver biops*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]<br>10. 8 or 9<br>11. exp hepatitis C/<br>12. (chronic hepatitis c or hep C).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]<br>13. 11 or 12<br>14. 4 and 7 and 10 and 13 |
| Science Citation Index Expanded | 1900 to the date of search. | #7 879 #6 AND #5 AND #4 AND #3<br>#6 48,967 TS=(chronic hepatitis c or hep C)<br>#5 28,567 TS=(liver biops*)<br>#4 83,012 TS=((hepatic or liver) and (fibrosis or cirrhosis))<br>#3 3,418 #2 OR #1<br>#2 613 TS=(fibrotest* or fibrosure*)<br>#1 3,091 TS=(transient elastograph* or fibroscan*) |

## Appendix 2. QUADAS-2

| Domain | Participant selection | Index test | Reference standard | Flow and timing |
|---|---|---|---|---|
| **Description** | **Describe methods of participant selection: describe included participants (prior testing, presentation, intended use of index test, and setting):**<br>The studies that fulfil the inclusion criteria of this review should have included participants of any sex and ethnic origin, > 18 years old, and diagnosed with chronic hepatitis C. The participants could have been | **Describe the index test and how it was conducted and interpreted:**<br>FibroTest, transient elastography method, and combined FibroTest and transient elastography method - no matter the sequence of application - for diagnosing fibrosis and cirrhosis, conducted either before or after liver biopsy | **Describe the reference standard and how it was conducted and interpreted:**<br>The morphological interpretation of the liver biopsy samples is reported with semi-quantitative scores such as METAVIR, Knodell, Ishak, Kleiner, Scheuer, or Brunt (see Table 1). | **Describe any people who did not receive the index test(s) or reference standard (or both) or who were excluded from the 2 × 2 table (refer to flow diagram) : describe the time interval and any interventions between index test(s) and reference standard:**<br>We will exclude participants if the time interval between diagnostic liver |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| | hospitalised or managed as outpatients. The diagnosis of chronic hepatitis C in the study participants had to be established based on the detection of both anti-hepatitis C virus antibodies and hepatitis C virus ribonucleic acid in the presence of biological or histological signs of chronic hepatitis<br>The participants could have had any stage of fibrosis, including cirrhosis<br>We will not consider participants who had received a liver transplant and with recurrent hepatitis C infection as well as participants with aetiologies of liver diseases other than chronic hepatitis C virus infection<br>To ascertain the diagnosis of chronic hepatitis C and study the presence of cirrhosis, any or both of our index tests (FibroTest, transient elastography method, and combined FibroTest and transient elastography method) as well as the reference standard had to be performed, irrespective of the sequence | | | biopsy and any of the review index tests is > 6 months<br>We will exclude participants from studies who underwent combined FibroTest and transient elastography method if data from both tests were missing or if data on liver biopsy were missing |
| **Signalling questions: yes/no/unclear** | **Was a consecutive or random sample of participants enrolled?**<br>Yes: all consecutive participants or random sample of people diagnosed with chronic hepatitis C were enrolled in the study | **Were the index test results interpreted without knowledge of the results of the reference standard?**<br>Yes: FibroTest, transient elastography<br>method, and combined FibroTest and transient | **Is the reference standard likely to classify the target condition correctly?**<br>Yes: if participants have undergone liver biopsy and the liver tissue specimen was deemed adequate for confident his- | **Was there an appropriate interval between index test(s) and reference standard?**<br>Yes: the interval between the FibroTest, transient elastography method, and combined FibroTest and transient elastogra- |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| | No: selected participants were not included.<br>Unclear: insufficient data were reported to permit a judgement | elastography method results were interpreted without knowledge of the results of the liver biopsy<br>No: FibroTest, transient elastography method, and combined FibroTest and transient elastography method results were interpreted with knowledge of the results of the liver biopsy<br>Unclear: insufficient data were reported to permit a judgement | tological assessment<br>No: the liver tissue specimen was not deemed adequate for confident histological assessment<br>Unclear: insufficient data were reported to permit a judgement | phy method and liver biopsy was ≤ 6 months<br>No: the interval between the FibroTest, transient elastography method, and combined FibroTest and transient elastography method and liver biopsy was > 6 months<br>Unclear: insufficient data were reported to permit a judgement |
| | **Was a patient-control design avoided?**<br>Yes: patient-control design was avoided.<br>No: patient-control design was not avoided.<br>Unclear: insufficient information was reported to permit a judgement | **If a threshold was used, was it pre-specified?**<br>Yes.<br>No.<br>Unclear: it was not reported or not clearly described. | **Were the reference standard results interpreted without knowledge of the results of the index test?**<br>Yes: liver biopsy results were interpreted without knowledge of the results of the ultrasonography test<br>No: liver biopsy results | **Did all participants receive the reference standard?**<br>Yes: all participants underwent the reference standard, liver biopsy<br>No: not all participants underwent liver biopsy.<br>Unclear: insufficient data were reported to permit a judgement |
