Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Comparison of liver fibrosis blood tests developed for HCV with new specific tests in HIV/HCV co-infection

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See Editorial, pages 222–224

Background & Aims: We compared 5 non-specific and 2 specific blood tests for liver fibrosis in HCV/HIV co-infection.

Methods: Four hundred and sixty-seven patients were included into derivation ($n = 183$) or validation ($n = 284$) populations. Within these populations, the diagnostic target, significant fibrosis (Metavir F $\geqslant$2), was found in 66% and 72% of the patients, respectively. Two new fibrosis tests, FibroMeter HICV and HICV test, were constructed in the derivation population.

Results: Unadjusted AUROCs in the derivation population were: APRI: 0.716, Fib-4: 0.722, Fibrotest: 0.778, Hepascore: 0.779, FibroMeter: 0.783, HICV test: 0.822, FibroMeter HICV: 0.828. AUROCs adjusted on classification and distribution of fibrosis stages in a reference population showed similar values in both populations. FibroMeter, FibroMeter HICV and HICV test had the highest correct classification rates in F0/1 and F3/4 (which account for high predictive values): 77–79% vs. 70–72% in the other tests ($p = 0.002$). Reliable individual diagnosis based on predictive values $\geqslant$90% distinguished three test categories: poorly reliable: Fib-4 (2.4% of patients), APRI (8.9%); moderately reliable: Fibrotest (25.4%), FibroMeter (26.6%), Hepascore (30.2%); acceptably reliable: HICV test (40.2%), FibroMeter HICV (45.6%) ($p < 10^{-3}$ between tests). FibroMeter HICV classified all patients into four reliable diagnosis intervals ($\leqslant$F1, F1 ± 1, $\geqslant$F1, $\geqslant$F2) with an overall accuracy of 93% vs. 79% ($p < 10^{-3}$) for a binary diagnosis of significant fibrosis.

Conclusions: Tests designed for HCV infections are less effective in HIV/HCV infections. A specific test, like FibroMeter HICV, was the most interesting test for diagnostic accuracy, correct classification profile, and a reliable diagnosis. With reliable diagnosis intervals, liver biopsy can therefore be avoided in all patients.

Keywords: Liver fibrosis; Blood test; Hepatitis C virus; Human immunodeficiency virus; Metavir staging; Diagnostic test.

Received 8 October 2009; received in revised form 13 March 2010 ; accepted 17 March 2010; available online 18 April 2010

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Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; CLD, chronic liver disease; HCV, hepatitis C virus; HICV, human immunodeficiency and C virus (used for HICV fibrosis test); HIV, human immunodeficiency virus; NPV, negative predictive value; PPV, positive predictive value.

Introduction

All HIV-infected patients should be screened for hepatitis A, B and C, and liver fibrosis must be evaluated in those with chronic hepatitis according to 2008 European AIDS Clinical Society guidelines [1]. Blood tests for liver fibrosis have been mainly evaluated in chronic viral hepatitis C. However, in a previous study, we observed that the cause of chronic liver disease (CLD) was an independent predictor of fibrosis and thus that it was preferable to develop specific tests for alcoholic, viral or metabolic CLD to improve accuracy

[2,3]. Similarly, several blood tests (e.g. Fib-4, SHASTA) have been specifically designed for co-infection, in particular human immunodeficiency virus (HIV)/hepatitis C virus (HCV) co-infection [4,5]. However, these tests appeared to provide suboptimal diagnostic performance or inhomogeneous results in some studies [6–9]. This was also the case for tests originally constructed in mono-infected patients, e.g. aspartate aminotransferase to platelet ratio index (APRI) [4,10,11] and Fibrotest [12], when they were evaluated in co-infected patients [13]. This observation was recently confirmed in a large independent study, which concluded that FibroMeter, Hepascore and Fibrotest outperformed other blood tests (SHASTA, APRI, Forns index and Fib-4) for the prediction of significant liver fibrosis in HIV/HCV co-infected patients [14]. Also, by using the weighted area under the receiver operating characteristic (AUROC), two tests that include hyaluronic acid and α-2-macroglobulin – FibroMeter and Hepascore – outperformed the others [14]. Taken together, these results suggest that tests based on simple non-specific biomarkers are hampered by confounding factors in HIV co-infection. Moreover, the use of specific biomarkers, like α-2-macroglobulin and/or hyaluronic acid, may explain the high diagnostic accuracy of FibroMeter and Hepascore. Finally, a recent systematic review concluded that additional studies are necessary to identify optimal measurements [15].

The main aim of the present prospective study was to develop a blood test for significant liver fibrosis, specifically designed to optimize diagnostic performance in HIV co-infection by using biomarkers included in the best performing usual blood tests. Thus, we compared seven blood tests in HIV/HCV co-infected patients.

Patients and methods

Centers

Four tertiary centers, Angers, Paris Hôpital Européen Georges Pompidou, Rennes and Tours, and one secondary center, La Roche sur Yon, provided a total of 183 patients in the derivation (or testing) population. Thus, individual patient data were available from five centers, independent for patient recruitment, blood marker analysis and interpretation of liver histology. The validation population, including 284 patients, was established from the Ribavic and Hepavih cohorts issued from multicentric studies supported by the French National Agency for AIDS and Viral Hepatitis (ANRS HC02 and Co13) [14,16].

