Clinical and Experimental
HEPATOLOGY

Original paper

Non-invasive evaluation of the liver disease severity in children with chronic viral hepatitis using FibroTest and ActiTest – comparison with histopathological assessment

Maria Pokorska-Śpiewak, Barbara Kowalik-Mikołajewska, Małgorzata Aniszewska, Magdalena Pluta, Magdalena Marczyńska

Department of Children's Infectious Diseases, Medical University of Warsaw, Poland
Hospital of Infectious Diseases in Warsaw, Poland

Abstract

Aim of the study was to evaluate liver disease severity in children with chronic viral hepatitis using the FibroTest and ActiTest (FT/AT), and compare the results with the liver biopsy.

Material and methods: The study included 11 treatment-naïve children [mean age, 9.0 ± 3.0 years, 10 infected with hepatitis C virus (HCV), and 1 with hepatitis B virus (HBV)] who underwent an FT/AT. Ten of the children underwent a liver biopsy. The histopathological evaluation was based on the METAVIR scoring system. The FT/AT and METAVIR scores were considered concordant if the necroinflammatory activity or the fibrosis did not differ by more than one grade or stage. To analyze the agreement between the FT/AT and the histopathological evaluation, the inter-rater agreement (kappa) was used.

Results: In the histopathological evaluation, most children presented with mild necroinflammatory activity (METAVIR A1) and with minimal to mild fibrosis (METAVIR F1-2). Both the AT and FT values did not show any linear increases with advancing METAVIR scores A and F, respectively. A discordance between the FT and METAVIR scores was observed in 3/10 (30%) cases; concordance between the AT and METAVIR scores was found in 9/10 cases. The inter-rater agreement test showed poor agreement between the FT/AT and the histopathological evaluation (kappa for AT: 0.0667, and kappa for FT: 0.176).

Conclusions: The FT and AT values poorly correlate with histopathological evaluation. Further studies on non-invasive methods to evaluate liver disease severity in children with chronic viral hepatitis are needed.

Key words: ActiTest, FibroTest, chronic hepatitis, children, liver biopsy.

Address for correspondence

Maria Pokorska-Śpiewak, Department of Children's Infectious Diseases, Medical University of Warsaw, 37 Wolska St., 01-201 Warsaw, Poland, phone: +48 22 335 52 50, e-mail: mpspiewak@gmail.com

Introduction

Liver biopsy is a standard method used for obtaining liver tissue to assess necroinflammatory activity and fibrosis [1, 2]. Liver biopsy remains the gold standard for the evaluation of liver disease in patients with chronic viral hepatitis B and C despite its invasive procedure [1]. Limitations of the liver biopsy in pediatric patients (e.g., its invasive nature requiring general anesthesia, complications, sampling errors, and intra-observer variability) have paved the way for the development of non-invasive methods to assess liver disease severity. Therefore, so far, the following two main types of non-invasive methods have been studied: novel imaging studies (e.g., elastography) and combinations of biomarkers. Several combinations of serum biomarkers were evaluated for their

ability to determine the grade of necroinflammatory activity and stage of fibrosis [3]. In the case of chronic viral hepatitis, the most commonly used tests are the FibroTest (FT) and ActiTest (AT) [4]. FibroTest and AT (BioPredictive, Paris, France) are non-invasive biomarkers used for the evaluation of liver fibrosis and necroinflammatory activity, respectively. FT combines the quantitative results of the following five serum markers: alpha-2-macroglobulin, haptoglobin, apolipoprotein-A1, bilirubin, and gamma-glutamyl-transpeptidase. ActiTest supplementary includes alanine aminotransferase (ALT) as the sixth marker. These biomarkers are combined with the patient's age and sex data in a patented artificial algorithm to provide the results of the FT and AT, which range between 0.00 and 1.00 [5]. Then, the results are converted to METAVIR grades and stages, which are calculated from the median scores and 95% confidence intervals (CI) [4, 6].

