Int J Clin Exp Med 2018;11(2):792-798
www.ijcem.com /ISSN:1940-5901/IJCEM0060921

Original Article

Evaluation of non-invasive methods in hepatitis B virus (HBV)-infected patients with normal liver function

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Received April 16, 2017; Accepted December 14, 2017; Epub February 15, 2018; Published February 28, 2018

Abstract: Background: We aimed to compare the diagnostic performance of four non-invasive methods in detecting liver injury in chronic hepatitis B (CHB) patients with persistently normal liver function, and to develop a combined algorithm to improve current assays for liver injury evaluation. Materials and Methods: We obtained the results of aspirate aminotransferase (AST)-to-platelet ratio index (APRI), fibrosis index based on the 4 factors (FIB-4), red blood cell distribution width (RDW) to platelets ratio (RPR) and Fibroscan in a cohort of 58 CHB patients who underwent liver biopsy (LB). Then we combined serum markers with Fibroscan and evaluated their performance in detecting significant fibrosis. Results: The areas under the receiver operating characteristic curve (AUROC) for APRI, FIB-4, RPR and Fibroscan were 0.696, 0.708, 0.736 and 0.756 respectively for detecting significant liver fibrosis. An improved performance was obtained by combining Fibroscan and RPR, AUROC of whom was 0.836, reducing liver biopsy required for detection of significant fibrosis in 37.9% of patients with an accuracy of 95%. Conclusion: APRI, FIB-4, RPR and Fibroscan show moderate clinical value for detecting significant fibrosis in chronic HBV patients with normal liver function. The combination of Fibroscan and RPR improved diagnostic performance and reduced the number of patients who need liver biopsy.

Keywords: Aspirate aminotransferase-to-platelet ratio index, fibrosis index based on the 4 factors, red blood cell distribution width to platelets ratio, Fibroscan, Hepatitis B virus, normal liver function

Introduction

Hepatitis B virus (HBV) infection is a serious global health problem. Approximately 240 million people are chronically infected with HBV, and are at high risk for developing cirrhosis and hepatocellular carcinoma (HCC) [1]. And chronic HBV infection causes a wide spectrum of clinical manifestations. The guidelines for managing HBV infection do not recommend antiviral treatment for patients with normal alanine aminotransferase (ALT) levels. There is an increasing concern about accuracy to reflect liver injury by ALT. Recent studies have found significant necroinflammation and/or fibrosis in 28-37% of HBV patients with persistent normal ALT [2, 3]. High risk for advanced fibrosis and cirrhosis in patients can be delayed or prevented by antiviral therapy if significant fibrosis is detected earlier [4, 5].

Liver biopsy (LB) based evaluation of liver histology has been the most reliable detection of liver inflammation, stage of liver fibrosis and treatment efficiency. However LB is invasive, expensive and inconvenient for patients. Furthermore, sampling error occurs if histological alterations are non-homogenous, and variations between intra and inter-observers pose another challenge [6-9].

There is a strong interest in the field for developing non-invasive methods for accurately detecting liver injury. Fibroscan, a new imaging technique, measures the velocity of an elastic shear wave through the liver that reflects liver stiffness, resulting in an estimate of liver fibrosis [10, 11]. Some researchers have developed a mathematical formula that calculates a value for fibrosis using routine laboratory tests in chronic hepatitis C (CHC). Aspirate aminotransferase (AST)-to-platelet ratio index (APRI) and fibrosis index based on the 4 factors (FIB-4) are examples of routine serum tests [12-14]. Previous analyses suggested that FIB-4 and APRI are strong predictors for liver fibrosis in

HBV-infected patients with elevated ALT [15, 16]. Red blood cell distribution width (RDW) to platelets ratio (RPR) is an index of platelet morphology, which can predict significant fibrosis and cirrhosis in CHB patients with relatively high accuracy [17, 18].

However, few studies evaluated detection of significant fibrosis in HBV patients with PNALT using Fibroscan, APRI, FIB-4 and RPR. The aim of this study was to evaluate these four non-invasive assays for detecting significant liver fibrosis using liver histology as a reference in CHB patients with persistent normal liver function. We also tested the models that combined different non-invasive methods to evaluate whether the combination can decrease the rate of LB and have a high accuracy for detecting significant fibrosis in these patients.

Materials and methods

Patients

We retrospectively enrolled 58 patients who were previously diagnosed as CHB and underwent LB from January 2010 to December 2015 in the First Hospital of Jilin University. The purpose of LB was to assess severity of liver fibrosis and inflammation and to determine whether antiviral treatment is warranted. Inclusion criteria were as follows: 1) Patients were hepatitis B surface antigen (HBsAg) positive for at least 6 months and chronic hepatitis was confirmed by histology, HBV DNA positive (HBV DNA > 50 IU/ml by PCR); 2) Liver function was normal, as determined by ALT, AST, and total bilirubin (TBIL), for at least two consecutive measurements over a period of 6 months [19], the upper limit of normal (ULN) of ALT, AST and TBIL are 50 U/L for male/40 U/L for female, 40 U/L for male/35 U/L for female and 30 μ mol/L, respectively; 3) Age of patients ≥ 18 years old. Exclusion criteria were: 1) Patients who were co-infected with hepatitis C virus or human immunodeficiency virus (HIV), auto-immune hepatitis, Wilson disease, hepatocelluar carcinoma, chronic ethanol consumption (> 20 g/day for female, > 40 g/day for male). 2) A history of previous antiviral treatment or a history of malignant disorder. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee in the First Hospital of Jilin University.

