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Non-invasive biomarkers of liver fibrosis in haemophilia patients with hepatitis C: can you avoid liver biopsy?

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Summary. *Introduction:* Liver biopsy remains the gold standard for the evaluation of fibrosis despite its risks and limitations, especially in haemophilia patients. Recently, non-invasive biomarkers have been used to assess histological features. The most thoroughly evaluated biomarker is the FibroTest (FT) (AUROC 0.80 for fibrosis stages F2F3F4 vs. F0F1). *Aim:* To estimate liver fibrosis in haemophilia patients infected with hepatitis C (HCV) using non-invasive biomarkers without liver biopsy. *Methods:* One hundred and thirty-two haemophilia patients (124 male, mean age 38 ± 14 years) with anti-HCV antibodies were evaluated. These patients were stratified into several groups: patients with features of advanced liver disease – seven, persistently HCV RNA-negative – 21, persistently normal liver function tests (LFTs) – 24, HCV/HIV co-infected – 27. The following biomarkers of fibrosis were used: FT, AST-to-platelet ratio index (APRI), Forns index, age-platelet index and hyaluronic acid. The obtained scores were correlated with the clinical features of the patients. *Results:* Estimated by the FT, the distribution of the stage of fibrosis in the 132 patients was F0F1 = 65% (86/132), F2 = 5% (7/132), F3 = 13% (17/132) and F4 = 17% (22/132). Using FT, all patients with clinical suspicion of advanced liver disease were classified as F3F4, whereas patients with persistently HCV RNA-negative were all classified as F0F1. Twenty-one per cent (5/24) of the patients with persistently normal LFTs had fibrosis stage F3F4. The proportion of F3F4 among HCV/HIV co-infected patients was significantly higher than among HCV mono-infected (52% vs. 33%; $P = 0.05$). Concordance of three or more biomarkers was present in 43% (57/132) of the patients. Liver biopsy could be avoided in 70% (92/132) using a practical assumption that if FT is in concordance with APRI and/or Forns, then we may confidently rely on the biomarker. Concordance rate for patients with presumably advanced or minimal liver disease was excellent (100% and 95% respectively). *Conclusions:* In our HCV-infected haemophilia patients, FT correctly identified clinically advanced or minimal liver disease. Discordance among the various biomarkers of fibrosis was considerate; nevertheless, practical combination of FT, APRI, and Forns may predict stage of fibrosis with accuracy, potentially avoiding liver biopsy in the majority of the patients.

Keywords: fibrosis, FibroTest, haemophilia, hepatitis C, liver biopsy, non-invasive biomarkers

Introduction

The majority of haemophilia patients who received coagulation factor concentrates that were not virally inactivated acquired hepatitis C (HCV) and many contracted HIV as well, becoming HCV/HIV co-infected [1–3]. Factor concentrates are routinely undergoing virucidal process since the mid-1980s; therefore, HCV-positive haemophilia patients are considered to harbour infection for 20 years or more [4]. During this time interval, 20% to 25% of patients infected with HCV will develop cirrhosis and its complications [3,5,6].

Staging liver fibrosis enables assessment of prognosis as well as therapeutic decision making in patients with HCV. Liver biopsy is currently considered the 'gold standard' for the assessment of liver

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histology [7], but it is occasionally prone to limitations. These limitations include its highly invasive nature and a risk of complications with morbidity between 0.3% and 0.6% and mortality of 0.05% [8]. 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 [9,10].

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 haemophiliacs, and no major complications of the procedure were reported in these studies [11–16]. However, coagulation factor replacement is required; therefore, the cost of liver biopsy is substantially higher in haemophilia patients.

Many studies have been performed to evaluate the use of readily available laboratory tests to predict significant fibrosis or cirrhosis in patients with HCV and substantially reduce the number of biopsies performed for the management of HCV infection [17–21]. The FibroTest score (FT) is computed with the patient's age, sex, and results of analyses of serum haptoglobin, α2-macroglobulin, apolipoprotein A1, gamma-glutamyl transpeptidase (GGT), and bilirubin levels. In the original studies as well as the validation cohorts, FT was found to have an area under the receiver operator curve (ROC) for the diagnosis of bridging fibrosis, F2F3F4 vs. F0F1 [17,22–25]. Wai *et al.* [18] developed the aspartate aminotransferase (AST) to platelets ratio index (APRI), which is the ratio between AST and platelet count. Forns *et al.* [19] developed the Forns score, which is an algorithm including platelet count, GGT, age and cholesterol level. A very simple index includes only age and platelet count [20]. Hyaluronic acid (HA) is a high molecular weight polysaccharide, which is an essential component of extracellular matrix in virtually every tissue in the body. It was shown that serum HA level increases with the development of liver fibrosis [21].