Patients

Inclusion and exclusion criteria were very similar at all five centers for the derivation population. Patients with chronic viral hepatitis C and HIV infection were prospectively included from April 1997 to August 2007 if they had anti-HCV and anti-HIV antibodies, and HCV RNA in serum. For the present study, in all recruitment centers, we selected patients who had available liver biopsy and all blood markers needed to calculate blood tests. Fasting blood samples were collected immediately before or no more than 3 months after liver biopsy. Exclusion criteria comprised additional causes of liver disease, particularly HBV co-infection, complicated cirrhosis, anti-fibrotic treatment in the previous 6 months, alcohol consumption of more than 30 g/day in the five years prior to inclusion, and inclusion in the Ribavic or Hepavih cohorts. In all, the centers provided 467 patients, of whom 23 were excluded because of missing criteria or data. This left a core population of 444. The study protocol conformed to the ethical guidelines of the current Declaration of Helsinki and received the approval of local ethics committees.

Blood measurements

Blood samples were processed independently at each center. Determined variables were: platelet count, urea, bilirubin, γ-glutamyl transpeptidase, aspartate (AST) and alanine aminotransferases, prothrombin index, apolipoprotein A1, haptoglobin, hyaluronic acid, and α-2-macroglobulin. Direct markers were measured in either fresh blood or a frozen sample of serum stored at $\leqslant -20$ °C. Indirect markers were usually measured in fresh blood. Automation and assay techniques varied between the centers (details not provided), with the exceptions of apolipoprotein A1 and α-2-macroglobulin (Dade Behring) and hyaluronic acid (Corgenix). Original blood tests were calculated according to the most recent published formulas [5,17–19] or patent for Fibrotest (WO 02/16949). The list of variables included in each test is detailed in the Appendix. AST used in APRI was divided by a common upper limit of normal, as several studies performed in numerous laboratories in France have shown high inter-laboratory reproducibility [20].

Liver biopsy

Liver biopsies were performed using Menghini's technique with a 1.4–1.6 mm diameter needle. Biopsy specimens were fixed in a formalin–alcohol–acetic solution and embedded in paraffin; 5 μm thick sections were then cut and stained with hematoxylin–eosin–saffron. Liver fibrosis was staged from F0 to F4 according to the Metavir staging system [21]. The diagnostic target, significant fibrosis, was defined as follows: F2 + F3 + F4. Readings were performed by independent, senior pathologists specialized in hepatology. These pathologists were blinded for blood tests.

Outcomes

The main objective of the present study was to develop a blood test for significant liver fibrosis specifically designed for co-infection. Two approaches were used. One included the biomarkers of the best performing test; this has the advantage to handle a test already validated in other causes; the inconvenience is to force the entry of biomarkers into the model and thus not necessarily to select independent biomarkers. The second one included all the biomarkers available in the present study; the advantage is to select only independent biomarkers; the inconvenience is to produce a completely new test, i.e. with no evaluation background.

Secondary objectives were to evaluate:

- the comparison of this specific test to previous blood tests, called here HCV fibrosis tests, either simple or sophisticated as distinguished in the previous large comparative study [14];
- the best overall diagnostic performance by calculating the diagnostic cut-off values of HCV fibrosis tests adapted for the diagnosis of significant liver fibrosis in this HIV/HCV population [10,19];
- the *single* performance of blood tests for each fibrosis stage [22], which allows for the circumvention of prevalence bias, by calculating the rates of correct classification of the blood tests (especially worthwhile in patients with no fibrosis or with cirrhosis);
- the *individual* performance (the most useful for the clinician) by calculating:
  - the proportion of patients with high predictive values [23], implying a reliable individual diagnosis for the diagnostic target, significant fibrosis, in low and high blood test values,
  - the intervals of reliable individual diagnosis for all patients, i.e. in all blood test values, by using additional diagnostic targets [23]; this outcome was restricted to the most accurate blood test.

Statistical analysis

Data were reported according to STARD statements [24]. Quantitative variables were expressed as mean ± SD, unless otherwise specified. Stepwise binary logistic regression was used especially for the determination of new tests as described elsewhere [3]. The performance of each test was mainly expressed either by the overall accuracy (i.e., true positives and negatives) and the AUROC, or with more detailed diagnostic indices [25]. Among these indices, kappa index was determined to reflect the agreement between the blood test diagnosis and histological diagnostic target. Unadjusted AUROCs were compared by the Delong test. In addition to unadjusted AUROCs, *adjusted AUROCs* and *Obuchowski indexes* were also measured [26] using a recently-described population of 3567 HIV/HCV patients as a reference [27]. In addition, the misclassification rate of blood tests for significant fibrosis, also called *test performance profile* [22], was calculated in each Metavir F stage(s). Detailed definitions are listed in the Appendix. The level of type I error was fixed at $p < 0.05$.

The size of the exploratory population was determined to show a significant difference between FibroMeter and the new test. With α risk: 0.05, β risk: 0.2, significant fibrosis prevalence: 0.65, AUROC correlation: 0.75, and a bilateral test, the sample size was 166 patients for the following hypothesis of AUROC: FibroMeter: