Research Article

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Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease

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Background & Aims: NAFLD is highly prevalent but only a small subset of patients develop advanced liver fibrosis with impaired liver-related prognosis. We aimed to compare blood fibrosis tests and liver stiffness measurement (LSM) by FibroScan for the diagnosis of liver fibrosis and the evaluation of prognosis in NAFLD.

Methods: Diagnostic accuracy was evaluated in a cross-sectional study including 452 NAFLD patients with liver biopsy (NASH-CRN fibrosis stage), LSM, and eight blood fibrosis tests (BARD, NAFLD fibrosis score, FibroMeter^{NAFLD}, aspartate aminotransferase to platelet ratio index (APRI), FIB4, FibroTest, Hepascore, FibroMeter^{V2G}). Prognostic accuracy was evaluated in a longitudinal study including 360 NAFLD patients.

Results: LSM and FibroMeter^{V2G} were the two best-performing tests in the cross-sectional study: AUROCs for advanced fibrosis (F3/4) were, respectively, 0.831 ± 0.019 and 0.817 ± 0.020 ($p \leqslant 0.041$ *vs.* other tests); rates of patients with ⩾90% negative/-positive predictive values for F3/4 were 56.4% and 46.7% ($p < 0.001$ *vs.* other tests); Obuchowski indexes were 0.834 ± 0.014 and 0.798 ± 0.016 ($p \leqslant 0.036$ *vs.* other tests). Two fibrosis classifications were developed to precisely estimate the histological fibrosis stage from LSM or FibroMeter^{V2G} results without liver biopsy (diagnostic accuracy, respectively: 80.8% *vs.* 77.4%, $p = 0.190$). Kaplan-Meier curves in the longitudinal study showed that both classifications categorised NAFLD patients into subgroups with significantly different prognoses ($p < 0.001$): the higher was the class of the fibrosis classification, the worse was the prognosis.

Conclusions: LSM and FibroMeter^{V2G} were the most accurate of nine evaluated tests for the non-invasive diagnosis of liver fibrosis in NAFLD. LSM and FibroMeter^{V2G} fibrosis classifications help physicians estimate both fibrosis stage and patient prognosis in clinical practice.

Lay summary: The amount of liver fibrosis is the main determinant of the liver-related prognosis in patients with non-alcoholic fatty liver disease (NAFLD). We evaluated eight blood tests and FibroScan in a cross-sectional diagnostic study and found that FibroScan and the blood test FibroMeter^{V2G} were the two most accurate tests for the non-invasive evaluation of liver fibrosis in NAFLD. A longitudinal prognostic study showed these two tests initially developed for the diagnosis are also prognostic markers as they allow for the stratification of NAFLD patients in several subgroups with significantly different prognosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the manifestation of metabolic syndrome in the liver. With the worldwide burden of obesity, NAFLD has reached a median prevalence of 20–30% in the general population and thus become the most prevalent cause of chronic liver disease worldwide [1,2]. NAFLD is a heterogeneous entity that covers a wide spectrum of liver lesions ranging from bland steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and finally cirrhosis with its life-threatening complications. Several pathological lesions are used to describe NAFLD severity [3–5], but convergent findings from recent longitudinal studies indicate that liver fibrosis amount is the main determinant of patient outcome [6–8]. Consequently, as in other causes of chronic liver disease, liver fibrosis in NAFLD patients must be accurately evaluated in clinical practice. Liver biopsy currently

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; LSM, liver stiffness measurement; AUROC, area under the receiver operating characteristics; NPV, negative predictive value; PPV, positive predictive value.

remains the reference method to evaluate liver lesions and liver fibrosis in NAFLD [9]. However, this invasive procedure, with potentially severe or fatal complications [10], appears unsuitable for evaluating prognosis in NAFLD, as it would induce a large number of biopsies completely disproportionate to the low rate of patients who develop advanced fibrosis [11].

The NAFLD fibrosis score (NFS) is a blood test specifically developed for the non-invasive diagnosis of advanced fibrosis in NAFLD [12]. Numerous studies have validated its diagnostic accuracy [13], and consequently the American Association for the Study of Liver Diseases recently recommended its use as 'a clinically useful tool for identifying NAFLD patients with higher likelihood of having bridging fibrosis and/or cirrhosis' [9]. Nevertheless, the NFS has two main limitations. First, it is used with two diagnostic cut-offs, one to exclude and another to affirm advanced fibrosis. This leaves one third of patients in a 'grey zone' where liver biopsy is still required [14]. Second, the NFS includes only indirect markers of liver fibrosis. In chronic hepatitis C, it has been shown that blood tests including both direct and indirect markers of liver fibrosis are more accurate than tests including only indirect markers [15,16]. Liver stiffness measurement (LSM) by transient elastography (FibroScan) is another accurate method for the non-invasive diagnosis of liver fibrosis in NAFLD [14]. However, only a few, small studies have investigated LSM specifically in NAFLD [17].

According to the 2015 guidelines from the European Association for the Study of the Liver, LSM and blood fibrosis tests are less validated in NAFLD than in chronic hepatitis C [17]. In addition, among the different existing blood fibrosis tests, only NFS and FIB4 have been externally validated more than once, in different NAFLD populations and with consistent results [17]. Finally, recent longitudinal studies have suggested that blood fibrosis tests are prognostic markers predictive of overall mortality [18,19], mortality from liver-related complication [19] and mortality from extra-hepatic cause [19]. However, data are lacking in this area, especially for LSM. Overall, LSM and blood fibrosis tests require further validation in NAFLD, with direct comparison of their diagnostic accuracy and evaluation of their prognostic significance in large samples of patients.

In the present study, we evaluated and directly compared the accuracy of eight blood tests and LSM for the non-invasive diagnosis of liver fibrosis in a large population of NAFLD patients. For the most accurate fibrosis tests, we developed fibrosis classifications that allow for a precise estimation of the histological fibrosis stage, without the need for any liver biopsy. Finally, we validated the clinical relevance of these fibrosis classification by evaluating their prognostic accuracy in a longitudinal cohort, thereby performing the first prognostic evaluation of LSM in NAFLD.

Patients and methods

The study protocol of the present study conformed to the ethical guidelines of the current declaration of Helsinki. All patients included in the cross-sectional population and the longitudinal cohort gave informed written consent to participate.

Cross-sectional population

The purpose of the cross-sectional population was to evaluate and compare the diagnostic accuracy of the non-invasive fibrosis tests, and to develop fibrosis classifications.

Patients

Patients with biopsy-proven NAFLD were consecutively included from January 2004 to June 2014 at Angers University Hospital and from October 2003 to April 2014 at Bordeaux University Hospital. NAFLD was defined as liver steatosis on liver biopsy after exclusion of concomitant steatosis-inducing drugs, excessive alcohol consumption (>210 g/week in men or >140 g/week in women), chronic hepatitis B or C infection, and histological evidence of other concomitant chronic liver disease. Patients were excluded if they had liver complications (ascites, variceal bleeding, systemic infection, or hepatocellular carcinoma).

Liver biopsy

In each centre, pathological examination was performed by a senior expert specialized in hepatology and blinded for patient data. Liver fibrosis was evaluated according to the NASH-CRN scoring system [3]: F0 = no fibrosis; F1 = perisinusoidal or portal/periportal fibrosis, F2 = perisinusoidal and portal/periportal fibrosis, F3 = bridging fibrosis, and F4 = cirrhosis. Inter-observer reproducibility for liver fibrosis evaluation according to the NASH-CRN scoring system has been previously shown to be excellent between two experts with Kappa index = 0.84 [3]. Significant fibrosis was defined as F $\geqslant$2, advanced fibrosis as F $\geqslant$3, and cirrhosis as F4. Because previous longitudinal studies have demonstrated that liver-related prognosis is impaired when advanced fibrosis occurs [6–8,13], and as recommended by the latest EASL guidelines [17], we chose advanced fibrosis as our primary diagnostic target.

Blood fibrosis tests

Fasting blood samples were taken the day of or within the week preceding liver biopsy. Eight blood fibrosis tests were calculated according to published or patented formulas: NFS [12], BARD [20], FibroMeter^{NAFLD} [21], APRI [22], FIB4 [23], FibroTest [24], Hepascore [25], and FibroMeter^{V2G} [26]. BARD, NFS and FibroMeter^{NAFLD} were specifically developed for liver fibrosis assessment in NAFLD, whereas the five other tests were developed in patients with chronic viral hepatitis. In addition to indirect markers, FibroMeter^{V2G} and Hepascore also include two direct markers of liver fibrosis (hyaluronate and alpha 2-macroglobulin), while FibroTest includes only one (alpha 2-macroglobulin). The five other blood tests evaluated here include only indirect markers of liver fibrosis. All blood assays were performed in the laboratories of the Angers or Bordeaux centres. We have previously demonstrated the excellent inter-laboratory reproducibility of blood fibrosis tests [27].

Liver stiffness measurement

In each centre, LSM with FibroScan was performed using the standard M probe by a specialized nurse experienced with the procedure (>500 examinations) and who was blinded for patient data. LSM was performed in fasting condition, the day of liver biopsy or no more than three months before or after. Examination conditions were those recommended by the manufacturer [28]. LSM was stopped when 10 valid measurements were recorded and the result (kilo Pascal: kPa) was expressed as the median of these valid measurements. According to recently published [29] and independently validated [30] criteria, LSM was considered unreliable if LSM median was $\geqslant$7.1 kPa with an interquartile range/median (IQR/M) ratio >0.30. LSM failure was defined as LSM with no valid measurement (0% success rate) or LSM with only one valid measurement with thus no IQR calculated by the device.

Longitudinal cohort

The purpose of the prognostic longitudinal cohort was to validate the clinical significance of the fibrosis classifications developed in the cross-sectional population. All NAFLD patients seen between January 2005 and December 2009 in the Hepatology Department of the Angers University Hospital for a non-invasive evaluation of liver fibrosis were retrospectively included. Follow-up started the day of the non-invasive evaluation of liver fibrosis and ended November 15th, 2014. Dates and causes of death were obtained from the computerised National Registry of Individuals (CepiDC-Inserm, France). For those patients who could not be matched individually within the national registry, mortality data were obtained from the hospital database, or from the concerned general practitioner. All deaths related to primary liver cancer or cirrhosis complications were defined as "deaths related to liver complications", and all the others as "deaths related to extra-hepatic causes". All-cause mortality was the primary outcome of the prognostic study. Secondary outcomes were mortality from liver-related complication and mortality from extra-hepatic cause.