Among the aforementioned markers of fibrosis, FT has been more intensely studied and validated [17,22–25]. In addition, it has the advantage of having a corresponding stage of fibrosis for each FT score with minimal overlap among the various stages; therefore, it is devoid of the indeterminate zone in which the stage of fibrosis cannot be defined, an inherent characteristic of each of the other biomarkers of fibrosis.

Our aim was to assess liver fibrosis in haemophilia patients infected with HCV using non-invasive biomarkers without liver biopsy with reference to available clinical features. Moreover, we examined the utility of combining scores to improve the accuracy of predicting fibrosis, and to reduce the need of liver biopsy.

Materials 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 antiretroviral 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.

Virologic 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) with a dynamic range of 600 to <500 000 IU mL^{-1} [26]. Genotyping was preformed by direct sequencing of polymerase chain reaction generated amplicons from the 5′ non-coding region, followed by sequence comparison with a reference sequence database [27]. Persistently negative HCV RNA was defined based on at least three measurements 6 months apart. All viral studies were performed before the initiation of any anti-hepatitis 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 perform FT, serum samples were taken for determination of five serum biochemical markers: alpha2-macroglobulin, haptoglobin, GGT, total bilirubin and apolipoprotein A1. Biochemical marker analysis was performed in accredited laboratories following the guidelines recommended for FT assessment by the authors of the initial publication [17]. GGT, and total bilirubin levels were measured by Hitachi 917 Analyzer and Roche Diagnostics reagents (both Mannheim, Germany). Alpha2-macroglobulin, apolipoprotein A1, and haptoglobin were measured using a Modular analyser (BNII, Dade Behring; Marburg, Germany). Platelets were measured by Beckman Coulter LH 750. All biochemical analyses were performed on serum samples stored at −80°C and shipped to the Laboratoire ALPHABIO, Marseille, France. FT was calculated using the Biopredictive website according to the manufacturer's instructions. FT formula is available on the USPTO website (http://www.uspto.gov; Patent no. 6,631,330) [28]. Hyaluronate levels were measured with the Corgenix Hyaluronic Acid Test Kit, Corgenix Inc., CO, following the manufacturer's instructions (patients in fasting conditions, no physical effort). HA level was measured in duplicate (range 1–871 $\mu g\ L^{-1}$) and a pool control set was used [21]. Moreover, stored blood samples were collected for further biochemical determination and further evaluations of non-invasive fibrosis markers. All biochemical parameters and FT determinations were done without knowledge of the patients' clinical characteristics. The sera were collected and stored after the patients gave their informed consent. The study was approved by our Institutional Ethics Committee.

Predictive models

FibroTest, APRI, Forns, age-platelet index and HA were assessed and compared in this study for the patients with complete serum biochemical markers. FT calculations were assessed through the internet link of the Biopredictive group using FT formula described above. APRI score formula and Forns score age-platelet formula and measurement of HA were taken from respective publications [18–21]. We compared fibrosis determined by FT on a scale of 0–4 with respect to METAVIR fibrosis staging as described by the authors [22] (METAVIR is a cooperative French grading and staging system for HCV). For FT score from 0 to 0.21 fibrosis was staged F0, from 0.22 to 0.27 F0F1, from 0.28 to 0.31 F1, from 0.32 to 0.48 F1F2, from 0.49 to 0.58 F2, from 0.59 to 0.72 F3, from 0.73 to 0.74 F3F4 and from 0.75 to 1 F4.

The cut-off values for the biomarkers used were as suggested by the authors [18–21]:

Forns: Forns <4.2 indicated no fibrosis and Forns >6.9 indicated fibrosis.

APRI: APRI ≤0.5 indicated no fibrosis and APRI >1.5 indicated fibrosis. APRI ≤1 indicated no cirrhosis and APRI >2 indicated cirrhosis.

Age-platelet index: age-platelet ≤2 indicated no fibrosis and age-platelet >7 indicated fibrosis.

HA: HA <30 indicated no fibrosis and HA >90 indicated fibrosis.

The obtained scores were correlated with the aforementioned clinical features of the patients.

Discordance determination

Significant discordance between FT and APRI, Forns, age-platelet score, and HA was defined as discordance between the result of the test according to the respective cut-off value of each test and the result of fibrosis staging in the METAVIR scoring system. Concordance was defined when three or more biomarkers of fibrosis were in concordance with the FT. Discordance was defined when three or more test were disconcordant, were indeterminate for the particular biomarker (e.g. Forns index >4.2 and <6.9), or were not available. Non-conclusive for concordance was considered when two tests were concordant for the FT and the other two were discordant, indeterminate, or not available. Bourliere *et al.* have recently shown that if FT = APRI and/or Forns, then we may confidently rely on the FT and avoid the need of liver biopsy [29].

We considered that discordance was considered highly attributable to FT, APRI, Forns, or age-platelet score failure if one of the components of each test had an abnormal value attributable to a clinically identified condition. We considered FT failure to be an isolated abnormal value of one of the five FT components attributable to a clinically identified condition such as haemolysis with haptoglobin <0.30 $g\ L^{-1}$, inflammation or sepsis with haptoglobin >2 $g\ L^{-1}$ and/or alpha2-macroglobulin >3 $g\ L^{-1}$, Gilbert's disease, or an extra-hepatic cholestasis with elevated bilirubin and/or GGT. We considered APRI failure to be an abnormal value of one of the two components, especially normal AST value, that can

lead to underestimation of fibrosis. We considered Forns score failure to be an isolated abnormal value of one of the three biological Forns score components attributable to a clinically identified condition such as hypercholesterolemia, drug-induced GGT elevation or inflammation leading to high platelet value.

Statistical analysis

Continuous variables were compared by student *t*-test. Frequencies were compared using the two-tailed Fisher's exact test. A value of $P < 0.05$ was considered statistically significant. 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. There were 124 males (94%), the mean age was 39 ± 14 years. Haemophilia was diagnosed in 90% (119/132) of patients. Seventy-eight per cent (84/108) were infected with genotype 1. Estimated by the FT, the distribution of fibrosis in the 132 patients was F0F1 = 65% (86/132), F2 = 5% (7/132), F3 = 13% (17/132) and F4 = 17% (22/132). Given the small proportion of patients estimated F2 by the FT we combined F0F1 (minimal fibrosis) and F3F4 (advanced fibrosis) for further analysis.

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

Variable	Mean (±SD)
Age (years)	39 (14)
Male gender, *n* (%)	124 (94)
Coagulation disorder	
Haemophilia	119 (90.1)
Von Willebrand	7 (5.3)
Glanzmann	3 (2.3)
Other	3 (2.3)
Genotype, *n* (%)	
1	84 (77.8)
2/3	17 (15.7)
Other	7 (6.5)
AST (IU L^{-1})	39.1 (31.4)
ALT (IU L^{-1})	58.9 (47.5)
Gamma-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* (%)*	
0/1	86 (65)
2	7 (5)
3	17 (13)
4	22 (17)

*Estimated by the FibroTest.

Using the FT, all seven patients with *a priori* clinical suspicion of advanced liver disease were classified as F3F4, whereas all 21 patients with persistently HCV RNA-negative were classified as F0F1. Of the patients with persistently normal LFTs 20.8% (5/24) had fibrosis stage F3F4. The proportion of F3F4 among HCV/HIV co-infected patients was 51.9% (14/27), while in HCV mono-infected the rate of advanced fibrosis was 32.8% (20/61) ($P = 0.05$).

Discordance

Table 2 compares FT, APRI, Forns, age-platelet index and HA in diagnosis of fibrosis. Compared with FT, other biomarkers were able to classify fewer patients with regards to the absence (F0F1) or presence (F2F3F4) of fibrosis. Furthermore, absence of fibrosis was detected by the various biomarkers in a larger proportion of patients than detection of its presence. The rate of discordance of the FT with each biomarker of fibrosis was APRI 41.1% (53/129), Forns index 41.7% (53/127), age-platelet index 50.8% (67/132) and HA 56.1 (74/132). The rate of indeterminate results for each biomarker was APRI 33.3% (43/129), Forns index 37.8% (48/127), age-platelet index 39.4% (52/132) and HA 44.7% (59/132) (Table 3).

The rate of concordance of three or more biomarkers was 43.2% (57/132), discordance 40.9% (54/132) and non-conclusive for concordance 15.9% (21/132). Discordance secondary to three or more disconcordant tests (not considering indeterminate scores) occurred only in 4.5% (6/132). The rate of advanced fibrosis (F3F4) among concordant was 14% (8/57), among discordant 48.1% (26/54) and among non-conclusive 23.8% (5/21) ($P < 0.01$ for discordant vs. concordant, and $P = 0.03$ for discordant vs. non-conclusive). Among the clinically defined groups, the concordance rate was advanced liver disease – 85.7% (6/7), persistently HCV RNA-negative – 71.4% (15/21), persistently normal LFTs – 45.8% (11/24), HCV/HIV co-infection – 29.6% (8/27) (Table 4).

Table 2. FibroTest, APRI, Forns, Age-PLT and HA in the diagnosis of fibrosis.

Biomarker	No fibrosis (*N*)	Fibrosis (*N*)	Concordance with: [*N* (no fibrosis/fibrosis)] FT	APRI	Forns	Age-PLT
FT	86	46	–	–	–	–
APRI	73	13	77 (65/12)	–	–	–
Forns	66	13	72 (62/10)	63 (55/8)	–	–
Age-PLT	69	11	65 (58/7)	55 (51/4)	62 (54/8)	–
HA	37	35	55 (36/19)	36 (28/8)	40 (32/8)	34 (28/6)

FT, FibroTest; APRI, AST-to-platelet ratio index; Age-PLT, age-plateler index; HA, hyaluronic acid.

Table 3. Discordance and indeterminate rates among FT, Forns, APRI, Age-PLT, and HA.

Biomarker	Discordance, *n* (%)	Indeterminate, *n* (%)
APRI	53 (41.1)	43 (33.3)
Forns	53 (41.7)	48 (37.8)
Age-PLT	67 (50.8)	52 (39.4)
HA	74 (56.1)	59 (44.7)

FT, FibroTest; APRI, AST-to-platelet ratio index; Age-PLT, age-platelet index; HA, hyaluronic acid.

Table 4. Concordance among FT, Forns, APRI, Age-PLT, and HA in the clinically defined groups.

Group	Concordance, *n* (%) (≥3 Biomarkers)	Concordance, *n* (%) (FT = APRI and/or Forns)
Advanced liver disease ($n = 7$)	6 (85.7)	7 (100)
RNA-negative ($n = 21$)	15 (71.4)	20 (95.2)
Normal LFT ($n = 24$)	11 (45.8)	17 (70.8)
HCV/HIV ($n = 27$)	8 (29.6)	14 (51.9)

FT, FibroTest; APRI, AST-to-platelet ratio index; Age-PLT, age-platelet index; HA, hyaluronic acid.

Using Bourliere's assumption [29], that if FT = APRI and/or Forns, then we may rely on the FT, 69.7% (92/132) patients could be correctly staged and liver biopsy avoided. In 30.3% (40/132), FT staging was not satisfactory. The rate of advanced fibrosis (F3F4) among concordant was 16.3% (15/92), among discordant 60% (24/40) ($P < 0.01$ for concordant vs. discordant). With this assumption, the accuracy of staging among the aforementioned clinically defined groups was advanced liver disease – 100% (7/7), persistently HCV RNA-negative – 95.2% (20/21), persistently normal LFTs – 70.8% (17/24), HCV/HIV co-infection – 51.9% (14/27) (Table 4).

Isolated extreme values of serum bilirubin of 20.5 and 22.1 mmol mL^{-1} were observed in two patients in whom FT indicated F0F1 and F0 respectively. All other biomarkers were concordant with the FT stage of fibrosis in these patients.

Discussion

Liver biopsy is still considered the 'gold standard' for determining the stage of hepatic fibrosis [7]. Recently, non-invasive biomarkers of fibrosis have been developed [17–21]. Indications for the use of these biomarkers are still poorly defined. One of the more appealing indications for such biomarkers is for patients with absolute or relative contraindications for liver biopsy, e.g. congenital or acquired disorders of coagulation. In our patient population mainly with haemophilia, one of the biomarkers, the FT correctly identified advanced or minimal liver disease in HCV-infected patients without the use of liver biopsy.

The main limitation of the study was the absence of liver biopsies in our patients. Liver biopsy in patients with disorders of coagulation raises complex and sometimes emotional issues. Nonetheless, liver biopsies have been performed in haemophilia patients and are a common practice in some centres [11–13]. The procedure was performed per-cutaneously, trans-jugular, and even on an out-patient basis [14–16]. No major complications of liver biopsy were reported in these studies [11–16]. However, coagulation factor administration to achieve 100% replacement is required before and 12–36 h following the procedure. Therefore, the cost of liver biopsy is substantially higher in haemophilia patients. Furthermore, the procedure is precluded in those patients who have inhibitors to factor VIII.

Recent studies suggest that staging fibrosis based on liver biopsy is prone to sampling error. This can be minimized with adequate biopsy length, containing enough portal spaces [9,10]. In our experience trans-jugular biopsies are less likely to be of sufficient accuracy to stage fibrosis. Several studies indicate that biomarkers assessed the stage of fibrosis with a higher accuracy than liver biopsy [22,29]. One study found discordance attributable to biopsy failure in 18%, whereas failure of biomarkers was considered in 2% [22]). In another study, cirrhosis was diagnosed in 17 patients by the FT, while biopsy

determined cirrhosis only in four [29]. They recommended that non-invasive markers should be used as first-line assessment of liver fibrosis in patients with HCV. Weighing the risk–benefit ratio of this procedure, we decided against liver biopsy in our patient population.

Our cohort of haemophilia patients has been using non-viral inactivated concentrated coagulation factors until the mid-1980s, when inactivation became universal. Therefore, we can assume that these patients were infected with HCV or HCV/HIV for 20 years or more. This period allowed the natural history of HCV infection to diverge according to the patient's individual characteristics into a slow or a rapid fibroser [30]. In 65%, probably those defined as slow fibrosers, liver disease did not progress beyond F0F1 stage of fibrosis when assessed by the FT. At the other end of the spectrum, those defined as rapid fibrosers had by this time developed advanced fibrosis (F3F4). This is especially evident in the HCV/HIV co-infected patients where about half were found to be F3F4 by the FT assay. This leaves only a minority to be classified as F2.

The FT is computed with the patient's age, sex and results of analyses of serum haptoglobin, α2-macroglobulin, apolipoprotein A1, GGT and bilirubin levels. In the original studies as well as the validation cohorts, the FT was found to have an AUROC of 0.80 for the diagnosis of bridging fibrosis, F2F3F4 vs. F0F1. The FT gave a 100% negative predictive value (NPV) for the absence of significant fibrosis and a 91% positive predictive value (PPV) for its presence [17,22–25]. Large prospective studies demonstrated only ~18% discordance between FT and liver biopsy [22,23,29]. The main advantage of the FT over the other biomarkers of fibrosis is that for each FT score derived from the aforementioned variables there is a corresponding fibrosis stage with very little overlap between stages. The other biomarkers of fibrosis have a specific cut-off defined for the presence of fibrosis (F2F3F4), and another cut-off value for the absence of fibrosis (F0F1), with an indeterminate zone in between, where the stage of fibrosis cannot be defined. For most biomarkers the indeterminate zone is wide [18,19]. In our study, the indeterminate rates ranged from 33% for APRI to 45% for HA. The main shortcoming of FT is that some of its variables are influenced by conditions other than the liver disease itself. For example, a haematoma in patients with haemophilia, or haemolysis occurring with some of the active anti-retroviral medications, may cause an isolated increase of bilirubin or drop in haptoglobin levels, resulting in overestimation of the stage of fibrosis. In our cohort, there were only two cases of isolated elevation of bilirubin. Nevertheless, the FT stage of fibrosis was in concordance with all the other biomarkers of fibrosis. We routinely avoided FT determination in an advent of haematoma or fever.

The concordance rate among three or more biomarkers was relatively low at 43%. This high discordance rate was mainly because of indeterminate scores of APRI, Forns, age-platelet index and HA rather than true discordance among these biomarkers and the FT. True discordance occurred only in 4.5% of our population.

Furthermore, the proportion of advanced fibrosis (F3F4) was significantly lower (14%) among the concordant scores than among discordant ones (48%). Therefore, our data suggest a significantly lower PPV of FT for advanced fibrosis (F3F4) than the PPV for absence of fibrosis (F0F1).

Recently, Bourliere *et al.* have shown that if FT is in concordance with APRI and/or Forns, then FT correlates well with the stage of fibrosis based on liver biopsy [29]. In their series, 81% of patients could be correctly staged, and liver biopsy avoided. Liver biopsy remained mandatory for the evaluation of fibrosis in 19%. Application of this assumption to our patient population resulted in a more modest figure with 70% in whom liver biopsy could be avoided using this practical approach.

The concordance rates among the aforementioned clinically defined groups differed greatly. In patients in whom advanced liver disease was anticipated, the concordance rate among biomarkers of fibrosis was high with both a concordance of three or more biomarkers or with the Bourliere's assumption (6/7 and 7/7 respectively). Such was the case for the patients with persistently negative-HCV RNA, in whom minimal or mild disease was suspected (15/21 and 20/21 respectively). In contrast, for patients with persistently normal LFTs and more so for HCV/HIV co-infected patients, concordance rates among biomarkers of fibrosis were low. The concordance rate reached only about 50% for the co-infected patients even with the use of the Bourliere's approach. One study which evaluated FT in HCV/HIV co-infected patients found FT to be accurate in this population, with an AUROC of 0.86 for F2F3F4 vs. F0F1. However, in this study FT was evaluated in a larger cohort, and was analysed against liver biopsy [31]. Concomitant use of anti-retroviral drugs, liver steatosis, immunological factors and other variables associated with co-infection may account for the low concordance rate in this group in our study.

In our haemophilia patients infected with HCV, FT correctly identified clinically advanced or minimal liver disease without the use of liver biopsy. Discordance among the various biomarkers of fibrosis was considerate; nevertheless, practical combination of FT, APRI, and Forns may predict stage of fibrosis with accuracy, potentially avoiding liver biopsy in the majority of patients. Strategy combining FibroScan [32] and FT should further evaluate haemophilia patients.

In conclusion, we believe that biomarkers such as FT as first-line non-invasive assessment of liver fibrosis are particularly useful in HCV-infected haemophilia patients and should be considered in this population.

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Non-invasive biomarkers of liver fibrosis in haemophilia patients with hepatitis C: can you avoid liver biopsy?

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Summary. *Introduction:* Liver biopsy remains the gold standard for the evaluation of fibrosis despite its risks and limitations, especially in haemophilia patients. Recently, non-invasive biomarkers have been used to assess histological features. The most thoroughly evaluated biomarker is the FibroTest (FT) (AUROC 0.80 for fibrosis stages F2F3F4 vs. F0F1). *Aim:* To estimate liver fibrosis in haemophilia patients infected with hepatitis C (HCV) using non-invasive biomarkers without liver biopsy. *Methods:* One hundred and thirty-two haemophilia patients (124 male, mean age 38 ± 14 years) with anti-HCV antibodies were evaluated. These patients were stratified into several groups: patients with features of advanced liver disease – seven, persistently HCV RNA-negative – 21, persistently normal liver function tests (LFTs) – 24, HCV/HIV co-infected – 27. The following biomarkers of fibrosis were used: FT, AST-to-platelet ratio index (APRI), Forns index, age-platelet index and hyaluronic acid. The obtained scores were correlated with the clinical features of the patients. *Results:* Estimated by the FT, the distribution of the stage of fibrosis in the 132 patients was F0F1 = 65% (86/132), F2 = 5% (7/132), F3 = 13% (17/132) and F4 = 17% (22/132). Using FT, all patients with clinical suspicion of advanced liver disease were classified as F3F4, whereas patients with persistently HCV RNA-negative were all classified as F0F1. Twenty-one per cent (5/24) of the patients with persistently normal LFTs had fibrosis stage F3F4. The proportion of F3F4 among HCV/HIV co-infected patients was significantly higher than among HCV mono-infected (52% vs. 33%; $P = 0.05$). Concordance of three or more biomarkers was present in 43% (57/132) of the patients. Liver biopsy could be avoided in 70% (92/132) using a practical assumption that if FT is in concordance with APRI and/or Forns, then we may confidently rely on the biomarker. Concordance rate for patients with presumably advanced or minimal liver disease was excellent (100% and 95% respectively). *Conclusions:* In our HCV-infected haemophilia patients, FT correctly identified clinically advanced or minimal liver disease. Discordance among the various biomarkers of fibrosis was considerate; nevertheless, practical combination of FT, APRI, and Forns may predict stage of fibrosis with accuracy, potentially avoiding liver biopsy in the majority of the patients.

Keywords: fibrosis, FibroTest, haemophilia, hepatitis C, liver biopsy, non-invasive biomarkers

Introduction

The majority of haemophilia patients who received coagulation factor concentrates that were not virally inactivated acquired hepatitis C (HCV) and many contracted HIV as well, becoming HCV/HIV co-infected [1–3]. Factor concentrates are routinely undergoing virucidal process since the mid-1980s; therefore, HCV-positive haemophilia patients are considered to harbour infection for 20 years or more [4]. During this time interval, 20% to 25% of patients infected with HCV will develop cirrhosis and its complications [3,5,6].

Staging liver fibrosis enables assessment of prognosis as well as therapeutic decision making in patients with HCV. Liver biopsy is currently considered the 'gold standard' for the assessment of liver

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