| | **Did the study avoid inappropriate exclusions?**<br>Yes: the study avoided inappropriate exclusions (e.g., difficult to diagnose participants, failure at liver biopsy, failure on ultrasonography)<br>No: the study excluded participants inappropriately. | | | **Did all participants receive the same reference standard?**<br>Yes: all participants received the same reference standard, i.e., liver biopsy<br>No: not all participants received the same reference standard, i.e., liver biopsy<br>Unclear: insuffi- |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| | Unclear: insufficient data were reported to permit a judgement | | | cient data were reported to permit a judgement<br><br>**Were all participants included in the analysis?**<br>Yes:<br>all participants meeting the selection criteria (selected participants) were included in the analysis, or data on all the selected participants were available so that a 2 × 2 table including all selected participants could be constructed<br>No: not all participants meeting the selection criteria were included in the analysis or the 2 × 2 table could not be constructed using data on all selected participants<br>Unclear: insufficient data were reported to permit a judgement |
| **Risk of bias: high/low/ unclear** | **Could the selection of participants have introduced bias?**<br>High risk of bias: if the answer to any of the 3 signalling questions on the participant selection was 'no'<br>Low risk of bias: if the answers to the 3 signalling questions on the participant selection was 'yes'<br>Unclear risk of bias: if the answers to the 3 signalling questions on the participant selection was either 'unclear' or any combination of 'unclear' | **Could the conduct or interpretation of the index test have introduced bias?**<br>High risk of bias: if the answer to any of the 2 signalling questions on the conduct or interpretation of the index test was 'no'<br>Low risk of bias: if the answer to the 2 signalling questions on the conduct or interpretation of the index test was 'yes'<br>Unclear risk of bias: if the answers to the 2 signalling questions on the conduct or interpre- | **Could the reference standard, its conduct, or its interpretation have introduced bias?**<br>High risk of bias: if the answer to any of the 2 signalling questions on the reference standard, its conduct, or its interpretation was 'no'<br>Low risk of bias: if the answer to the 2 signalling questions on the reference standard, its conduct, or its interpretation was 'yes'<br>Unclear risk of bias: if the answers to the 2 | **Could the participant flow have introduced bias?**<br>High risk of bias: if the answer to any of the 4 signalling questions on flow and timing was 'no'<br>Low risk of bias: if the answer to the 4 signalling questions on flow and timing was 'yes'<br>Unclear risk of bias: if the answers to the 4 signalling questions on flow and timing was either 'unclear' or any combination of 'unclear' with 'yes' |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| | with 'yes' | tation of the index test was either 'unclear' or any combination of 'unclear' with 'yes' | signalling questions on the reference standard, its conduct, or its interpretation was either 'unclear' or any combination of 'unclear' with 'yes' | |
| **Concerns regarding applicability: high/low/unclear** | **Are there concerns that the included participants do not match the review question?**<br>High concern: there was high concern that the included participants did not match the review question<br>Low concern: there was low concern that the included participants did not match the review question<br>Unclear concern: if it was unclear. | **Are there concerns that the index test, its conduct, or interpretation differ from the review question?**<br>High concern: there was high<br>concern that the conduct or interpretation of the FibroTest, transient elastography method, and combined FibroTest and transient elastography method differed from the way it is likely to be used in clinical practice<br>Low concern: there was low concern that the conduct or interpretation of the conduct or interpretation of the FibroTest, transient elastography method, and combined FibroTest and transient elastography method differed from the way it is likely to be used in clinical practice<br>Unclear concern: if it was unclear. | **Are there concerns that the target condition as defined by the reference standard did not match the review question?**<br>High concern: all participants did not undergo liver biopsy for cirrhosis<br>Low concern: all participants underwent liver biopsy for cirrhosis<br>If it was unclear. | -- |

## CONTRIBUTIONS OF AUTHORS

Chavdar S Pavlov: generation of idea, drafted and revised the protocol, and is a guarantor of the protocol.

Giovanni Casazza: drafted and revised the protocol.

Dimitrinka Nikolova: drafted and revised the protocol.

Emmanuel Tsochatzis: revised the protocol.

Vladimir T Ivashkin: revised the protocol.

Christian Gluud: drafted and revised the protocol.

All authors agreed on the final version of the review protocol.

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