2016

The ability of Hepascore to predict liver fibrosis in chronic liver disease: A meta-analysis

Yi Huang

Leon A. Adams

John Joseph

Max Bulsara
University of Notre Dame Australia, max.bulsara@nd.edu.au

Gary P. Jeffrey

This article was originally published as:
Huang, Y., Adams, L. A., Joseph, J., Bulsara, M., & Jeffrey, G. P. (2016). The ability of Hepascore to predict liver fibrosis in chronic liver disease: A meta-analysis. *Liver International, Early View (Online First)*.

Original article available here:
http://onlinelibrary.wiley.com/doi/10.1111/liv.13116/abstract

Received Date : 10-Feb-2016

Revised Date : 22-Feb-2016

Accepted Date : 08-Mar-2016

Article type : Original Articles

The Ability of Hepascore to Predict Liver Fibrosis in Chronic Liver Disease: a Meta-analysis

Yi Huang[1,2], Leon A Adams[1,2], John Joseph[3], Max Bulsara[4], Gary P Jeffrey[1,2]

1. School of Medicine and Pharmacology, University of Western Australia, Perth, Australia. 2. Department of Gastroenterology and Hepatology, Sir Charles Gairdner Hospital, Perth, Australia. 3. Department of Biochemistry, PathWest Laboratory Medicine, QEII Medical Centre, Perth, Australia. 4. Institute of Health Research, University of Notre Dame, Perth, Australia.

Contact information: Prof Gary P Jeffrey MB BS, MD, FRACP, FRCP

Address: School of Medicine and Pharmacology, University of Western Australia, 5th Floor, Harry Perkins Institute of Medical Research, 6 Verdun Street, Nedlands, 6009.

Email: gary.jeffrey@uwa.edu.au.

Phone: +61 8 6151 0917

List of Abbreviations: HCV: chronic hepatitis C virus; HBV: chronic hepatitis B; ALD: alcoholic liver disease; NAFLD: non-alcoholic fatty liver disease; AUROC: area under ROC curve; CI: confidence interval; QUADAS: quality assessment of diagnostic accuracy studies; DANA: difference between the mean fibrosis stages in advanced fibrosis and non-advanced fibrosis groups; PPV: positive predictive value; NPV: negative predictive value; HIV: human immunodeficiency virus;

Conflict of interest: The University of Western Australia (employer of YH, LAA and GPJ) hold the patent for Hepascore and have a licencing agreement with Quest Diagnostics.

Financial support: Nil

ABSTRACT

Background & Aims: Hepascore is a serum model that was developed to assess the severity of liver fibrosis. It has been well validated in common causes of chronic liver disease. This study performed a meta-analysis to evaluate the pooled diagnostic performance of Hepascore and to compare it for different aetiologies of chronic liver disease.

Methods: Two reviewers searched electronic databases from October 2005 to September 2015 for studies that evaluated the diagnostic performance of Hepascore for liver fibrosis in chronic liver disease.

Results: 21 studies were included. The AUROC was adjusted according to the distribution of fibrosis stages. The mean adjusted AUROC was 0.83 (95%CI, 0.81-0.85) for significant fibrosis, 0.89 (95%CI, 0.85-0.92) for advance fibrosis and 0.93 (95%CI, 0.91-0.95) for

cirrhosis. A cut point of 0.50-0.55 achieved a summary sensitivity of 70% and a summary specificity of 79% to predict significant fibrosis. A cut point of 0.50-0.61 had a summary sensitivity of 81% and a summary specificity of 74% to predict advanced fibrosis. A cut point of 0.80-0.84 had a summary sensitivity of 72% and a summary specificity of 0.88% to predict cirrhosis. The accuracy of Hepascore was similar among all disease aetiologies for the prediction of cirrhosis. However, Hepascore had better diagnostic ability for significant and advanced fibrosis in chronic hepatitis C, chronic hepatitis B and alcoholic liver disease than for non-alcoholic fatty liver disease and HIV co-infected viral hepatitis.

Conclusions: Hepascore is a clinically useful measure of liver fibrosis in patients with common causes of chronic liver disease.

Key words: Hepascore; DANA; liver fibrosis; AUROC

KEY POINT BOX

- Hepascore has been validated in chronic hepatitis C, chronic hepatitis B, alcoholic liver disease and non-alcoholic fatty liver disease.
- Hepascore had an excellent accuracy to exclude cirrhosis in all four common causes of chronic liver disease.
- Hepascore had good diagnostic performance for significant fibrosis and advanced fibrosis in chronic hepatitis C, chronic hepatitis B and alcoholic liver disease.
- A cut point of 0.50-0.61 is predictive of significant fibrosis and advanced fibrosis and a cut point of 0.80-0.84 is predictive of cirrhosis.

INTRODUCTION

Chronic liver disease is a major global health problem. Chronic hepatitis C virus (HCV) infection, chronic hepatitis B virus (HBV) infection, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) are the main causes of chronic liver disease. Most chronic liver diseases have a similar clinical course with a prolonged asymptomatic early phase during which liver damage progresses silently and variable late clinical presentation of decompensated cirrhosis. The disease prognosis is closely associated with the severity of liver fibrosis and the majority of adverse outcomes occur after the development of liver cirrhosis [1]. As a result, liver fibrosis severity is currently the most reliable patient prognostic measure. Additionally, the measurement of liver fibrosis can help to guide important clinical management decisions that include the need for treatment and the initiation of hepatocellular carcinoma and liver decompensation surveillance.

Histopathological staging of liver biopsy has been used as the gold standard for liver fibrosis measurement. However liver biopsy is limited in its use due to its invasive nature, sampling error and risk of serious complications or death [2]. Furthermore, liver biopsy is inconvenient, expensive and not widely accessible to a large number of patients or physicians. Surrogate serum fibrosis models that can accurately predict the severity of liver fibrosis are of great clinical significance. Compared to liver biopsy, serum fibrosis models have advantages of low cost, wide availability, high reproducibility and non-invasive nature. During the last two decades, a number of serum fibrosis models have been developed and a few of them have been well validated and used in routine clinical practice.

Hepascore is a serum model that was developed to predict the severity of liver fibrosis in patients with chronic hepatitis C [3]. Hepascore includes four biomarkers, namely: alpha2-macroglobulin, hyaluronic acid, bilirubin and gamma-glutamyl transpeptidase, as well as age and gender [3]. After the initial development and validation of Hepascore in chronic hepatitis

C, Hepascore has been more widely validated in chronic hepatitis B, ALD and NAFLD to detect significant liver fibrosis (Metavir F2, F3, F4), advanced fibrosis (Metavir F3, F4) and cirrhosis (Metavir F4). However, no meta-analysis of these studies has been performed. This study performed a meta-analysis of all Hepascore validation studies to evaluate the summary diagnostic performance of Hepascore and to compare it across different aetiologies of chronic liver disease.

MATERIALS AND METHODS

Literature search strategy

An electronic search was performed on PubMed and Cochrane library using the key word "Hepascore" from October 2005 to September 2015. Studies were included if they met the following inclusion criteria: 1) The whole population or a sub-group of patients who had chronic hepatitis C with or without HIV co-infection, chronic hepatitis B with or without HIV co-infection, ALD, NAFLD or mixed aetiology of chronic liver disease could be extracted from the study. 2) Both liver biopsy and Hepascore were performed for patients and the diagnostic performance of Hepascore was evaluated. 3) Studies provided the area under ROC curve (AUROC) (95% CI) of Hepascore for different fibrosis stages and/or the true positive, true negative, false positive and false negative of at least one cut point could be calculated from the data. Exclusion criterial included: 1) non-English literature. 2) reviews. 3) duplicated cohorts. 4) The target population were patients with liver disease other than chronic hepatitis C, chronic hepatitis B, ALD and NAFLD.

Study inclusion and quality assessment

The studies were assessed by two independent reviewers using predetermined inclusion and exclusion criteria. The quality of each study was determined by using the validated quality assessment of diagnostic accuracy studies (QUADAS) questionnaire [4]. The questionnaire included 14 questions that covered use of appropriate patient population and reference standard, disease progression bias, verification bias, review bias, clinical review bias, incorporation bias, test execution, study withdrawals and indeterminate bias [4]. For each question, yes, no or unclear was scored. Disagreements between the two reviewers were resolved through further review.

Statistical analysis

Two approaches were used to evaluate the summary diagnostic performance of Hepascore. Firstly, meta-analysis was performed using the random effects model for all included studies that provided both AUROC and 95% CI. Previous studies found that the AUROC was significantly influenced by the distribution of fibrosis stages [5]. Hence, the AUROC was standardized according to the difference between the mean fibrosis stages in advanced fibrosis and non-advanced fibrosis groups (DANA) using the formula: adAUROC = obAUROC + (0.1056) * (2.5 - DANA) [5]. Meta-analysis was performed for both observed AUROC (obAUROC) and adjusted AUROC (adAUROC). ObAUROC and adAUROC of Hepascore was compared between different aetiologies of chronic liver disease. Meta-regression analysis and sensitivity analysis were used to evaluate the influence of seven characteristics of individual studies on AUROC, namely: patient inclusion methods (single centre vs multicentre), mean biopsy length (<20mm vs ≥20mm), biopsy evaluation (blinded vs not blinded), interval time between biopsy and serum collection (within one month vs >1

month), serum collection (fasting vs non-fasting), Hyaluronic acid test kit (Corgenix vs other kit) and study quality (all question score yes vs one or more questions scored no or unclear).

Secondly, a summary ROC (SROC) model was calculated for all included studies from which at least one 2X2 table containing true positive, true negative, false positive and false negative could be created [6]. Summary sensitivity and specificity of validated cut points to predict significant fibrosis, advanced fibrosis and cirrhosis were calculated. The estimated positive predictive value (PPV) and negative predictive value (NPV) of each cut point was calculated using the observed prevalence.

RESULTS

Literature search results

A total of 55 articles were identified from the literature search. 45 were original studies and were published in English. 24 studies were excluded for the following reasons: 14 studies did not evaluate the diagnostic performance of Hepascore or did not provide sufficient data for the meta-analysis; six studies evaluated the utility of Hepascore in other types of liver disease; four studies had duplicated cohorts. 21 studies were included in the final analysis. The main characteristics of these studies were shown in table 1 [3, 7-27]. A total of 5686 patients were included: 3523 had HCV infection, 441 had HCV/HIV co-infection, 588 had HBV infection, 108 had HBV/HIV co-infection, 321 had ALD, 242 had NAFLD and 463 had mixed aetiology of chronic liver disease. 11% patients had Metavir F0 (range: 0-44%), 34% had Metavir F1 (range: 11-47%), 25% had Metavir F2 (range 17-40%), 15% had Metavir F3 (range: 3-25%), 15% had Metavir F4 (range: 6-32%). According to the QUADAS

questionnaire, the qualities of the final 21 studies were good to excellent. (table 2). Twenty of these used the Metavir staging system as the reference standard.

Diagnostic performance of Hepascore for significant fibrosis

19 cohorts reported AUROC and 95%CI for significant fibrosis. The mean obAUROC of Hepascore was 0.78 (95%CI, 0.76-0.80) and the mean adAUROC was 0.83 (95%CI, 0.81-0.85) (figure 1). Hepascore had an increased diagnostic accuracy in HCV, HBV, ALD and mixed aetiology with a mean obAUROC of 0.80 (95%CI, 0.77-0.82), 0.79 (95%CI, 0.75-0.83), 0.82 (95%CI, 0.76-0.87) and 0.80 (95C%CI, 0.74-0.86) respectively. Less diagnostic accuracy of Hepascore was found for patients with HIV co-infection and NAFLD, with a mean obAUROC of 0.73 (95%CI, 0.67-0.79) and 0.73 (95%CI, 0.66-0.80) respectively. Compared to obAUROC, most adAUROC's for different causes of liver disease increased, with adAUROC of 0.85 (95%CI, 0.82-0.87) in HCV, 0.84 (95%CI, 0.80-0.88) in HBV, 0.83 (95%CI, 0.76-0.89) in ALD, 0.80 (95%CI, 0.76-0.84) in HIV co-infection and 0.81 (95%CI, 0.75-0.87) in mixed aetiologies (figure 1). The diagnostic accuracy for NAFLD remained less than other forms of liver disease with adAUROC of 0.74 (95%CI, 0.67-0.81). Significant heterogeneity was observed for obAUROC (I^2=55.6%, p=0.002) and for adAUROC (I^2=47%, p=0.013). Sub-group analysis of heterogeneity was performed according to the causes of chronic liver disease. Non-significant heterogeneity of obAUROC was found in chronic hepatitis B (I^2=6.8%, p=0.359), HIV co-infection (I^2=47%, p=0.152) and ALD (I^2=7.4%, p=0.299), while significant heterogeneity was found in chronic hepatitis C (I^2=60%, p=0.015). Non-significant heterogeneity of adAUROC was found in all analysed causes: chronic hepatitis C (I^2=48.8%, p=0.057), chronic hepatitis B (I^2=6.5%, p=0.361), HIV co-infection (I^2=0.0%, p=0.625) and ALD (I^2=27.9%, p=0.239). Sub-group analysis was not performed

for NAFLD and mixed aetiologies due to only one study of each cause was available for analysis.

The diagnostic performance of at least one cut point was reported in 18 cohorts and these were included in the SROC analysis (table 3). The average prevalence of significant fibrosis was 52% (ranged: 41% - 66%). The summary AUROC for all causes of liver disease was 0.79 (95%CI, 0.76-0.83) and this was similar to the AUROC calculated using the random effects model (figure 2A). The summary diagnostic odds ratio (DOR) was 7.3 (5.9-9.1). A cut point of 0.50-0.55 was validated in 10 cohorts with a summary sensitivity of 0.70 (95% CI, 0.60-0.78) and summary specificity of 0.79 (95% CI, 0.72-0.85). A lower cut point of 0.31-0.34 was validated in four cohorts with a summary sensitivity of 0.75 (95%CI, 0.60-0.86) and a summary specificity of 0.65 (95%CI, 0.57-0.73). Using the average observed prevalence of significant fibrosis (52%) in the included studies, the cut point of 0.5-0.55 had an estimated PPV of 0.78 and an estimated NPV of 0.71. The cut point of 0.31-0.34 had an estimated PPV of 0.70 and an estimated NPV of 0.71.

Diagnostic performance of Hepascore for advanced fibrosis

16 cohorts reported AUROC and 95%CI for advanced fibrosis. The mean obAUROC was 0.84 (95%CI, 0.81-0.87) and the mean adAUROC was 0.89 (95%CI, 0.85-0.92) (figure 3). Hepascore achieved a higher diagnostic accuracy in HCV and HBV with a mean obAUROC of 0.85 (95%CI, 0.80-0.90) and 0.86 (95%CI, 0.79-0.94) respectively. The obAUROC was 0.78 (95%CI, 0.72-0.85) for HIV co-infection, 0.83 (95%CI, 0.74-0.93) for ALD and 0.81 (95%CI, 0.73-0.90) for NAFLD. A similar pattern was found using adAUROC, with the mean adAUROC of 0.90 (95%CI, 0.86-0.95) in HCV, 0.91 (95%CI, 0.84-0.98) in HBV, 0.86 (95%CI, 0.82-0.90) in HIV co-infection, 0.84 (95%CI, 0.74-0.93) in ALD, 0.83 (95%CI, 0.74-0.91) in NAFLD and 0.73 (95%CI, 0.62-0.83) in mixed liver disease aetiologies.

Significant heterogeneity was observed for both obAUROC (I^2=83.5%, p<0.001) and adAUROC (I^2=81.1%, p<0.001). Sub-group analysis showed that significant heterogeneity of both obAUROC and adAUROC was found in HCV (obAUROC: I^2=89%, p<0.001, adAUROC: I^2=85.7%, p<0.001) and HBV (obAUROC: I^2=80.8%, p=0.001, adAUROC: I^2=76.9%, p=0.005) but not for HIV co-infection (obAUROC: I^2=43.7%, p=0.183, adAUROC: I^2=0.0%, p=0.646). Sub-group analysis of heterogeneity was not performed for ALD, NAFLD and mixed aetiologies due to the presence of one study in each subgroup.

14 cohorts validated cut points to predict advanced fibrosis and these were included in the SROC analysis (table 3). The average prevalence of advanced fibrosis was 27% (ranged: 19% - 38%). The summary AUROC was 0.84 (95%CI, 0.81-0.87) and summary DOR was 11.9 (95%CI, 9.0 - 15.7) (figure 2B). A cut point of 0.50-0.61 was validated in seven cohorts with a summary sensitivity of 0.81 (95% CI, 0.71-0.87) and a summary specificity of 0.74 (95% CI, 0.72-0.77). Using the average observed prevalence of advanced fibrosis (27%), the same cut point had an estimated PPV of 0.54 and an estimated NPV of 0.91.

Diagnostic performance of Hepascore for cirrhosis

15 cohorts reported AUROC and 95%CI for cirrhosis. The mean obAUROC was 0.88 (95%CI, 0.86-0.90) and the mean adAUROC was 0.93 (95%CI, 0.91-0.95) (figure 4). Excellent accuracy was observed in all aetiologies of chronic liver disease with obAUROC of 0.89 (95%CI, 0.88-0.91) in HCV, 0.88 (95%CI, 0.83-0.92) in HBV, 0.87 (95%CI, 0.79-0.96) for HIV co-infection, 0.85 (95%CI, 0.70-1.00) for ALD, 0.91 (95%CI, 0.83-0.99) for NAFLD. The adAUROC was 0.95 (95%CI, 0.93-0.97) in HCV, 0.92 (95%CI, 0.87-0.98) in HBV, 0.95 (95%CI, 0.90-0.99) for HIV co-infection, 0.86 (95%CI, 0.70-1.00) for ALD and 0.92 (95%CI, 0.84-1.00) for NAFLD. Hepascore had less diagnostic accuracy in patients with

mixed aetiologies with obAUROC of 0.81 (95%CI, 0.75-0.86) and adAUROC of 0.82 (95%CI, 0.76-0.88). Significant heterogeneity was observed for both obAUROC (I^2=44.2%, p=0.034) and adAUROC (I^2=62.2%, p=0.001). Sub-group analysis found significant heterogeneity of obAUROC in HIV co-infection (I^2=82.2%, p=0.018) and ALD (I^2=78.9%, p=0.029) but not in HCV (I^2=0.0%, p=0.671) and HBV (I^2=0.0%, p=0.380). Significant heterogeneity of adAUROC was only found in ALD (I^2=81.2%, p=0.021) but not in chronic hepatitis C (I^2=29.1%, p=0.217), chronic hepatitis B (I^2=22.3%, p=0.276) and HIV co-infection (I^2=26.1%, p=0.245). Sub-group analysis of heterogeneity was not performed for NAFLD and mixed aetiologies due to the presence of one study in each subgroup.

13 cohorts validated cut points to predict cirrhosis and were included in the SROC analysis (table 3). The average prevalence of advanced fibrosis was 13% (ranged: 6% - 31%). The summary AUROC was 0.90 (95%CI, 0.88-0.93) and the summary DOR was 25.3 (95%CI, 17.1-37.5) (figure 2C). A cut point of 0.80-0.84 was validated in seven cohorts with a summary sensitivity of 0.72 (95% CI, 0.64-0.79) and a summary specificity of 0.88 (95% CI, 0.0.85-0.91). Using the average observed prevalence of cirrhosis (13%), the same cut point had an estimated PPV of 0.47 and an estimated NPV of 0.95.

Sensitivity analysis

A sensitivity analysis was performed to test the effect of study characteristics on the adAUROC of Hepascore. No significant effect on adAUROC was found when patient inclusion methods, mean biopsy length, biopsy evaluation, interval time between biopsy and serum collection, timing of serum collection, hyaluronic acid test kit and study quality were analysed (table 4).

DISCUSSION

This meta-analysis reviewed 21 studies that evaluated the diagnostic performance of Hepascore for measuring the severity of liver fibrosis in chronic liver disease. Duplicate cohorts were excluded. The strength of this meta-analysis was that these studies included worldwide populations of the most common aetiologies of chronic liver disease, namely chronic hepatitis C, chronic hepatitis B, ALD and NAFLD. Standardization of the AUROC according to the distribution of liver fibrosis stages amongst cohorts allowed a more accurate comparison of the diagnostic performance of Hepascore for different aetiologies of chronic liver disease.

