Haemophilia (2007), **13**, 722–729 DOI: 10.1111/j.1365-2516.2007.01548.x

ORIGINAL ARTICLE *Transfusion transmitted infection*

Improving estimation of liver fibrosis using combination and newer noninvasive biomarker scoring systems in hepatitis C-infected haemophilia patients

Y. MAOR,* P. CALÈS,† D. BASHARI,‡ G. KENET,‡ A. LUBETSKY,‡ J. LUBOSHITZ,‡ J. M. SCHAPIRO,‡ G. PÉNARANDA,§ S. BAR-MEIR,* U. MARTINOWITZ‡ and P. HALFON¶
**Department of Gastroenterology and Hepatology, Sheba Medical Center, Tel-Hashomer, Israel; †Laboratoire HIFIH, UPRES 3859, IFR 132, Service d'Hépato-Gastroentérologie, Université Angers, France; ‡The Israeli National Hemophilia Center (INHC), Sheba Medical Center, Tel-Hashomer, Israel; §CDL Pharma, Marseille, France; and ¶Laboratoire Alphabio, Marseille, France*

Summary. Non-invasive biomarkers have gained popularity for estimating fibrosis stage. In our hepatitis C-infected haemophilia patients, Fibrotest (FT) correctly identified clinically advanced or minimal liver disease. More accurate tests, like the FibroMeters, have recently been validated. The aim of the study was to improve the estimation of liver fibrosis in hepatitis C-infected haemophiliacs using a combination of biomarkers and FibroMeters. One hundred and thirty-two hepatitis C-infected haemophilia patients (124 male, mean age: 39 ± 14 years) were evaluated. The following biomarkers were used: FT, AST-to-platelet ratio index (APRI), Forns index, hyaluronic acid and FibroMeter. We applied a published algorithm suggesting that if FT is in concordance with APRI and/or Forns score, then the FT concurs with liver biopsy for estimation of fibrosis. Concordance of three or more biomarkers was present in 43.2% (57/132) of the patients. This high discordance rate was mainly because of indeterminate scores. Significant fibrosis (F2–F4) was estimated at 34.8% (46/132) and 37.9% (50/132) by the FT and FibroMeter respectively. The discordance rate between the FT and FibroMeter was 16.7% (22/132), ($P < 0.01$ vs. other biomarkers). Using the algorithm, liver histology could be confidently estimated in 69.7% (92/132) of the patients. Concordance between the FT and FibroMeter in those patients who met the terms of the algorithm was 90.2% (83/92). Discordance between biomarkers is significant, and is mainly because of biomarkers with indeterminate results. The concordance rate between FT and FibroMeter is higher compared with the other biomarkers. Practical combination of tests may potentially limit the need of liver biopsy in the majority of haemophilia patients.

Keywords: FibroMeter, Fibrotest, haemophilia, hepatitis C, liver biopsy, noninvasive biomarkers of fibrosis

Introduction

Haemophiliacs belong to a distinct group in whom the prevalence of hepatitis C (HCV) is highest, ranging from 70% to 90% [1–3]. Liver disease in HCV patients may progress to liver cirrhosis in variable rates depending on the host and viral factors [4,5]. Staging liver fibrosis enables assessment of prognosis as well as therapeutic decision-making in patient with HCV. Liver biopsy is currently considered the 'gold standard' for the assessment of liver histology [6]. However, liver biopsy is of a highly invasive nature and harbours the risk of complications with morbidity from 0.3% to 0.6% and mortality of 0.05% [7]. Moreover, the interpretation of liver biopsy is prone to sampling error, and an appropriate amount of tissue needs to be obtained to stage liver fibrosis correctly [8,9]. Not unexpectedly, there is reluctance to perform liver biopsy in patients with disorders of coagulation because of concerns about the safety of the procedure in this population. Nonetheless, liver biopsies have been performed in

Correspondence: Yaakov Maor, MD, Department of Gastroenterology and Hepatology, Sheba Medical Center, 52621 Tel-Hashomer, Israel.
Tel.: +972 3 5302694; fax: +972 3 5303160;
e-mail: yaakov@sheba.health.gov.il

Accepted after revision 8 July 2007

haemophiliacs, and no major complications of the procedure were reported in these studies [10,11].

Noninvasive biomarkers estimate liver fibrosis using scores formulated from several blood tests. Biomarkers are gaining increased acceptance for estimation of fibrosis, limited by methodological and analytical factors. Fibrotest (FT) is the most widely validated and used biomarker [12–16]. We recently described the distribution of fibrosis stage estimated by the FT in an HCV-infected haemophilia cohort and found scores compatible with F0–F1 and F2–F4 in 65% and 35% of the patients respectively. In clinically defined subgroups, FT score estimated advanced fibrosis (F3–F4) in all patients with clinically suspected advanced liver disease, whereas in all HCV RNA-negative patients the scores correlated with minimal fibrosis (F0–F1). Nevertheless, the concordance between FT and other biomarkers of fibrosis was only 40% [17].

The main approaches to minimize discordance rates among biomarkers and improve estimation of liver histology include combination of biomarkers or the use of newer more accurate biomarkers. Halfon *et al.* described an algorithm suggesting that if FT is in concordance with aspartate aminotransferase (AST)-to-platelet ratio index (APRI) [18] and/or Forns index [19], then we may confidently estimate the stage of fibrosis by the FT, and liver biopsy may be avoided [20]. A recently described biomarker, the FibroMeter is formulated from platelet count (PLT), prothrombin index (PI), AST, α2-macroglobulin, hyaluronic acid (HA), urea and age. This biomarker estimated clinically significant fibrosis (F2–F4) with high accuracy (AUROC of 0.883). Furthermore, FibroMeter is devoid of indeterminate scores and may also assess the area of fibrosis [21].

We sought to improve estimation of liver fibrosis by a combination of noninvasive biomarkers, and evaluate fibrosis in HCV-infected haemophilia patients by the FibroMeter.

Patients and methods

Population

Consecutive patients with haemophilia and other coagulation disorders with follow-up at the Israeli National Hemophilia Center (INHC) who had anti-HCV antibodies were evaluated between July 2004 and July 2005. All patients had not previously received anti-hepatitis therapy. Patients with HCV/HIV co-infection were maintained on their anti-retroviral therapy as indicated by the HIV specialist.

Patient evaluation

Patient evaluation included clinical assessment, biochemical tests and imaging studies consisting of abdominal ultrasonography with Doppler of the hepatic vessels and ^{99}Tc liver/spleen scan. Persistently, normal liver function tests (LFTs) were defined based on at least three measurements 6 months apart. Advanced liver disease was defined as decompensated liver disease (ascites, hepatic encephalopathy, jaundice or bleeding varices), signs of hypersplenism, ultrasonography suggesting the presence of cirrhosis or its complications, or signs of portal hypertension (varices on upper gastrointestinal endoscopy, diagnostic ^{99}Tc liver/spleen scan). Liver biopsies were not performed in any of the haemophilia patients.

Virological studies

Anti-HCV antibodies were detected by third-generation enzyme immunoassay (Abbott HCV MEIA version 3.0; Abbott Diagnostic, Wiesbaden, Germany). Viral load quantification was preformed using the Cobas Amplicor HCV Monitor, v2.0 (Roche Diagnostics, Branchburg, NJ, USA) [22]. Genotyping was preformed by direct sequencing [23]. Persistently, HCV RNA-negative was defined based on at least three measurements 6 months apart.

All viral studies were performed before the initiation of any anti-HCV therapy. According to this evaluation, these patients were stratified into several clinically distinctive groups: patients with features of advanced liver disease, those with persistently HCV RNA-negative, considered to harbour only minimal to mild liver fibrosis, patients with persistently normal LFTs and HCV/HIV co-infected.

Biomarkers of fibrosis

To determine the various biomarkers designed to estimate the stage of fibrosis, serum samples were obtained for the following variables: α2-macroglobulin, total bilirubin, gamma-glutamyl transpeptidase (GGT), apolipoprotein A1, haptoglobin, urea, alkaline phosphatases, PI, AST, PLT, total cholesterol and HA. Total bilirubin, GGT, alkaline phosphatases, AST and urea levels were measured using a Hitachi 917 Analyzer and Roche Diagnostics reagents (both Mannheim, Germany). α2-Macroglobulin, apolipoprotein A1, haptoglobin and total cholesterol levels were measured using a Modular analyzer (BNII; Dade Behring, Marburg, Germany). Platelets were measured using a Beckman Coulter LH 750 (Beckman Coulter France S. A., Roissy, France). The method for

PI (Neoplastin CI plus; Diagnostica Stago, Asnières, France) has been previously described [24]. HA levels were measured with the Corgenix Hyaluronic Acid Test Kit (Corgenix Inc., Broomfield, CO, USA) [25]. All biochemical analyses were performed on serum samples stored at −80°C and shipped to Laboratoire Alphabio (Marseilles, France).

Fibrotest was calculated using the Biopredictive websiste according to the manufacturer's instructions. The following formula is available on the USPTO website (http://www.uspto.gov; Patent no. 6,631,330): $f = 4.467 \times \log$ [α_2-macroglobulin (g L^{-1})] − $1.357 \times \log$ [haptoglobin (g L^{-1})] + $1.017 \times \log$ [γ-glutamyl transpeptidase (IU - L^{-1})] + $0.0281 \times$ [age (years)] + $1.737 \times \log$ [bilirubin (μmol L^{-1})] − $1.184 \times$ [apolipoprotein A1 (g L^{-1})] + $0.301 \times$ sex (female = 0; male = 1) − 5.540 [26]. APRI score formula and Forns' score were taken from respective publications [18,19].

FibroMeter test values were determined using the following regression function: − 0.007 PLT (g L^{-1}) − 0.049 PI (%) + 0.012 AST (IU L^{-1}) + 0.005 α_2-macroglobulin (mg dL^{-1}) + 0.021 HA (μg L^{-1}) − 0.270 urea (mmol L^{-1}) + 0.027 age (years) + 3.718. This algorithm was used to determine score probability ranging from 0 to 1, with a cutoff fixed at 0.5 for a clinically significant fibrosis (F2–F4). The AUROC curve of derived score probability (FibroMeter test) was 0.883 ± 0.019 (95% CI: 0.846–0.921) [21].

The area of fibrosis, expressed as the percentage of liver specimen area, was estimated using the respective regression functions: 0.015 HA (g L^{-1}) + 0.091 bilirubin (mol L^{-1}) − 1.666 apolipoprotein A1 (g L^{-1}) − 0.034 GGT (IU L^{-1}) + 3.037 GAPRI [(GGT/PLT)×100] + 9.491 and 0.090 HA (g L^{-1}) + 0.028 α_2-macroglobulin (mg dL^{-1}) − 0.009 PLT (g L^{-1}) − 0.017 HAPRI [(HA/PI)×100] + 2.166.The correlation between the area of fibrosis measured via image analysis and estimated via blood test was ${}_aR^2 = 0.645$ [21].

All biochemical parameters, FT and FibroMeter determinations were performed without the knowledge of the patients' clinical characteristics. The sera were collected and stored after the patients informed consent. The study was approved by our Institutional Ethics Committee.

Discordance determination

Significant discordance between FT, FibroMeter, APRI, Forns and HA was defined as discordance between the results of the test according to the respective cut-off value of each test and the result of fibrosis stage estimation in the METAVIR scoring system set by a French Cooperative Study Group of expert pathologists. We considered that discordance was highly attributable to FT, FibroMeter, APRI and Forns failure if one of the components of each test had an abnormal value attributable to a clinically identified condition (e.g. haemolysis). We have to emphasize that, when using different noninvasive biomarkers we are comparing scores, which estimate fibrosis, and not comparing measurement of fibrosis.

Halfon's algorithm

As a means to improve estimation of liver fibrosis in the advent of discordant scores, we applied a published algorithm suggesting that if FT is in concordance with APRI and/or Forns score, then the FT concurs with liver biopsy for estimation of fibrosis [20]. In the original publication, the stage of fibrosis could be correctly estimated in 81% of patients and liver biopsy avoided. Liver biopsy remained mandatory for the evaluation of fibrosis in the remaining 19% [20].

Statistical analysis

Quantitative variables were expressed as the mean ± SD unless otherwise specified. Continuous variables were compared by Student's *t*-test. Frequencies were compared using the two-tailed Fischer exact test or chi-squared test when suitable. In these cases, *P*-values of <0.05 were considered significant. Unpaired chi-squared test (McNemar) was also used to compare marginal distributions in contingency table, i.e. to determine whether disagreement rates were different. In this case, a *P*-value of <0.05 means that distributions are not significantly different. Kappa (κ)-score was used to measure the agreement between FT and FibroMeter; a $\kappa < 0.20$ means poor agreement, κ in (0.21;0.40) means fair agreement, κ in (0.41;0.60) means moderate agreement, κ in (0.61;0.80) means good agreement, κ in (0.81;1.00) means very good agreement. All were calculated using SAS v8.0 software.

Results

Patients and stage of fibrosis

One hundred and thirty-two consecutive patients with haemophilia and other coagulation disorders with anti-HCV antibodies were evaluated. Demographic, clinical, biochemical, virological data and fibrosis stage estimated by the FT are presented in

Table 1. Characteristics of 132 patients with coagulation disorders and chronic hepatitis C.

Variable	Distribution, mean (± SD) or % (*n*)
Age	39 (14)
Male gender, % (*n*)	94 (124)
Coagulation disorder, % (*n*)	
Haemophilia	90.1 (119)
von Willebrand	5.3 (7)
Glanzmann	2.3 (3)
Other	2.3 (3)
Genotype, % (*n*)	
1	77.8 (84)
2/3	15.7 (17)
Other	6.5 (7)
AST (IU L^{-1})	39.1 (31.4)
ALT (IU L^{-1})	58.9 (47.5)
γ-GT (IU L^{-1})	65.2 (63.1)
Bilirubin (μmol L^{-1})	9.6 (4.6)
α2-Macroglobulin (g L^{-1})	2.4 (0.9)
Platelets (g L^{-1})	227.7 (89)
Hyaluronate (μg L^{-1})	90.8 (122.6)
Stage of fibrosis, % (*n*)*	
F0–F1 (≤0.31)	47.7 (63)
F1–F2 (0.32;0.48)	17.4 (23)
F2 (0.49;0.58)	5.3 (7)
F3 (0.59;0.72)	11.4 (15)
F3–F4 (0.73;0.74)	1.5 (2)
F4 (≥0.75)	16.7 (22)

*Estimated by the Fibrotest.
GGT, Gamma-glutamyl transpeptidase.

Table 1. There were 124 males (94%), the mean age was 39 ± 14 years. Haemophilia was the coagulation disorder in 90% (119/132) of the patients. Seventy-eight per cent (84/108) were infected with genotype 1. Estimated by the FT, the distribution of fibrosis in 132 patients was: F0–F1 (FT ≤ 0.31): 47.7% (63/132), F1–F2 [FT(0.32;0.48)]: 17.4% (23/132), F2 [FT(0.49;0.58)]: 5.3% (7/132), F3 [FT(0.59;0.72)]: 11.4% (15/132), F3–F4 [FT(0.73;0.74)]: 1.5% (2/132), and F4 (FT ≥ 0.75): 16.7% (22/132). Therefore, estimated significant fibrosis (F2–F4) was present in 34.8% (46/132) of HCV-infected haemophilia patients. Using FibroMeter assessment, the rate of estimated significant fibrosis was not significantly different at 37.9% (50/132) (κ 0.57 and McNemar test, $P < 0.0001$).

Discordance between FT, FibroMeter and other biomarkers of fibrosis

The Fibrotest score agreed with all the other tested biomarkers in 43.2% (57/132) of patients. Among the conventional scores, the discordance rates were between 41.1% (53/129) for APRI and 56.1% (74/132) for HA. Most of the discordant results were because of a wide range of indeterminate scores inherent to all the aforementioned biomarkers ranging from 33.3% (43/129) for APRI to 44.7% (59/132) for HA. The discordance rate between FibroMeter and FT was only 16.7% (22/132), a significantly lower rate than the other biomarkers ($P < 0.01$). Moreover, FibroMeter is devoid of an indeterminate zone, enabling fibrosis staging in all patients (Table 2).

The concordance rates between the various biomarkers of fibrosis and FibroMeter were similar to those observed with FT, and were again highest with APRI at 59.7% (77/129) and lowest with HA at 49.2% (65/132). Again, most discordant results were secondary to indeterminate scores. When indeterminate results of other biomarkers were excluded, the agreement between FibroMeter and the other tests reached 93% for all the tests (Table 3). Clinically significant fibrosis (F2–F4) estimated by FibroMeter was significantly less frequent among the patients with concordant than those with discordant scores [23.6% (26/110) vs. 59.1% (13/22), $P < 0.001$].

Table 2. Discordance and indeterminate rates between Fibrotest and other biomarkers of fibrosis.

Biomarker	Discordance, % (*n*)	Indeterminate, % (*n*)
APRI	41.1 (53/129)	33.3 (43/129)
Forns	41.7 (53/120)	37.8 (48/120)
HA	56.1 (74/132)	44.7 (59/132)
FibroMeter	16.7 (22/132)	0

APRI, AST-to-platelet ratio index; HA, hyaluronic acid.

Table 3. Concordance between FibroMeter and other biomarkers of fibrosis.

	FibroMeter, % (*n*)
FT	83.3 (110/132)
Algorithm +*	90.2 (83/92)
Algorithm − *	67.5 (27/40)
APRI	
Indeterminate included	59.7 (77/129)
Indeterminate not included	93 (120/129)
Forns index	
Indeterminate included	56.7 (68/120)
Indeterminate not included	93.3 (112/120)
HA	
Indeterminate included	49.2 (65/132)
Indeterminate not included	93.9 (124/132)

*Halfon's algorithm: FT ≈ APRI and/or Forns: FT ≈ liver biopsy.
FT, Fibrotest; APRI, AST-to-platelet ratio index; HA, hyaluronic acid.

Practical algorithm of combined biomarkers

We applied a practical algorithm assuming that if FT is in concordance with either APRI or Forns index, then FT correctly estimated the stage of fibrosis. In our cohort of haemophilia patients with HCV, 69.7% (92/132) met the terms of the algorithm. Among the patients in whom FT and APRI or Forns index agreed, the concordance between FT and FibroMeter was even higher at 90.2% (83/92) than in those with disagreement between FT and APRI or Forns (Table 3). Clinically significant fibrosis (F2–F4) estimated by FibroMeter was significantly less frequent among the patients in whom FT and APRI or Forns index agreed than in the other discordant patients: 16.3% (15/92) vs. 60% (24/40), respectively, $P < 0.001$.

Fibrosis staging by FT and FibroMeter in clinically defined subgroups

Fibrosis stage F3–F4 was estimated by both FT and FibroMeter scores in all patients with clinically suspected advanced liver disease. In HCV RNA-negative patients, both biomarkers scores estimated F0–F1 (Table 4a). In HCV/HIV co-infected patients, clinically significant fibrosis was estimated in 51.9% (14/27) and 55.6% (15/27) by FT and FibroMeter respectively. The rate of F2–F4 fibrosis in patients with persistently normal LFT was estimated at 20.8% (5/24) and 16.7% (4/24) by FT and FibroMeter respectively (Table 4a). The concordance (per cent crude agreement) between FT and FibroMeter was perfect in the groups with advanced liver disease and in those who tested HCV RNA-negative. The agreement was lower for HCV/HIV co-infected patients and for those with persistently normal LFT, 74.1% (20/27) and 75% (18/24) respectively (Table 4b). Application of Halfon's algorithm in the two later clinically defined groups augmented concordance between FT and FibroMeter only in those patients with persistently normal LFT, from 75% to 88.3% (15/17), but not in HCV/HIV co-infected patients (Table 4b).

Assessment of the area of fibrosis

The FibroMeter system is able to assess the area of fibrosis with good correlation between the area of fibrosis measured via image analysis and estimated via blood test ($R^2_a = 0.645$). In our cohort of HCV-infected haemophilia patients, the estimated area of fibrosis was significantly higher for fibrosis stage F4 vs. F0–F3 (16.94% vs. 9.03%, $P < 0.001$), and F2–F4 vs. F0–F1 (10.82% vs. 8.24%, $P < 0.0001$). To validate the area of fibrosis in the present population, we estimated METAVIR classes from the box plots of FibroMeter against METAVIR F determined by liver biopsy in the original population [21] with 0.5 meaning an intermediate stage, e.g. 2.5: stage 2 or 3. We observed a progressive increase in the estimated area of fibrosis as a function of those estimated METAVIR stages (Fig. 1).

Discussion

In our cohort of HCV-infected haemophilia patients, we estimated clinically significant fibrosis (F2–F4) in over one-third of the patients using either FT or FibroMeter. The concordance rate between FT and FibroMeter was significantly higher than between each of these noninvasive

Table 4a. Fibrosis stage estimated by Fibrotest and by FibroMeter in clinically defined groups.

	F-stage by blood test	Fibrotest, % (*n*)	FibroMeter, % (*n*)	*P*-value	κ-value
Cirrhosis	F3–F4	100 (7/7)	100 (7/7)	*ns**	NA
HCV RNA-negative	F0–F1	100 (21/21)	100 (21/21)	*ns**	NA
HIV	F2–F4	59 (16/27)	63 (17/27)	*ns*†	0.77
LFT-Nl	F2–F4	25 (6/24)	16.7 (4/24)	*ns*†	0.50

*Chi-squared test; †Fischer exact test; NA, not available (calculation impossible).

Table 4b. Concordance between Fibrotest (FT) and FibroMeter in clinically defined groups.

	F-stage by blood test	Concordance, % (*n*)		
		Crude	Halfon's algorithm*	*P*-value†
Cirrhosis	F3–F4	100 (7/7)	100 (6/6)	*ns*
HCV RNA-negative	F0–F1	100 (21/21)	100 (19/19)	*ns*
HIV	F2–F4	74.1 (20/27)	73.3 (11/15)	*ns*
LFT-Nl	F2–F4	75 (18/24)	88.25 (15/17)	*ns*

*FT ≈ AST-to-platelet ratio index and/or Forns: FT ≈ liver biopsy.
†Fischer exact test.

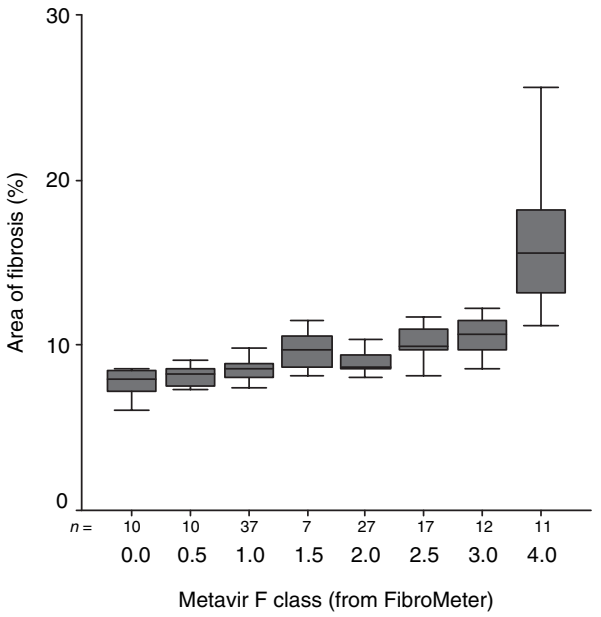

Fig. 1. Box plots (median, quartiles and extremes) of the area of fibrosis as a function of fibrosis stages estimated by original FibroMeter.

biomarkers of fibrosis and APRI, Forns index or HA. Discordance between scores stems mainly from indeterminate scores inherent to these first-generation tests.

The main limitation of the study was the absence of liver biopsies in our patients. Liver biopsies have been performed in haemophilia patients, and are a common practice in some centres. No major complications were reported with the procedure in haemophiliacs [10,11]. Nevertheless, the cost involved with liver biopsy is substantially higher in haemophilia patients, given the excessive requirement for coagulation factors. Furthermore, the procedure is precluded in those patients who have inhibitors to FVIII. Several studies pointed out the sampling error occurring in up to a third of biopsies, mostly in relation to an inadequate biopsy size [8,9]. In our experience, *trans*-jugular biopsies (commonly practised in patients with coagulation disorders) are less likely to be of sufficient length to stage fibrosis correctly. Several studies indicated that biomarkers estimated the stage of fibrosis with a higher accuracy than liver biopsy [14,20]. One study found discordance attributable to biopsy failure in 18%, whereas failure of biomarkers was considered in 2% [14]. In another study, cirrhosis was diagnosed in 17 patients by the FT, while the biopsy-determined cirrhosis was diagnosed only in four patients [20]. They recommended that noninvasive markers should be used as first-line assessment of liver fibrosis in patients with HCV. Furthermore, a recent report applied the FT without biopsies to follow the histological progression of lamivudine-treated hepatitis B patients [27]. Weighing the risk–benefit ratio of this procedure, we decided against liver biopsy in our patient population.

We have to emphasize that when using different noninvasive biomarkers we are comparing scores which estimate fibrosis, and not comparing measurement of fibrosis.

To overcome the significant discordance rate between FT and other biomarkers, along with the high proportion of patients in whom APRI, Forns index and HA are not able to estimate the stage of fibrosis (indeterminate zone), the use of combination of biomarkers has been suggested. Castéra *et al.* [28] have shown that a combination of FT and Fibroscan, a method based on the correlation between the amount of fibrotic tissue and elasticity, estimated fibrosis more accurately than each method separately, within an AUROC of 0.9 for the combination of tests. Recently, Halfon *et al.* have shown that if FT is in concordance with APRI and/or Forns index, then FT correlates well with the stage of fibrosis based on liver biopsy [20]. In their series, the stage of fibrosis could be estimated correctly in 81% of patients, and liver biopsy avoided. Liver biopsy remained mandatory, however, for the evaluation of fibrosis in the remaining 19%. We applied the Halfon's algorithm in our cohort. In our HCV-infected patients with haemophilia and other disorders of coagulation, the assumption resulted in a more modest figure, with 70% in whom liver biopsy could be potentially avoided using this practical approach. This may represent differences between the two populations. Indeed, 27 of our haemophilia patients had HCV/HIV co-infection, a condition excluded from Halfon's study. However, further analysis of our cohort excluding these co-infected patients, did not change significantly the concordance rate between FT and FibroMeter or the frequency of patients meeting the terms of the Halfon's algorithm (data not shown). Furthermore, the various variables formulating the FT may be influenced by non-hepatic conditions, e.g. haematoma, more prevalent in haemophilia patients.

Sebastiani *et al.* [29] have employed several algorithms each devised for a specific group. In their study, the positive predictive value (PPV) for significant fibrosis or cirrhosis guided the sequence of the use of APRI, FT and Forns index in three clinically distinguished scenarios. These included estimation of significant fibrosis stage (METAVIR stage ≥F2) in HCV patients with elevated ALT, patients with

persistently normal ALT, or for the diagnosis of cirrhosis (METAVIR stage F4). Their rather complex algorithm resulted in 95% accuracy rate. In their training and validation cohort, however, the rate of significant fibrosis was relatively high, a characteristic that might influence the PPV. Nonetheless, the meticulous use of combination of noninvasive biomarkers of fibrosis is now suggested as a means to overcome the shortcomings of individual tests.

There is a need for newer, more accurate biomarkers, which are derived from commonly available tests. Calès *et al.* [21] have recently devised a combination of seven variables to form a novel scoring system, termed FibroMeter, which was found to be highly predictive of clinically significant fibrosis, with an AUROC of 0.883 and 0.892 in the training and validation population respectively. In addition to being highly accurate, the FibroMeter also has the advantage of lacking indeterminate results. Using the FibroMeter in our patient population, we estimated clinically significant fibrosis in close agreement with the FT. The high concordance rate between FT and FibroMeter does not automatically infer the correctness of staging, even the higher concordance rate observed in those patients who fulfilled the terms of Halfon's algorithm (83–90%) supports this notion. Further studies, e.g. a recent study by Halfon *et al.* [30], comparing and combining FT and FibroMeter in independent cohorts, will lead to the eventual validation of the clinical application of these biomarkers.

There are several other newer biomarkers of fibrosis. One of these tests termed Hepascore is derived from bilirubin, GGT, HA, α2-macroglobulin, age and sex [31]. Although in our cohort Hepascore demonstrated agreement with FT not different from the concordance between FibroMeter and FT, Hepascore tended to overestimate fibrosis stage compared with the other biomarkers. Furthermore, application of Halfon's assumption did not augment the concordance between Hepascore and FT (data not shown).

FibroMeter is also the first noninvasive system to predict the area of fibrosis [21]. Currently, image analysis, the method to assess area of fibrosis, is considered a research tool. Area of fibrosis enables precise quantification of fibrosis specifically in cirrhosis, however, because of marked overlap it may not distinguish between F0 and F3 stages of fibrosis. In our patients, the interquartile range for estimating the area of fibrosis in the cirrhotic stage (F4) (FibroMeter score 0.99–1.00) was 13.2–18.6%. The relationship between the estimated area of fibrosis and estimated METAVIR stages in the present series (Fig. 1) supports the accuracy of the blood determination of the area of fibrosis. Indeed, whereas those two variables were independently determined, the shape of the relationship closely resembled that observed when the two variables were determined on liver specimens by histological determination [32].

Clinically significant fibrosis estimated by FibroMeter was significantly less common among those with concordant scores than those with discordant scores (24% vs. 59%). The rate of estimated F2–F4 was also lower among those complying than those who did not comply with the Halfon's assumption (16% vs. 60%). Therefore, our data suggest a significantly lower PPV of FibroMeter for significant fibrosis than PPV for absence or minimal fibrosis. Similarly, low PPV for clinically significant fibrosis was observed with FT evaluation of the same population [17]. Several components of the FibroMeter are influenced by the conditions other than the liver disease itself. Conditions such as haematoma, frequent in our population, with resultant high total bilirubin levels and low haptoglobin, may overestimate fibrosis scores. Only two patients had isolated elevation of bilirubin, in both FibroMeter score was in concordance with all other biomarkers. In addition, we routinely avoided FibroMeter determination in case of haematoma or fever.

In conclusion, discordance between biomarkers is significant, and is mainly because of indeterminate results. The concordance rate between FT and FibroMeter is higher compared with the other biomarkers. A practical combination of tests may potentially limit the need of liver biopsy in the majority of haemophilia patients.

References

1 Yee TT, Griffioen A, Sabin CA, Dusheiko G, Lee CA. The natural history of HCV in a cohort of haemophilic patients infected between 1961 and 1985. *Gut* 2000; **47**: 845–51.
2 Lee C, Dusheiko G. The natural history and antiviral treatment of hepatitis C in Haemophilia. *Haemophilia* 2002; **8**: 322–9.
3 Maor Y, Bashari D, Kenet G *et al.* Hepatitis C at the israeli national hemophilia center. *Haemophilia* 2006; **12**: 68–74.
4 Seeff LB, Hollinger FB, Alter HJ *et al.* Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: a National Heart, Lung, and Blood Institute collaborative study. *Hepatology* 2001; **33**: 455–63.
5 Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic

hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825–32.
6 Dienstag JL. The role of liver biopsy in chronic hepatitis C. *Hepatology* 2002; **36**(Suppl.): S152–60.
7 Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; **32**: 477–81.
8 Regev A, Berho M, Jeffers LJ *et al.* Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614–8.
9 Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449–57.
10 Theodore D, Fried MW, Kleiner DE *et al.* Liver biopsy in patients with inherited disorders of coagulation and chronic hepatitis C. *Haemophilia* 2004; **10**: 413–21.
11 Shin JL, Teitel J, Swain MG *et al.* Virology and Immunology Committee of the Association of Hemophilia Clinic Directors of Canada. A Canadian multicenter retrospective study evaluating transjugular liver biopsy in patients with congenital bleeding disorders and hepatitis C: is it safe and useful? *Am J Hematol* 2005; **78**: 85–93.
12 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T, for the MULTIVIRC group. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069–75.
13 Halfon P, Imbert-Bismut F, Messous D *et al.* A prospective assessment of the inter-laboratory variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) in patients with chronic liver disease. *Comp Hepatol* 2002; **1**: 3.
14 Poynard T, Munteanu M, Imbert-Bismut F *et al.* Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. *Clin Chem* 2004; **50**: 1344–55.
15 Le Calvez S, Thabut D, Messous D *et al.* The predictive value of fibrotest vs. APRI for the diagnosis of fibrosis in chronic hepatitis C. *Hepatology* 2004; **39**: 862–3.
16 Halfon P, Bourlière M, Deydier R *et al.* Independent prospective multicenter validation of biochemical markers (fibrotest-actitest) for the prediction of liver fibrosis and activity in patients with chronic hepatitis C: the Fibropaca study. *Am J Gastroenterol* 2006; **101**: 547–55.
17 Maor Y, Bashari D, Kenet G *et al.* Non-invasive biomarkers of liver Fibrosis in haemophilia patients with hepatitis C: can you avoid Liver Biopsy? *Haemophilia* 2006; **12**: 372–9.
18 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518–26.
19 Forns X, Ampurdanes S, Llovet JM *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986–92.
20 Bourlière M, Pénaranda G, Renou C *et al.* Validation and comparison of indexes or fibrosis and cirrhosis prediction in chronic hepatitis C patients: proposal for a pragmatic approach classification without liver biopsies. *J Viral Hepat* 2006; **13**: 659–70.
21 Calès P, Oberti F, Michalak S *et al.* A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; **42**: 1373–81.
22 Gerken G, Rothaar T, Rumi MG *et al.* Performance of the Cobas Amplicor HCV Monitor test, version 2.0, an automated reverse transcription-PCR quantitative system for hepatitis C virus load determination. *J Clin Microbiol* 2000; **38**: 2210–4.
23 Halfon P, Trimoulet P, Bourlière M *et al.* Hepatitis C virus genotyping based on 5′ noncoding sequence analysis (Trugene). *J Clin Microbiol* 2001; **39**: 1771–3.
24 Croquet V, Vuillemin E, Ternisien C *et al.* Prothrombin index is an indirect marker of severe liver fibrosis. *Eur J Gastroenterol Hepatol* 2002; **14**: 1133–41.
25 Halfon P, Bourlière M, Pénaranda G *et al.* Accuracy of hyaluronic acid level for predicting liver fibrosis stages in patients with hepatitis C virus. *Comp Hepatol* 2005; **4**: 6.
26 Poynard T. Diagnosis method of inflammatory or cancerous disease using the biochemical markers. US Patent 6,631,330 B1, 2003.
27 Poynard T, Zoulim F, Ratziu V *et al.* Longitudinal assessment of histology surrogate markers (FibroTest–ActiTest) during lamivudine therapy in patients with chronic hepatitis B infection. *Am J Gastroenterol* 2005; **100**: 1970–80.
28 Castéra L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, Fibrotest, APRI and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343–50.
29 Sebastiani G, Vario A, Guido M *et al.* Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. *J Hepatol* 2006; **44**: 686–93.
30 Halfon P, Bacq Y, De Muret A *et al.* Comparison of test performance profile for blood tests of liver fibrosis in chronic hepatitis C. *J Hepatol* 2007; **46**: 395–402.
31 Adams LA, Bulsara M, Rossi E *et al.* Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; **51**: 1867–73.
32 Pilette C, Rousselet M, Bedossa P *et al.* Histopathological evaluation of liver fibrosis: quantitative image analysis vs. semi-quantitative scores: comparison with serum markers. *J Hepatol* 1998; **28**: 439–46.