Liver biopsy examination

Percutaneous LB was using an 18 G biopsy needle. LB was repeated for patients whose liver tissues were shorter than 13 mm to minimize the influence of the length of liver specimen on the accuracy of diagnosis. The liver tissues were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin. Every specimen was assessed by one pathologist who was blinded to clinical data. The Scheuer scoring system was used to evaluate the samples. Fibrosis was therefore scored on a scale from 0 to 4 (S0 = no fibrosis, S1 = enlarged portal tracts, S2 = periportal or portoportal septa, S3 = fibrosis with architectural distortion, S4 = cirrhosis). Significant fibrosis was defined as Scheuer score ≥ 2 [20].

Liver stiffness measurement by Fibroscan

Fibroscan (Fibroscan, Echosens SA, Paris, France) was performed by an experienced operator (more than 100 determinations in patients with chronic liver diseases) within 1 week before LB. Per instruction, 10 validated measurements were taken for each patient. The median value (in kilopascal, kPa) was considered representative of the liver elastic modulus. The measurement was considered reliable unless the interquartile range/median was < 30% and success rate was > 60% as suggested by the manufacturing company [21].

Non-invasive biomarkers

Fasting blood serum samples were used for laboratory tests within one week prior to LB. Platelet (PLT), RDW, AST, ALT, TBIL and gamma-glutamyl transpeptidase (GGT) were analyzed. HBV DNA level was determined with real time PCR with low detection limit of 50 IU/ml. HBsAg, anti-HBsAb, hepatitis B e-antigen (HBeAg), anti-HBe, hepatitis core B antibody (HBcAb), anti-HCV and anti-HIV were also assessed. APRI, FIB-4 and RPR were calculated using the principle reported formulas, as APRI = AST (U/L)/[PLT (10^9/L)], FIB-4 = Age (years) × AST (U/L)/[PLT (10^9/L) × $ALT^{1/2}$ (U/L)], RPR = RDW/PLT (10^9/L).

Cut-off values

Cut-off values of APRI, FIB-4, and RPR were selected from the published literatures while

Table 1. Characteristics of demography, laboratory index and histology

Variables	Total (58)
Male/Female	40/18
Age (Y)	40.22±9.74
Duration of infection (Y)	11.59±7.85
AST (U/L)	25.59±6.87
ALT (U/L)	29.58±11.25
GGT (U/L)	27.66±15.72
TBIL (μ mol/L)	14.14±6.04
PLT (× 10^9/L)	181.03±45.56
HBeAg-positive, n (%)	26 (44.8)
Median HBV DNA (log_{10} copies/mL)	7.83
Fibroscan	58
< F2 (< 7.3 kPa)	19 (32.8%)
≥ F2 (≥ 7.3 kPa)	39 (67.2%)
Liver biopsy	
Inflammation	55
G0	2 (3.6%)
G1	31 (56.4%)
G2	12 (21.8%)
G3	6 (10.9%)
G4	4 (7.3%)
Fibrosis stage	58
Non-significant fibrosis	36 (62.1%)
S0	15 (25.9%)
S1	15 (25.9%)
Significant fibrosis	15 (25.9%)
S2	10 (17.2%)
S3	10 (17.2%)
S4	10 (17.2%)

AST, aspirate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; PLT, platelet; TBIL, total bilirubin; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus. Data are expressed as mean ± standard deviation, or as number of cases (%).

the cut-off value of Fibroscan was derived from the Manual [12-14]. Patients with APRI > 0.5, FIB-4 > 1.45, RPR > 0.1 or Fibroscan ≥ 7.3 kPa were considered to have no significant fibrosis.

Evaluation of the combined non-invasive methods

Evaluation of non-invasive methods was designed for better detection of significant fibrosis by combining serum markers with Fibroscan, and the results were compared with histology diagnosis. In algorithm APRI+Fibroscan, if the results of APRI and Fibroscan were consist (APRI < 0.5 and Fibroscan < 7.3 kPa or APRI > 0.5 and Fibroscan ≥ 7.3 kPa), the patients were classified as significant fibrosis free(< S2) or to be positive for significant fibrosis (≥ S2). If the results were inconsistent, the patients were considered to need LB. The other algorithms were similar with APRI+Fibroscan.