FibroTest and AT have been validated in adult patients with chronic viral hepatitis B and C with prognostic values at least similar to that of liver biopsy [7]. Pediatric patients with chronic viral hepatitis could mostly benefit from the introduction of non-invasive methods to evaluate liver disease in routine medical practice. However, none of these methods have been validated in children so far; therefore, these methods cannot substitute for liver biopsy in pediatric patients with chronic viral hepatitis [1, 6]. Thus, the aim of this retrospective clinicopathological study was to evaluate the necroinflammatory activity and fibrosis in children with chronic viral hepatitis using non-invasive serum biomarkers tests, FT, and AT, and compare the results with the histopathological assessment.

Material and methods

Patient characteristics

We retrospectively analyzed the results of FT and AT performed between 2012-2013 in consecutive treatment-naïve patients chronically infected with hepatitis B virus HBV and hepatitis C virus HCV. Non-invasive serum biomarker analysis and liver biopsies were performed simultaneously as a part of qualification for the antiviral treatment, according to the current European recommendations [8, 9].

The diagnosis of chronic viral hepatitis was established in patients with at least 6-month history of hepatitis based on elevated ALT and aspartate aminotransferase (AST) serum levels and positive serological testing (HBsAg for HBV infection and anti-HCV for HCV infection), which was confirmed by nucleic acid testing (positive HBV DNA or HCV RNA real-time

polymerase chain reaction [RT-PCR] method; Amplicor, Roche and Cobas TaqMan, Roche). Biochemical and serological testing was performed using commercially available laboratory kits (Vitros, Ortho-Clinical Diagnostics, Johnson & Johnson).

Children with known contraindications for FT/AT (acute hepatitis, hemolysis, Gilbert's disease, or extrahepatic cholestasis), and patients with HBV/HCV coinfection, HDV or HIV infection, autoimmune hepatitis, Wilson's disease, or alpha-1 antitrypsin deficiency were excluded from this study. Patients' weights and heights were recorded on the day of the FT/AT examination. Body mass index standard deviation scores (BMI z-scores) were calculated according to the WHO Child Growth Standards and Growth reference data, using the WHO Anthropometric calculator AnthroPlus v. 1.0.4.

FibroTest and ActiTest evaluation

FibroTest and AT were performed according to the published laboratory analytic recommendations [5, 7]. Serum markers were assayed using either the spectrophotometric method (for ALT, bilirubin, gamma-glutamyltranspeptidase) or immunoturbidimetric analysis (alpha-2-macroglobulin, haptoglobin, apolipoprotein-A1) using a Cobas 6000 analyzer (Roche Diagnostics). The FT and AT scores were determined using the manufacturer's dedicated website (www.biopredictive.com). Fibrosis as determined by FT was staged with respect to the METAVIR scoring system according to the following cut-offs: F0: 0.00-0.21; F0-1: 0.22-0.27; F1: 0.28-0.31; F1-2: 0.32-0.48; F2: 0.49-0.58; F3: 0.59-0.72; F3-4: 0.73-0.74; and F4: 0.75-1.00. The grades of the necroinflammatory activity were as follows: A0: 0.00-0.17; A0-1: 0.18-0.29; A1: 0.30-0.36; A1-2: 0.37-0.52; A2: 0.53-0.60; A2-3: 0.61-0.62; and A3: 0.63-1.00 (www.biopredictive.com).

Histological evaluation

Percutaneous liver biopsy was performed using the Menghini needle (Hepafix kit 1.4 or 1.6 mm, Braun). A histopathological evaluation was performed by an experienced pathologist who was unaware of the clinical data. To histologically evaluate necroinflammation and fibrosis, the METAVIR scoring system was used [10]. The necroinflammatory activity was graded using a four-point scale, in which A0 indicates no activity; A1 indicates minimal or mild activity; A2 indicates moderate activity; and A3 indicates severe activity. Fibrosis was staged on a 5-point scale (F0 – no fibrosis; F1 – portal fibrosis without septa; F2 – portal fibrosis with few

septa; F3 – numerous septa without cirrhosis; and F4 – cirrhosis).

The FT/AT and METAVIR scores were considered concordant if the necroinflammatory activity or the fibrosis did not differ by more than one grade or stage.

Statistical analysis

Continuous variables were expressed as either mean ± SD or medians with interquartile ranges (IQR) as required and were tested for normal distribution with the Kolmogorov-Smirnov test. Data were compared using the χ^2 test or Fisher's exact test for categorical variables and Student's t-test or the Mann-Whitney test for continuous variables. To analyze agreement between the FT/AT and the histopathological evaluation, the inter-rater agreement (kappa) was used. A perfect agreement would equate to a kappa of 1, and a chance agreement would equate to 0. A linear regression analysis was conducted to identify factors associated with FT and AT, and Pearson correlation coefficients were obtained. A multiple regression was performed with the following variables (candidate predictors) entered into the model irrespective of the results of the univariate analysis: BMI z-score, age, sex (for male), and the duration of the infection. After entering the variables into the model, those variables that showed the least significant associations were subsequently excluded until all variables remained significant ($p < 0.05$). The model fit for the multiple regression was assessed with the coefficient of determination, R^2, and the adjusted R^2, the coefficient of determination adjusted for the number of independent variables in the model.

All statistical analyses were performed using Med-Calc Statistical Software, version 16.4.3 (MedCalc, Mariakerke, Belgium). A two-sided p-value of < 0.05 was considered significant.

Ethical statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients to be included in the study.

Results

Study population

FibroTest and AT were performed in 11 children (5 boys and 6 girls) aged 6-16 years. Ten were chronically infected with HCV, and 1 was infected with HBV. Most of the children were infected vertically. Liver biopsy was performed in all children, except a 7-year-old girl who was infected with HCV genotype 3. Baseline characteristics of the study group are presented in Table 1.

FibroTest and ActiTest evaluation

The mean FT value in this group was 0.18 ± 0.08, and, in all cases, the FT value classified patients into groups with no to minimal fibrosis (METAVIR F0-1). The mean AT score was 0.29 ± 0.12, which was interpreted as no to moderate necroinflammatory activity according to the METAVIR score (A0-A2) (Table 2).

Histopathological evaluation

All of the obtained liver biopsy specimens were of good quality for an accurate diagnosis. The mean length of the needle biopsies was 24.5 ± 7.0 mm (range, 15-36 mm), and the number of portal tracts was between 11 and 37. Most children presented with mild necroinflammatory activity (METAVIR A1 score in 80% of patients), and with minimal to mild fibrosis (METAVIR F1-2 in 80% of cases) (Table 2). We did not observe any case of liver cirrhosis in this group.

Comparison between FT/AT and histopathological evaluation

Neither the AT or FT values showed any linear increase with advancing METAVIR scores A and F, respectively (Fig. 1). A discordance between the FT and METAVIR scores was observed in 3/10 (30%) cases with FT showing no or only minimal fibrosis, while the histopathological evaluation revealed portal fibrosis with septa (METAVIR F2) (Fig. 2A). Concordance between the AT and METAVIR scores was found in 9 of 10 cases. In 1 patient, the AT result suggested more advanced necroinflammation compared to the liver biopsy (Fig. 2B). The inter-rater agreement test showed poor agreement between the FT/AT and the histopathological evaluation (kappa for AT: 0.0667; SE 0.05; 95% CI: –0.04-0.179, and kappa for FT: 0.176; SE 0.1; 95% CI: –0.01-0.372).

Factors associated with FT and AT

Both the univariate and multivariate linear regression analyses revealed that AT was positively associated with the patients' BMI z-scores (Table 3). In addition, the univariate analysis showed that the male sex and longer durations of infection were positively associated with the FT value ($p = 0.009$ and $p = 0.04$, respectively) (Table 4).

Table 1. Clinical and laboratory characteristics of the study group

Characteristics	Children with AT/FT	Children with LB
Number	11	10
Sex, male (%)/female (%)	5 (45)/6 (55)	5 (50)/5 (50)
Age at liver biopsy (years), mean ± SD	9.0 ± 3.0	9.4 ± 3.0
Duration of infection (years), mean ± SD	8.5 ± 2.1	9.0 ± 2.2
BMI z-score, mean ± SD	0.5 ± 1.0	0.6 ± 1.1
Mode of infection, vertical (%)	10 (91)	9 (90)
Type of infection		
HCV (%)/HBV (%)	10 (91)/1 (9)	9 (90)/1 (10)
HCV genotype (%)		
1b	9 (82)	9 (90)
3	1 (9)	
4	1 (9)	1 (10)
HBV genotype		
A/D	1	1
Viral load (IU/ml)		
HCV median (IQR)	8.48×10^5 ($2.66 \times 10^5 - 1.16 \times 10^6$)	1.10×10^6 ($3.0 \times 10^5 - 1.27 \times 10^6$)
HBV	2.82×10^8	2.82×10^8
Laboratory findings, mean ± SD		
Alpha-2-macroglobulin (g/l)	3.44 ± 0.43	3.44 ± 0.45
Haptoglobin (g/l)	0.92 ± 0.32	0.92 ± 0.34
Apolipoprotein-A1 (g/l)	1.55 ± 0.19	1.54 ± 0.20
Bilirubin (μmol/l)	8.54 ± 3.67	8.40 ± 3.80
Gamma-glutamyl transpeptidase (IU/l)	23.45 ± 14.19	24.40 ± 14.59
Alanine aminotransferase (IU/l)	57.54 ± 20.64	59.80 ± 20.28
FibroTest, mean ± SD	0.18 ± 0.08	0.18 ± 0.08
ActiTest, mean ± SD	0.29 ± 0.12	0.30 ± 0.12

There were no significant differences between the both groups. AT – ActiTest, FT – FibroTest, LB – liver biopsy, BMI – body mass index, HCV – hepatitis C virus, HBV – hepatitis B virus

Discussion

The role of FT and AT has been analyzed in several cohorts of patients, including children [6, 11-15]. A meta-analysis performed by Poynard *et al.*, which included 3,501 patients with chronic HCV infection and 1,457 with chronic HBV, revealed that FT was an effective alternative to liver biopsy in adult patients. The standardized sensitivity analyses of the FT diagnostic values (the area under the receiver operating characteristic curve, AUROC) were 0.85 (0.82-0.87) for HCV-infected patients, and 0.80 (0.77-0.84) for chronic HBV infections [15]. The results of the FT and AT studies in children are inconsistent. Described by de Ledinghen *et al.*, the largest cohort of children in which FT was performed comprised 116 participants with different liver diseases, including 20 children with

chronic hepatitis B and C [6]. The FT results were compared with transient elastography (FibroScan) and liver biopsy, which was performed in 33 children but only 2 children were from the hepatitis B and C subgroups. Both the FT and FibroScan results correlated with the METAVIR fibrosis scores. For the diagnosis of cirrhosis (METAVIR F4), the AUROC for FT and FibroScan were 0.73 and 0.88, respectively [6]. El-Shabrawi *et al.* examined 50 children who were chronically infected with HCV, and found a highly significant linear trend of a correlation between the FT results and the fibrosis stage evaluated on the histopathological evaluation [16]. The same observation was made for the necroinflammatory activity. In addition, the authors demonstrated that FT may successfully discriminate between patients with no fibrosis (F0) and those with fibrosis scored at F1-F4, as well as between patients with mild

fibrosis (F1) and those with advanced fibrosis (F2-F4). However, Hermeziu *et al.* demonstrated concordance between the FT-AT and histopathological METAVIR scores in 10 cases, and discordance in 11 pairs of examinations in children with chronic hepatitis C [13]. Sokucu *et al.* showed no correlation between the FT/AT scores and the histopathological findings in their 25 pediatric patients with chronic hepatitis B [14]. In addition, none of the 9 cases of significant histological fibrosis were predicted by FT, whereas 15/19 patients with a significant AT value had insignificant histological necroinflammatory activity. Similar observations were made in our group as follows: in the 3/10 cases of discordance between the FT value and histological fibrosis, where the FT result did not predict the METAVIR F2 fibrosis, and, in one case, the AT was significantly higher compared to the histological activity. This observation suggests that FT may not show significant fibrosis, whereas AT may overrate the necroinflammatory activity in pediatric patients.

One of the postulated limitations of the biomarkers analyses is that they identify cirrhosis or no fibrosis; however, according to the European Association for the Study of the Liver (EASL), they are less reliable in resolving intermediate degrees of fibrosis [1, 9]. Because histopathological features in pediatric patients with chronic hepatitis B and C are usually mild and cirrhosis or advanced fibrosis are rarely observed, this could be an explanation as to why the FT and AT do not correlate adequately with the histopathological evaluation.

The main limitation of this study was the small number of patients in the study group. However, liver biopsy is currently rarely performed in children, and, as stated above, there are only a few similar studies in

Table 2. Necroinflammatory activity and fibrosis according to the ActiTest/FibroTest and the histopathological evaluation (METAVIR scoring system)

Histopathological findings	AT/FT ($n = 11$) (%)	Liver biopsy (METAVIR) ($n = 10$) (%)
Grade of necroinflammatory activity		
A0	2 (19)	1 (10)
A0-1	3 (27)	–
A1	3 (27)	8 (80)
A1-2	3 (27)	–
A2	–	1 (10)
A2-3	–	–
A3	–	–
Stage of fibrosis		
F0	6 (55)	2 (20)
F0-1	4 (36)	–
F1	1 (9)	5 (50)
F1-2	–	–
F2	–	3 (30)
F3	–	–
F4	–	–

which non-invasive tests were compared with histopathological evaluations. Therefore, the results of even such a small study seem to have a significant value and, together with other published studies in this field, could influence the everyday medical practice with this group of patients. Another issue is the fact that all children in this group had no to mild histopathological changes in the liver tissue, which precluded analysis of the possibility of the FT and AT to distinguish between
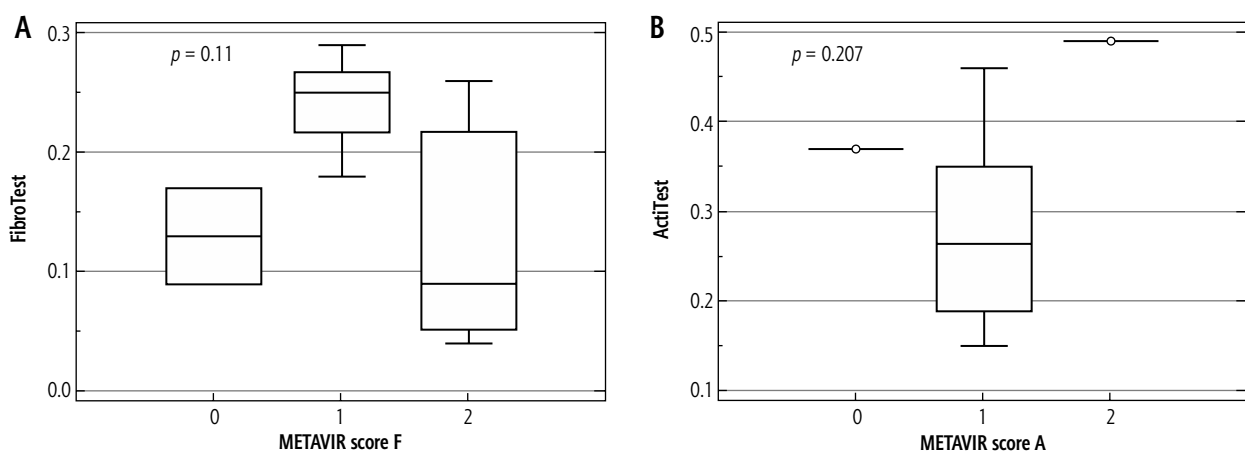

Fig. 1. A) Distribution of FibroTest values according to advancing METAVIR F scores. **B)** Distribution of ActiTest values according to advancing METAVIR A grades. Top and bottom of box plots are first and second quartiles. Line through the middle of the box represents the median. Error bars show the range (minimum and maximum values)
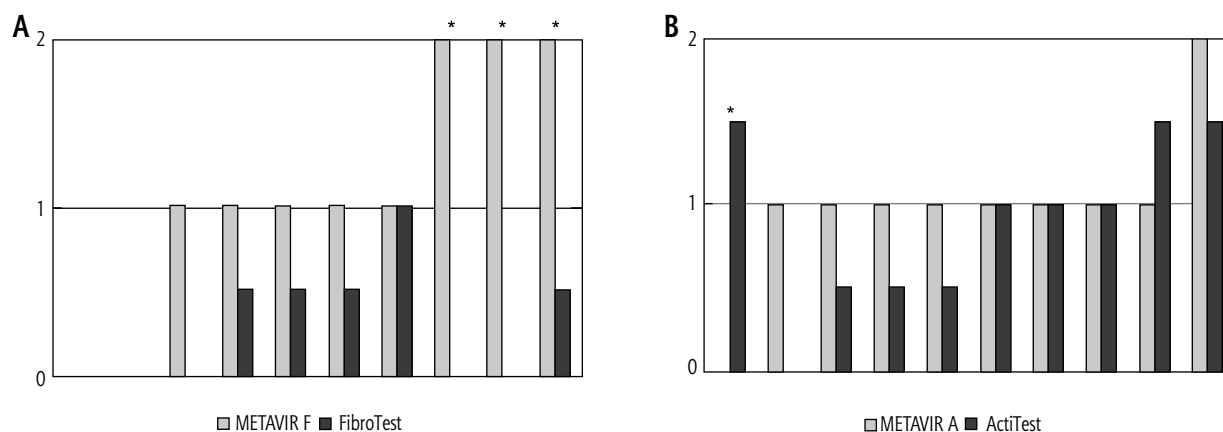
Maria Pokorska-Śpiewak, Barbara Kowalik-Mikołajewska, Małgorzata Aniszewska, Magdalena Pluta, Magdalena Marczyńska

Fig. 2. A) Comparison between METAVIR F scores resulting from the histopathological evaluation and the FibroTest results for each patient. Patients were ordered according to the advancing METAVIR histopathological scores. In the case of FibroTest F0-1, the result was presented as 0.5. (*) Indicates discordance between the histopathological evaluation and the FibroTest (difference by more than one stage). **B)** Comparison between METAVIR A grades resulting from the histopathological evaluation and the ActiTest results for each patient. Patients were ordered according to the advancing METAVIR histopathological grades. In the case of ActiTest A0-1, the result was presented as 0.5; in case of A1-2 as 1.5. (*) Indicates discordance between histopathological evaluation and ActiTest (difference by more than one grade)

Table 3. Factors associated with the ActiTest

Factor	Univariate analysis		Multivariate analysis	
	r (95% CI)	p	β (SE)	p
BMI z-score	0.75 (0.28-0.93)	0.007	0.08 (0.03)	0.04
Age	−0.46 (−0.83-0.18)	0.14	−0.004 (0.02)	0.83
Sex (for male)	−0.17 (−0.69-0.47)	0.61	0.09 (0.08)	0.28
Duration of infection	−0.45 (−0.83-0.19)	0.15	−0.01 (0.03)	0.60
			Model performance	
			R^2	0.65
			Adjusted R^2	0.42

Candidate predictors were entered into the model irrespective of the results of the univariate analysis. After entering all variables into the model, the variables that showed least significant associations were subsequently excluded until all variables remained significant ($p < 0.05$)
BMI – body mass index, β – coefficient, r – correlation coefficient, SE – standard error

Table 4. Factors associated with the FibroTest

Factor	Univariate analysis		Multivariate analysis	
	r (95% CI)	p	β (SE)	p
BMI z-score	−0.28 (−0.75-0.38)	0.40	−0.0004 (0.02)	0.98
Age	0.41 (−0.25-0.81)	0.20	−0.02 (0.01)	0.20
Sex (for male)	0.74 (0.25-0.92)	0.009	0.11 (0.05)	0.09
Duration of infection	0.61 (0.01-0.88)	0.04	0.02 (0.02)	0.23
			Model performance	
			R^2	0.67
			Adjusted R^2	0.45

Candidate predictors were entered into the model irrespective of the results of the univariate analysis. After entering all variables into the model, the variables that showed least significant associations were subsequently excluded until all variables remained significant ($p < 0.05$)
BMI – body mass index, β – coefficient, r – correlation coefficient, SE – standard error

patients with mild and advanced histopathological features. Due to these reasons, the AUROCs have not been constructed.

On the basis of our experience, we conclude that FT and AT correlated poorly with the histopathological evaluation in pediatric patients with chronic viral hepatitis. Further studies on non-invasive methods of liver disease severity evaluation in children with chronic viral hepatitis are needed.

Disclosure

Authors report no conflict of interest.

References

1. Pokorska-Śpiewak M, Kowalik-Mikołajewska B, Aniszewska M, et al. Is liver biopsy still needed in children with chronic viral hepatitis? World J Gastroenterol 2015; 21: 12141-12149.
2. Dezsőfi A, Baumann U, Dhawan A, et al. Liver biopsy in children: position paper of the ESPGHAN Hepatology Committee. J Pediatr Gastroenterol Nutr 2015; 60: 408-420.
3. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology 2012; 142: 1293-1302.
4. Imbert-Bismut F, Messous D, Thibault V, et al. Intra-laboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and reference ranges in healthy blood donors. Clin Chem Lab Med 2004; 42: 323-333.
5. Imbert-Bismut F, Ratziu V, Pieroni L, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001; 357: 1069-1075.
6. De Ledinghen V, Le Bail B, Rebouissoux L, et al. Liver stiffness measurement in children using FibroScan: feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. J Pediatr Gastroenterol Nutr 2007; 45: 443-450.
7. Halfon P, Munteanu M, Poynard T. FibroTest-ActiTest as a non-invasive marker of liver fibrosis. Gastroenterol Clin Biol 2008; 32 (6 Suppl 1): 22-39.
8. Sokal EM, Paganelli M, Wirth S, et al. Management of chronic hepatitis B in childhood: ESPGHAN clinical practice guidelines: consensus of an expert panel on behalf of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. J Hepatol 2013; 59: 814-829.
9. European Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol 2017; 66: 153-194.
10. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology (Baltimore) 1996; 24: 289-293.
11. Friedrich-Rust M, Rosenberg W, Parkes J, et al. Comparison of ELF, FibroTest and FibroScan for the non-invasive assessment of liver fibrosis. BMC Gastroenterol 2010; 10: 103.
12. Halfon P, Bourliere 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: 547-555.
13. Hermeziu B, Messous D, Fabre M, et al. Evaluation of FibroTest-ActiTest in children with chronic hepatitis C virus infection. Gastroenterol Clin Biol 2010; 34: 16-22.
14. Sökücü S, Gökçe S, Güllüoğlu M, et al. The role of the non-invasive serum marker FibroTest-ActiTest in the prediction of histological stage of fibrosis and activity in children with naive chronic hepatitis B infection. Scand J Infect Dis 2010; 42: 699-703.
15. Poynard T, Morra R, Halfon P, et al. Meta-analyses of FibroTest diagnostic value in chronic liver disease. BMC Gastroenterol 2007; 7: 40.
16. El-Shabrawi MH, Mohsen NA, Sherif MM, et al. Noninvasive assessment of hepatic fibrosis and necroinflammatory activity in Egyptian children with chronic hepatitis C virus infection using FibroTest and ActiTest. Eur J Gastroenterol Hepatol 2010; 22: 946-951.