ORIGINAL ARTICLE

Stage of liver fibrosis in patients with congenital bleeding disorders and hepatitis C virus infection

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KEY WORDS

FibroTest, hemophilia, hepatitis C, liver fibrosis, shear wave elastography

ABSTRACT

INTRODUCTION Hepatitis C virus (HCV) is the major cause of chronic liver disease in patients with hemophilia. However, since liver biopsy should not be routinely used in these patients, the accurate assessment of the stage of fibrosis has been limited so far.

OBJECTIVES The aim of this study was to determine the stage of liver fibrosis in HCV-infected patients with hemophilia by using noninvasive methods of fibrosis assessment, and to analyze the influence of risk factors on liver fibrosis.

PATIENTS AND METHODS The study included 71 HCV-infected patients with hemophilia and other congenital bleeding disorders. Patients were divided into 3 groups: HCV-RNA negative after successful treatment, HCV-RNA negative after spontaneous elimination of infection, and HCV-RNA positive. Liver fibrosis was measured with shear wave elastography and FibroTest. The risk factors for liver fibrosis were analyzed, including demographic factors, HCV genotype, coinfections, and comorbidities.

RESULTS Cirrhosis or significant fibrosis (METAVIR score >F2) was observed in 26.8% of the patients. The stage of fibrosis was associated with age and estimated duration of infection (P <0.001). Active and past HBV infection did not affect fibrosis. The stage of liver fibrosis was lower in patients with spontaneous clearance of HCV (P = 0.007).

CONCLUSIONS Patients in our study had a similar stage of liver fibrosis to that reported by other studies on hemophilia. The older age and long duration of infection are the main risk factors for advanced fibrosis. Noninvasive methods such as shear wave elastography and FibroTest may allow a proper assessment of the fibrosis stage in hemophilia patients, particularly when used together and in correlation with other clinical parameters. They may also be useful in other groups of HCV-infected patients.

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INTRODUCTION There is a high prevalence of hepatitis C virus (HCV) infection among patients with hemophilia and other congenital bleeding disorders. About 40% to 90% of these individuals are positive for anti-HCV antibodies in serum.[1-4] The incidence of liver disease in these patients is 17-fold higher than in the general population, and hepatocellular carcinoma is even 6-fold more common.[5,6] Moreover, complications of chronic hepatitis C, liver cirrhosis (eg, variceal hemorrhage, hepatic encephalopathy), and hepatocellular carcinoma are the second most common cause of death in patients with hemophilia.[7-9]

Progression of chronic liver disease is affected by such factors as age, sex, HCV genotype, inflammation activity, alcohol use, concomitant infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), other infections

associated with blood transfusions, the presence of metabolic disorders (diabetes, obesity, fatty liver, increased serum iron concentration), and cigarette smoking.[10-12]

The stage of liver fibrosis can be assessed by liver biopsy and histological examination of liver tissue or by new noninvasive methods. Recently, liver biopsy has become less popular because of potential complications and higher cost. According to current recommendations, in patients with chronic HCV infection, liver biopsy should not be used routinely but only in case of diagnostic doubts or if its result significantly affects the management.[13] The noninvasive methods include different types of elastography and mathematical models of serum fibrosis markers (eg, FibroTest [FT]).[14] The new noninvasive methods have enabled the assessment of liver fibrosis in patients in whom this measurement was so far not available due to contraindications to liver biopsy or refusal to perform the invasive procedure. These methods represent a new diagnostic approach, which is increasingly used by doctors of various specialties.

The aim of this study was to evaluate liver fibrosis in HCV-infected patients with bleeding disorders, with consideration of the factors that affect disease progression. To date, no study has been published assessing the stage of liver fibrosis in patients with congenital bleeding disorders in the Polish population.

PATIENTS AND METHODS

The study included 71 Polish individuals with congenital bleeding disorders and chronic HCV infection defined as positivity for anti-HCV antibodies in serum. All participants were adults and had signed a written consent form to participate in the study. Data were collected using the following procedures: history taking, physical examination, blood sampling for viral studies, and measurement of liver fibrosis using 2 methods: FT and shear wave elastography (SWE). Fibrosis was staged according to the METAVIR scoring system, from F0 (no fibrosis) to F4 (cirrhosis). In each patient, the examinations were conducted on the same day.

Shear wave elastography

Liver fibrosis was staged with real-time SWE, using the Aixplorer® US system (SuperSonic Imagine, Aix-en-Provence, France) with a convex broadband probe (SC6-1). Liver stiffness was measured in 5 different circular regions of interest on the right lobe of the liver, through intercostal spaces. Tissue elasticity was expressed in kilopascals; then, the mean value was calculated and presented using the METAVIR scoring system. The examinations were done in the fasting state.

FibroTest

The laboratory acted in compliance with the preanalytical and analytical recommendations required to achieve the fibrosis marker FT score (Biopredictive, Houilles, France). The FT score was calculated using the Biopredictive website at www.biopredictive.com. The results were analyzed according to a patented formula for fibrosis. The analysis was conducted in the fasting state. Conditions known to restrict the method accuracy were excluded (acute inflammation, hemolysis, acute hepatitis, massive liver necrosis, extrahepatic cholestasis, and hypercholesterolemia [with high values of high-density lipoprotein cholesterol]).

Patients had blood samples analyzed for serological tests for infections with hepatotropic viruses and bloodborne viruses. The following tests were performed: anti-HCV (Monolisa Anti-HCV Plus version 2, Bio-Rad, Marnes-la-Coquette, France), HIV Ag/Ab (Genscreen Ultra HIV Ag-Ab, Bio-Rad, Marnes-la-Coquette, France), HBsAg (Murex HBsAg version 3, Murex Biotech Limited, Dartford, United Kingdom), anti-HBc (ETI-AB-Corek Plus, DiaSorin S.p.A, Saluggia, Italy), and anti-HAV (ETI-AB-HAVK Plus Anti-HAV, DiaSorin S.p.A., Saluggia, Italy). Enzyme immunoassays were performed and interpreted according to the manufacturers' instructions. HCV RNA was determined by quantification technique, real-time PCR HCV (Real-TMQuant DX Sacace Biotechnologies, Como, Italy) using a Rotor-Gene 3000 analyzer (Corbett Research, Mortlake, New South Wales, Australia). The test was performed and interpreted according to the manufacturer's instructions. The HCV-RNA genotype was also determined (Versant HCV, HCV genotype 2.0 Assay [LiPA], Siemens Healthcare Diagnostics Inc, Tarrytown, New York, United States).

Statistical analysis

A statistical analysis was performed using the statistical package Statistica 64 v. 12 (StatSoft, Inc, Tulsa, Oklahoma, United States). Statistical tests were adjusted for the type of variables and analysis. For parametric comparisons, the analysis of variance (ANOVA) was used, and for nonparametric comparisons, the Mann–Whitney, Kruskal–Wallis ANOVA, and χ^2 tests were used. A P value of less than 0.05 was considered statistically significant.

RESULTS

The study group included 71 patients (67 men [94.4%]; mean [SD] age, 40.4 [12.5] years [range, 24–73 years]). Hemophilia A was reported in 60 patients (84.5%); hemophilia B, in 5 patients (7%); and von Willebrand disease and deficiencies of other plasma clotting factors, in 6 patients (8.5%). Severe hemophilia was diagnosed in 87.7% of the patients. Previous treatment with interferon alfa and ribavirin was reported in 28 patients; in 21 of these patients (75%), the treatment was successful.

Of 71 patients, 29 (40.8%) were positive for HCV RNA. Based on the presence of HCV RNA and a history of treatment, patients were divided into 3 groups: HCV-RNA negative after successful treatment (group 1); HCV-RNA negative after spontaneous HCV clearance (group 2), and HCV-RNA positive (no or failed treatment; group 3). The characteristics of the groups are presented in TABLE 1.

TABLE 1 Characteristics of the study groups: HCV-RNA negative after successful treatment (group 1); HCV-RNA negative after spontaneous HCV clearance (group 2), and HCV-RNA positive (group 3)

Variable		Group 1 n = 21 (100%)	Group 2 n = 21 (100%)	Group 3 n = 29 (100%)	*P* value
Age, y, mean (SD)		45.52 (2.71)	40.33 (2.71)	45.07 (2.31)	0.32
Male sex, n (%)		21 (100)	20 (95)	26 (90)	–
HCV genotypes[a], n (%)	Genotype 1	7 (33.33)	–	20 (68.96)	–
	Genotype 2	0 (0)		1 (3.44)	
	Genotype 3	8 (38.09)		4 (13.79)	
	Genotype 4	0 (0)		2 (6.89)	
	Mixed 1/4	0 (0)		1 (3.44)	
	Missing	6 (28.57)		1 (3.44)	
HCV viral load, IU/ml, n (%)	$\leq 8 \times 10^5$	–	–	11 (37.93)	–
	$>8 \times 10^5$			18 (62.07)	
HBsAg (+), n (%)		2 (9.52)	1 (4.76)	4 (13.79)	0.65[a] 0.29[b]
anti-HBc (+), n (%)		16 (76.19)	17 (80.95)	20 (68.96)	0.20[a] 0.095[b]
HBV DNA (+), n (%)		0	1(4.76)	2 (6.89)	–
anti-HAV (+), n (%)		6 (28.57)	4 (19.04)	11 (37.93)	0.49[a] 0.15[b]
HIV Ag/Ab, n (%)		0	0	1 (3.44)	–
BMI, kg/m^2		25.46 (1.05)	24.66 (1.05)	23.45 (0.89)	0.34
Alcohol consumption, n (%)[d]	0	6 (28.57)	6 (28.57)	10 (34.48)	<0.001[a] <0.001[b]
	1	10 (47.61)	6 (28.57)	14 (48.27)	
	2	3 (14.28)	6 (28.57)	2 (6.89)	
	3	2 (9.52)	3 (14.28)	3 (10.34)	

For age and BMI parametric comparisons, the analysis of variance was used; for nonparametric comparisons, the χ^2 test was used.

a Group 1 vs group 2

b Group 1 vs group 3

c The genotype was determined on the basis of medical records.

d Frequency of alcohol consumption: up to 4 standard drinks (a standard drink in Poland contains about 10 g of alcohol): 0, up to twice a month; 1, up to once a week; 2, every other day; 3, daily

Abbreviations: anti-HAV, anti-hepatitis A virus antibodies; anti-HBc, anti-hepatitis B core total antibodies; BMI, body mass index; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV Ag/Ab, human immunodeficiency virus antigen and antibodies

The results of SWE and FT were expressed as the METAVIR score of liver fibrosis. The mean fibrosis score for the entire study group was 0.84 for SWE and 1.21 for FT. The percentages of patients with significant fibrosis (METAVIR >F2) were 4.48% (3 of 67 patients) for SWE and 26.47% (18 of 68 patients) for FT. The results of liver fibrosis measurement differed significantly between the 2 methods. The results of fibrosis staging assessed with SWE and FT depending on the presence of HCV RNA are shown in TABLES 2 and 3, respectively.

Cirrhosis or significant fibrosis (METAVIR >F2) was observed in 19 patients (26.8%). Minimal or no fibrosis was observed in 52 patients (73.2%). A comparative analysis of the 2 groups depending on the factors that may have affected the progression of fibrosis is presented in TABLE 4. No cases of hepatocellular carcinoma or end-stage liver disease were diagnosed.

DISCUSSION An accurate assessment of liver fibrosis is essential for the proper management and prognosis in chronic liver disease. Liver biopsy is considered a reference method for the assessment of liver fibrosis, but limitations of this procedure (ie, invasiveness, sampling error, interobserver variability, and cost) have contributed to the development of noninvasive methods. Two different approaches, serum fibrosis markers and radiologic methods, have been used. The radiologic approach is based on ultrasonography, magnetic resonance imaging, and elastography, while serum biomarkers of fibrosis and their combinations with other serum tests and clinical parameters have been developed as complex algorithms. Currently, noninvasive methods are widely available and, in many cases, spare patients from liver biopsy. The advantages of noninvasive methods include the lack of contraindications and complications. They are also useful

TABLE 2 Shear wave elastography (SWE) results in the study groups (n = 67): HCV-RNA negative after successful treatment (group 1); HCV-RNA negative after spontaneous HCV clearance (group 2), and HCV-RNA positive (group 3)

Liver fibrosis (METAVIR score)	Group 1 (n = 20)	Group 2 (n = 19)	Group 3 (n = 28)
0	7 (35)	10 (52.63)	6 (21.4)
>0 – <2	11 (55)	8 (42.1)	17 (60.7)
2–3	2 (25)	1 (5.26)	3 (10.7)
>3–4	0	0	2 (7.1)

Data are presented as number (percentage) of patients. Kruskal–Wallis ANOVA: $P = 0.82$.

For SWE, the average liver fibrosis score in group 1, group 2, and group 3 was 0.72, 0.53, and 1.12, respectively.

TABLE 3 FibroTest (FT) results in the study groups (n = 67): HCV-RNA negative after successful treatment (group 1); HCV-RNA negative after spontaneous HCV clearance (group 2), and HCV-RNA positive (group 3)

Liver fibrosis (METAVIR score)	Group 1 (n = 20)	Group 2 (n = 19)	Group 3 (n = 28)
0	9 (42.85)	13 (68.42)	8 (28.6)
>0 – <2	6 (28.57)	5 (26.31)	8 (28.6)
2–3	0	1 (5.26)	6 (21.4)
>3–4	6 (28.57)	0	6 (21.4)

Data are presented as number (percentage) of patients. Mann–Whitney test: group 1 vs group 2, $P = 0.08$; group 1 vs group 3, $P = 0.17$; group 2 vs group 3, $P = 0.002$. Kruskal–Wallis ANOVA: $P = 0.005$.

For FT, the average liver fibrosis score in group 1, group 2, and group 3 was 1.19, 0.39, and 1.79, respectively.

to determine and differentiate between advanced fibrosis stages and allow the dynamic assessment of fibrosis over time. Simplicity and ease of use makes these methods an effective diagnostic tool in every day clinical practice.[14-16]

Chronic liver disease in individuals with hemophilia is associated mainly with HCV infection. HCV infection in these patients leads to liver cirrhosis in 10% to 30% of patients after 20 to 30 years.[17-21] In our study, we used the recent noninvasive methods of fibrosis assessment to evaluate the stage of fibrosis.[14-16] FT has been used for more than 10 years, while SWE is a new method that has been investigated in a limited number of studies in patients with hemophilia.

In our study, the stage of fibrosis in patients with hemophilia was similar to that reported by other studies (using FT): advanced fibrosis (MICROVIR >F2) was observed in 26.8% of the patients, determined with at least one of the methods (SWE or FT).[22,23] Only 1 patient with spontaneous clearance of HCV infection had advanced fibrosis, which was attributed to the presence of additional risk factors such as past HBV infection and regular alcohol consumption. There was no difference in the fibrosis stage between patients with hemophilia A, hemophilia B, and other coagulation disorders (however, 84.5% of our patients were diagnosed with hemophilia A).

The most important risk factor for advanced fibrosis in our study was age. Probably, our patients acquired HCV infection in the early years of life, when nonvirus-inactivated blood products were used. In young patients, fibrosis progresses at a much slower rate than in older patients, and in patients infected at a young age, the progression is slower regardless of the duration of the disease.[19,24-26] Poynard et al[24] demonstrated that after 20 years of infection, cirrhosis developed in 63% of patients infected at an age older than 50 years and only in 2% of patients infected before 20 years of age. Importantly, the progression of fibrosis is not linear.[24] On the other hand, the estimated duration of HCV infection was long. Our study group was still relatively young, and it is possible that the acceleration of fibrosis would be observed in the future.

Although there were differences in the distribution of HCV genotypes, the group with a known genotype was too small for proper interpretation. We also noted significant differences in the rate of past hepatitis A virus (HAV) infection markers; however, this finding was attributed to a much higher incidence of HAV infection in Poland until the 1980s, resulting in a higher rate of anti-HAV antibody positivity in older patients.[27-29]

Coinfections with HBV and HIV are well-established risk factors for faster progression of liver fibrosis.[30-35] Hence, it is important to vaccinate patients against HBV, which is highly effective.[36] In our group, 7 patients had an active HBV infection, but we did not show any association with advanced fibrosis. Only 1 person was infected with HIV. The number of coinfected patients was too low to show any impact on liver fibrosis.