Hepascore had an excellent accuracy to predict cirrhosis for all aetiologies of chronic liver disease with an adAUROC of 0.95 for chronic hepatitis C, 0.92 for chronic hepatitis B, 0.86 for ALD, 0.92 for NAFLD and 0.95 for HIV co-infected chronic hepatitis. Meta-analysis of chronic hepatitis C and chronic hepatitis B found that Hepascore also had an excellent adAUROC for significant fibrosis, 0.85 and 0.84 respectively and for advanced fibrosis, 0.90 and 0.91 respectively. In ALD, NAFLD and HIV co-infected chronic hepatitis the diagnostic performance of Hepascore was good for significant fibrosis and advanced fibrosis. The adAUROC for significant fibrosis in ALD, NAFLD and HIV co-infection hepatitis was 0.83, 0.74 and 0.80 respectively and for advanced fibrosis the adAUROC was 0.84, 0.83 and 0.86 respectively. Apart from NAFLD, all other chronic liver disease aetiologies had an increased adAUROC compared with obAUROC. The adAUROC according to DANA was developed in chronic hepatitis C, but we predict that the fibrosis distribution for other liver diseases has a similar effect on AUROC.

The diagnostic performance of validated cut points for significant fibrosis, advanced fibrosis and cirrhosis were also evaluated for all causes of chronic liver disease. The cut points proposed in the original study of Hepascore were those most commonly validated in subsequent studies. A cut point of 0.50-0.55 had a summary sensitivity of 70%, summary specificity of 79%, an estimated PPV of 0.78 and an estimated NPV of 0.71 to predict significant fibrosis. A cut point of 0.50-0.61 had a summary sensitivity of 81%, a summary specificity of 74%, an estimated PPV of 0.54 and an estimated NPV of 0.91 to predict advanced fibrosis. A cut point of 0.80-0.84 had a summary sensitivity of 72%, a summary specificity of 0.88%, an estimated PPV of 0.47 and an estimated NPV of 0.91 to predict cirrhosis. These results were similar to those reported in the original study. Hepascore had the most accurate ability to excluded advanced fibrosis and cirrhosis with an estimated NPV of 0.91 for both.

Significant heterogeneity of obAUROC was found between all studies. This was heterogeneity was reduced but still remained significant after adjustment of the AUROC for DANA. This suggested that heterogeneity might be partly caused by the different distribution of fibrosis stages between studies. Subgroup analysis of disease aetiology found that heterogeneity of adAUROC was no longer significant within: HCV, HBV, HIV co-infection and ALD to predict significant fibrosis; in HIV co-infection to predict advanced fibrosis and in HCV, HBV, HIV co-infection to predict cirrhosis. This suggests that in addition to fibrosis distribution different aetiologies of chronic liver disease was another cause of heterogeneity between studies. Meta-regression analysis and sensitivity analysis was performed using seven pre-defined study characteristics. However, none of these characteristics showed a significant effect on AUROC. Others had previously found that blinded biopsy reviewing and histological staging system had an effect on the AUROC of other serum models [28]. The small number of studies that included in each subgroup made further analysis of the source of

heterogeneity impossible. Another limitation of this study was the potential bias that may have been a result of only including published full length articles to ensure adequate and comprehensive assessment of study quality.

In summary, this study confirmed that Hepascore is a useful measure of the severity of liver fibrosis in patients with the common causes of chronic liver disease. Hepascore had an excellent accuracy to exclude cirrhosis in all four causes of chronic liver disease and had good diagnostic performance for significant fibrosis and advanced fibrosis in chronic hepatitis C, chronic hepatitis B and alcoholic liver disease.

REFERENCES:

[1] Seeff L B. Natural history of hepatitis C. Hepatology 1997;26:21S-28S.

[2] Rockey D C, Bissell D M. Noninvasive measures of liver fibrosis. Hepatology 2006;43:S113-20.

[3] Adams L A, Bulsara M, Rossi E, DeBoer B, Speers D, George J, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem 2005;51:1867-73.

[4] Whiting P, Rutjes A W, Reitsma J B, Bossuyt P M, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.

[5] Poynard T, Halfon P, Castera L, Munteanu M, Imbert-Bismut F, Ratziu V, et al. Standardization of ROC curve areas for diagnostic evaluation of liver fibrosis markers based on prevalences of fibrosis stages. Clin Chem 2007;53:1615-22.

[6] Littenberg B, Moses L E. Estimating diagnostic accuracy from multiple conflicting reports: a new meta-analytic method. Med Decis Making 1993;13:313-21.

[7] Cacoub P, Carrat F, Bedossa P, Lambert J, Penaranda G, Perronne C, et al. Comparison of non-invasive liver fibrosis biomarkers in HIV/HCV co-infected patients: the fibrovic study--ANRS HC02. J Hepatol 2008;48:765-73.

[8] Boursier J, Vergniol J, Sawadogo A, Dakka T, Michalak S, Gallois Y, et al. The combination of a blood test and Fibroscan improves the non-invasive diagnosis of liver fibrosis. Liver Int 2009;29:1507-15.

[9] Cales P, Halfon P, Batisse D, Carrat F, Perre P, Penaranda G, et al. Comparison of liver fibrosis blood tests developed for HCV with new specific tests in HIV/HCV co-infection. J Hepatol 2010;53:238-44.

[10] Lee S, Varano J, Flexman J P, Cheng W, Watson M W, Rossi E, et al. Decreased IP-10 and elevated TGFbeta1 levels are associated with viral clearance following therapy in patients with hepatitis C virus. Dis Markers 2010;28:273-80.

[11] Costelloe S J, Theocharidou E, Tsochatzis E, Thalassinos E, Martin N, Fede G, et al. Hepascore and hyaluronic acid as markers of fibrosis in liver disease of mixed aetiology. Eur J Gastroenterol Hepatol 2015;27:313-20.

[12] Nguyen-Khac E, Chatelain D, Tramier B, Decrombecque C, Robert B, Joly J P, et al. Assessment of asymptomatic liver fibrosis in alcoholic patients using fibroscan: prospective comparison with seven non-invasive laboratory tests. Aliment Pharmacol Ther 2008;28:1188-98.

[13] Guechot J, Lasnier E, Sturm N, Paris A, Zarski J P. Automation of the Hepascore and validation as a biochemical index of liver fibrosis in patients with chronic hepatitis C from the ANRS HC EP 23 Fibrostar cohort. Clin Chim Acta 2010;411:86-91.

[14] Becker L, Salameh W, Sferruzza A, Zhang K, ng Chen R, Malik R, et al. Validation of hepascore, compared with simple indices of fibrosis, in patients with chronic hepatitis C virus infection in United States. Clin Gastroenterol Hepatol 2009;7:696-701.

[15] Naveau S, Gaude G, Asnacios A, Agostini H, Abella A, Barri-Ova N, et al. Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. Hepatology 2009;49:97-105.

[16] Adams L A, George J, Bugianesi E, Rossi E, De Boer W B, van der Poorten D, et al. Complex non-invasive fibrosis models are more accurate than simple models in non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2011;26:1536-43.

[17] Boursier J, Bacq Y, Halfon P, Leroy V, de Ledinghen V, de Muret A, et al. Improved diagnostic accuracy of blood tests for severe fibrosis and cirrhosis in chronic hepatitis C. Eur J Gastroenterol Hepatol 2009;21:28-38.

[18] Bottero J, Lacombe K, Guechot J, Serfaty L, Miailhes P, Bonnard P, et al. Performance of 11 biomarkers for liver fibrosis assessment in HIV/HBV co-infected patients. J Hepatol 2009;50:1074-83.

[19] Basar O, Yimaz B, Ekiz F, Ginis Z, Altinbas A, Aktas B, et al. Non-invasive tests in prediction of liver fibrosis in chronic hepatitis B and comparison with post-antiviral treatment results. Clin Res Hepatol Gastroenterol 2013;37:152-8.

[20] Wu S D, Wang J Y, Li L. Staging of liver fibrosis in chronic hepatitis B patients with a composite predictive model: a comparative study. World J Gastroenterol 2010;16:501-7.

[21] Crisan D, Radu C, Lupsor M, Sparchez Z, Grigorescu M D, Grigorescu M. Two or more synchronous combination of noninvasive tests to increase accuracy of liver fibrosis assessement in chronic hepatitis C; results from a cohort of 446 patients. Hepat Mon 2012;12:177-84.

[22] Cales P, de Ledinghen V, Halfon P, Bacq Y, Leroy V, Boursier J, et al. Evaluating the accuracy and increasing the reliable diagnosis rate of blood tests for liver fibrosis in chronic hepatitis C. Liver Int 2008;28:1352-62.

[23] Zarski J P, Sturm N, Guechot J, Paris A, Zafrani E S, Asselah T, et al. Comparison of nine blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS HCEP-23 study. J Hepatol 2012;56:55-62.

[24] Leroy V, Sturm N, Faure P, Trocme C, Marlu A, Hilleret M N, et al. Prospective evaluation of FibroTest(R), FibroMeter(R), and HepaScore(R) for staging liver fibrosis in chronic hepatitis B: comparison with hepatitis C. J Hepatol 2014;61:28-34.

[25] Kalantari H, Hoseini H, Babak A, Yaran M. Validation of hepascore as a predictor of liver fibrosis in patients with chronic hepatitis C infection. Hepat Res Treat 2011;2011:972759.

[26] Bourliere M, Penaranda G, Ouzan D, Renou C, Botta-Fridlund D, Tran A, et al. Optimized stepwise combination algorithms of non-invasive liver fibrosis scores including Hepascore in hepatitis C virus patients. Aliment Pharmacol Ther 2008;28:458-67.

[27] Raftopoulos S C, George J, Bourliere M, Rossi E, de Boer W B, Jeffrey G P, et al. Comparison of noninvasive models of fibrosis in chronic hepatitis B. Hepatol Int 2011.

[28] Lin Z H, Xin Y N, Dong Q J, Wang Q, Jiang X J, Zhan S 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;53:726-36.

Table 1. Characteristics of studies included in the analysis.

Cohorts	year	aetiology	Country	centre	No.	mean age	male (%)	Staging system	F0 (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	Mean biopsy size (mm)	Serum collection	Interval days	DANA
Adams -training [3]	2005	HCV	Australia	Single centre	117	40	68	Metavir	20	37	25	13	6	13	no fasting	-	1.92
Adams-validation [3]	2005	HCV	Australia	Multi centre	104	41	73	Metavir	16	27	34	7	16	13	no fasting	-	2.07
Bourliere [26]	2008	HCV	France	Multi centre	467	47	59	Metavir	15	36	22	20	7	20	no fasting	0	2.00
Cales [22], Boursier [17]	2008-9	HCV	France	Multi centre	1056	46	60	Metavir	4	43	27	14	11	21	no fasting	-	1.79
Becker [14]	2009	HCV	US	Single centre	391	50	70	Metavir	16	34	15	16	19	16	no fasting	<90	2.41
Guéchot [13]	2010	HCV	France	Multi centre	512	50	60	Metavir	7	45	18	15	15	25	fasting	<60	2.06
Lee [10]	2010	HCV	Australia	Single centre	95	45	63	Scheuer	-	-	-	-	-	15	no fasting	< 210	-
Kalantari [25]	2011	HCV	Iran	Single centre	80	35	85	Metavir	15	31	25	9	20	-	no fasting	-	2.23
Crisan [21]	2012	HCV	Romania	Single centre	446	49	38	Metavir	7	30	36	18	9	11	fasting	0	1.76
Leroy [24]	2014	HCV	France	Single centre	255	47	57	Metavir	15	38	26	11	11	24	fasting	0	1.97
Cacoub [7]	2008	HCV/HIV	France	Multi centre	272	40	72	Metavir	0	25	40	25	10	19	fasting	-	1.60
Cales [9]	2010	HCV/HIV	France	Multi centre	169	41	65	Metavir	8	26	33	13	20	25	fasting	<90	2.03
Wu [20]	2010	HBV	China	Single centre	78	33	84	Metavir	17	42	17	13	12	18	no fasting	-	2.16
Raftopoulos [27]	2011	HBV	Australia	Multi centre	179	42	71	Metavir	15	43	20	14	8	21	no fasting	<180	1.99
Basar [19]	2013	HBV	Turkey	Single centre	76	45	45	Metavir	12	21	29	21	17	-	no fasting	0	2.18
Leroy [24]	2014	HBV	France	Single centre	255	40	72	Metavir	15	38	26	11	11	25	fasting	0	1.97
Bottero [18]	2009	HBV/HIV	France	Multi centre	108	42	90	Metavir	10	33	26	16	15	17	no fasting	<180	2.04
Naveau [15]	2008	ALD	France	Single centre	218	47	78	Metavir	7	30	22	10	31	15	no fasting	<30	2.34
Nguyen-Khac [12]	2008	ALD	France	Single centre	103	53	74	Metavir	8	17	23	19	32	12	no fasting	0	2.42
Adams [16]	2011	NAFLD	Australia	Multi centre	242	47	60	Metavir	36	24	18	12	10	-	no fasting	0	2.38
Boursier [8]	2009	Mixed	France	Multi centre	390	52	68	Metavir	7	18	23	20	31	-	no fasting	-	2.39
Costelloe [11]	2015	Mixed	UK	Single centre	73	51	62	Metavir	44	11	12	3	30	-	no fasting	0	3.19

Table 2. Quality assessment of included studies.

Author, year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14
	Spectrum bias	Selection criteria	Appropriate reference standard	Disease progression bias	Partial verification bias	Differential verification bias	Incorporation bias	Test execution details	Reference execution details	Test review bias	Diagnostic review bias	Clinical review bias	Intermediate results	withdraws
Adams, 2005[3]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Cales, 2008 [22]	yes	yes	yes	unclear	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes
Bourliere, 2008 [26]	yes	yes	unclear	yes	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes
Boursier, 2009 [17]	yes	yes	yes	unclear	yes	yes	yes	unclear	yes	yes	unclear	yes	yes	yes
Becker, 2009 [14]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Guéchot, 2010 [13]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Lee, 2010 [10]	yes	unclear	unclear	yes	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes
Kalantari, 2011 [25]	yes	no	yes	unclear	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes
Crisan, 2012 [21]	yes	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes	yes	yes	yes
Leroy, 2014 [24]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Cacoub, 2008 [7]	yes	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes	yes	yes	yes
Cales, 2010 [9]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Wu, 2010 [20]	yes	yes	unclear	unclear	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes
Raftopoulos, 2011 [27]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Basar, 2013 [19]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Bottero, 2009 [18]	yes	unclear	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes	yes	yes
Naveau, 2008	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Nguyen-Khac, 2008 [12]	no	yes	yes	yes	yes	yes	yes	unclear	yes	yes	yes	yes	yes	yes
Adams, 2011 [16]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Boursier, 2009 [8]	no	unclear	yes	yes	yes	yes	yes	unclear	yes	yes	unclear	yes	yes	yes
Costelloe, 2015 [11]	no	no	unclear	unclear	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes

Table 3. Validated cut points of Hepascore by included studies.

Cohorts	Year	Aetiology	Significant fibrosis			Advanced fibrosis			Cirrhosis		
			Cut point	Sen	Spe	Cut point	Sen	Spe	Cut point	Sen	Spe
Adams -training [3]	2005	HCV	0.5	0.67	0.92	0.50	0.95	0.81	0.84	0.71	0.84
Adams-validation [3]	2005	HCV	0.5	0.63	0.89	0.50	0.88	0.74	0.84	0.71	0.89
Bourliere [26]	2008	HCV	0.5	0.63	0.86	-	-	-	0.84	0.71	0.88
Cales [22], Boursier [17]	2008-9	HCV	0.47	0.66	0.79	0.50	0.82	0.71	0.80	0.80	0.83
Becker [14]	2009	HCV	0.55	0.82	0.65	0.80	0.72	0.77	-	-	-
Guéchot [13]	2010	HCV	0.5	0.77	0.70	0.60	0.80	0.70	0.75	0.86	0.74
Lee [10]	2010	HCV	0.5	0.77	0.79	-	-	-	-	-	-
Kalantari [25]	2011	HCV	0.34	0.67	0.56	0.61	0.82	0.86	0.84	1.00	0.97
Crisan [21]	2012	HCV	0.34	0.57	0.72	0.61	0.61	0.73	-	-	-
Leroy [24]	2014	HCV	0.5	0.52	0.85	0.47	0.79	0.85	0.84	0.60	0.89
Cales [9]	2010	HCV/HIV	0.31	0.90	0.59	-	-	-	-	-	-
Wu [20]	2010	HBV	0.5	0.88	0.50	-	-	-	-	-	-
Raftopoulos [27]	2011	HBV	0.52	0.77	0.76	0.72	0.68	0.85	0.88	0.87	0.86
Basar [19]	2013	HBV	0.32	0.78	0.68	0.50	0.71	0.79	0.52	0.85	0.75
Leroy [24]	2014	HBV	0.5	0.42	0.84	0.42	0.75	0.71	0.84	0.56	0.92
Bottero [18]	2009	HBV/HIV	0.48	0.67	0.68	0.76	0.72	0.87	0.9	0.80	0.89
Naveau [15]	2008	ALD	0.25	0.90	0.37	-	-	-	0.97	0.90	0.87
Adams [16]	2011	NAFLD	0.44	0.51	0.88	0.37	0.76	0.84	0.7	0.87	0.89
Costelloe [11]	2015	Mixed	-	-	-	0.99	0.79	0.74	-	-	-

Table 4. Sensitivity analysis of study characteristics.

Characteristic	Significant fibrosis		Advanced fibrosis		Cirrhosis	
	No.	AdAUROC (95%CI)	No.	AdAUROC (95%CI)	No.	AdAUROC (95%CI)
All	19	0.83 (0.81-0.85)	16	0.89 (0.85-0.92)	15	0.93 (0.91-0.95)
Patients inclusion						
single centre	9	0.82 (0.80-0.85)	8	0.87 (0.81-0.94)	6	0.92 (0.87-0.97)
multicentre	10	0.83 (0.81-0.86)	8	0.89 (0.87-0.91)	9	0.93 (0.91-0.96)
Mean biopsy length						
<20 mm	9	0.82 (0.79-0.85)	7	0.90 (0.84-0.97)	6	0.94 (0.90-0.98)
≥20 mm	7	0.85 (0.84-0.87)	6	0.93 (0.88-0.89)	6	0.95 (0.93-0.97)
Biopsy evaluation						
blinded	15	0.83 (0.81-0.84)	13	0.87 (0.83-0.91)	12	0.93 (0.91-0.96)
not blinded	4	0.86 (0.84-0.88)	3	0.93 (0.88-0.98)	3	0.92 (0.85-0.99)
Interval time						
≤1 month	9	0.81 (0.78-0.84)	8	0.86 (0.83-0.89)	7	0.93 (0.90-0.95)
> 1 month	10	0.85 (0.83-0.87)	8	0.91 (0.87-0.96)	7	0.94 (0.90-0.98)
Serum collection						
fasting	6	0.82 (0.79-0.85)	5	0.86 (0.82-0.90)	4	0.92 (0.90-0.95)
non-fasting	13	0.84 (0.82-0.86)	11	0.90 (0.86-0.94)	11	0.93 (0.90-0.96)
HA test kit						
Corgenix	11	0.84 (0.81-0.86)	9	0.91 (0.88-0.94)	9	0.96 (0.94-0.97)
other kits	4	0.85 (0.82-0.88)	4	0.86 (0.76-0.96)	3	0.92 (0.90-0.95)
QUADAS score						
all scored yes	10	0.84 (0.81-0.86)	7	0.90 (0.85-0.95)	8	0.94 (0.91-0.96)
not all scored yes	9	0.82 (0.80-0.85)	9	0.87 (0.83-0.92)	7	0.92 (0.88-0.96)

FIGURE LEGENDS:

Figure 1. ObAUROC and adAUROC of Hepascore to predict significant fibrosis.

Figure 2. SROC curves of Hepascore. (A): SROC curve for significant fibrosis. (B): SROC curve for advanced fibrosis. (C): SROC curve for cirrhosis.

Figure 3. ObAUROC and adAUROC of Hepascore to predict advanced fibrosis.

Figure 4. ObAUROC and adAUROC of Hepascore to predict cirrhosis.