The stage of liver fibrosis differed between the subgroups classified according to HCV elimination and successful treatment.[37] Regardless

TABLE 4 Comparison of the group with significant fibrosis (METAVIR >F2) with the group with minimal or no fibrosis (METAVIR, F0–F2)

Variable		METAVIR F0–F2 n = 52 (100%)	METAVIR >F2 n = 19 (100%)	*P* value
Age, y, mean (SD)		39.56 (10.4)	55.42 (10.13)	<0.001
Male sex, n (%)		48 (92.3)	19 (100)	0.21
Hemophilia A, n (%)		45 (86.53)	15 (78.94)	0.13
Hemophilia B, n (%)		3 (5.76)	2 (10.52)	
Other coagulation disorders, n (%)		4 (7.69)	2 (10.52)	
HCV RNA (+), n (%)		17 (32.69)	12 (63.15)	0.021
Spontaneous clearance, n (%)		20 (38.46)	1 (5.26)	0.007
Successful HCV treatment, n (%)		15 (28.84)	6 (31.57)	0.072
HCV genotypes[a], n (%)	Genotype 1	13 (76.47)	7 (58.33)	–
	Genotype 2	0 (0)	1 (8.33)	
	Genotype 3	2 (11.76)	2 (16.66)	
	Genotype 4	1 (5.88)	1 (8.33)	
	Mixed 1/4	0 (0)	1 (8.33)	
	Missing	1 (5.88)	0	
HCV viral load, IU/ml, n (%)	$>8\times10^5$, IU/ml	9/17 (52.94)	9/12 (75.00)	0.56
	$<8\times10^5$, IU/ml	8/17 (47.05)	3/12 (25.00)	0.41
anti-HBc (+), n (%)		38 (73.07)	15 (78.94)	0.16
HBsAg (+), n (%)		4 (7.69)	3 (15.78)	0.31
anti-HAV (+), n (%)		2 (3.84)	9 (47.36)	0.0001
HIV Ag/Ab (+), n (%)		0	1 (5.26)	0.096
BMI, kg/m^2, mean (SD)		24.55 (4.19)	24.00 (6.32)	0.67
ALT, U/l, mean (SD)		30.4 (21.22)	36.33 (26.84)	0.67
GGTP, U/l, mean (SD)		33.00 (11.68)	38.83 (22.57)	0.60
Lipids, mg/dl, mean (SD)		96.94 (44.12)	92.47 (33.98)	0.69

Abbreviations: ALT, alanine transaminase; GGTP, γ-glutamyl transpeptidase; others, see **TABLE 1**

a The genotype was determined on the basis of medical records.

of the method used, the lowest stage was observed in patients with spontaneous elimination of HCV infection, and the highest stage, in untreated individuals.

The significant discrepancies between the results of FT and SWE, particularly in the scope of advanced fibrosis (>2), require a comment. FT is registered for use in patients with hemophilia and was performed in a certified laboratory, according to the manufacturer's recommendations.[23,38,39] SWE has been used in hemophilia patients in several studies.[40,41] We cannot exclude that the higher fibrosis stage in FT may be caused by additional factors associated with hemophilia itself. For example, Maor et al[23] discussed the effect of some pathological states in hemophilia that may explain the overestimation of liver fibrosis by FT.[23] The detailed analysis of the discrepancies will be the subject of a separate publication.

We could not refer the results of SWE and FT in our patients to those of liver biopsy; however, we assume that these methods may properly assess the stage of liver disease in hemophilia patients, particularly when used together and in correlation with other clinical parameters.

In conclusion, our study showed that the stage of liver fibrosis in Polish patients with congenital bleeding disorders and HCV infection is similar to that reported in other studies on hemophilia. Considering the presence of multiple risk factors for the progression of liver disease, we were expecting to confirm advanced fibrosis in most patients; meanwhile, the percentage of patients with significant fibrosis was 26.8%. Older age and estimated duration of infection are the main risk factors for advanced fibrosis. On the other hand, the lowest stages of fibrosis were observed in patients with spontaneous elimination of HCV. Our results emphasize the role of noninvasive methods of fibrosis assessment, SWE and FT. Both methods may properly assess the stage of liver disease in patients with hemophilia, particularly when used together and in relation to other clinical parameters. However, the significant discrepancies between the results of both methods require further studies. The noninvasive techniques of liver fibrosis assessment may also be useful in other patients with contraindications to liver biopsy.

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