Statistical analysis

All statistical analyses were processed using SPSS, version 22.0 (SPSS Inc, Chicago, IL). Continuous variables are expressed as mean ± standard deviations (SD) and the difference was determined by student *t* test. Categorical variables are expressed as percentages and the difference was determined by the chi-square test. A *P* value < 0.05 was considered significant. The diagnostic performance of APRI, FIB-4, RPR and Fibroscan and their combinations were assessed by sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic accuracy, receiver operating characteristic curves (ROC) and AUROC. McNemar's test was performed to evaluate the agreement between the combination with best accuracy and liver histology.

Results

Characteristics of enrolled patients

A total of 58 patients were enrolled in this study. The demographic, laboratory, Fibroscan results and histological features are summarized in **Table 1**. Median years of HBV infection were 11.6, 44.8% of the patients were HBeAg positive. There was no significant difference in the years of HBV infection (*P* = 0.74) between HBeAg positive and negative patients. Significant fibrosis was detected in 37.3% patients with normal liver function, 8.6% of whom had liver cirrhosis.

Performance of individual non-invasive methods

The diagnostic performance of the four non-invasive methods were evaluated for their ability to detect significant fibrosis (Scheuer fibrosis stage ≥ S2). Cut-off values for APRI, FIB-4, RPR and Fibroscan were 0.5, 1.45, 0.1 and 7.3 kPa respectively. RPR had the highest specificity, LR+, diagnostic accuracy and the lowest LR- (**Table 2**). ROC curves of these methods for detecting significant fibrosis were presented in **Figure 1**, AUROC of APRI, FIB-4, RPR and

Table 2. Performance of APRI, FIB-4, RPR and Fibroscan in excluding significant fibrosis in livers

Method	Cut-off	Sensitivity (%)	Specificity (%)	LR+	LR-	DA (%)	AUROC (95% CI)
APRI	0.5	31.8	86.1	2.29	0.79	65.5	0.696 (0.55-0.84)
FIB-4	1.45	59.1	80.6	3.05	0.51	72.4	0.708 (0.56-0.86)
RPR	0.1	45.5	91.7	5.48	0.02	74.1	0.736 (0.62-0.89)
Fibroscan	7.3	44.4	86.4	3.26	0.64	60.3	0.756 (0.60-0.87)

APRI: aspirate aminotransferase to platelet ratio index; FIB-4: fibrosis index based on the 4 factors; RPR: red blood cell distribution width to platelets ratio; LR+: positive likelihood ratio; LR-: negative likelihood ratio; DA: diagnostic accuracy; AUROC: area under receiver operating characteristic curve.

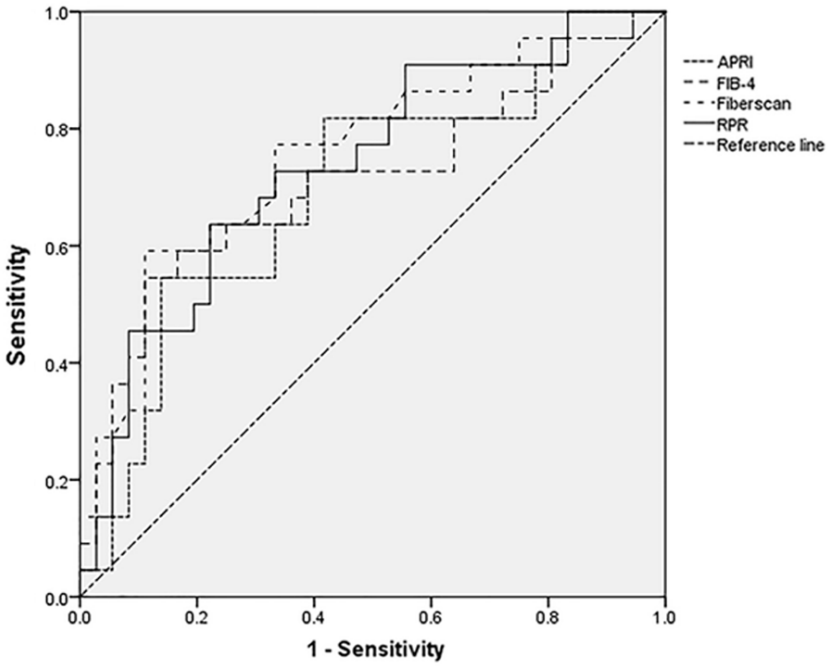

Figure 1. AUROC curves for fibrosis scores (APRI, FIB-4, RPR, Fibroscan) at different stages of fibrosis: S0-1 vs S ≥ 2, with an area under the receiver operating characteristic curve (AUROC) of 0.696, 0.708, 0.736 and 0.756 respectively.

Table 3. Performance of combinations of APRI, FIB-4, RPR and Fibroscan in detecting significant fibrosis in livers

Method	Sensitivity (%)	Specificity (%)	LR+	LR-	DA (%)	AUROC (95% CI)
APRI+Fibroscan	70.0	82.4	3.98	0.25	77.8	0.779 (0.65-0.91)
FIB-4+Fiberscan	91.7	80.0	4.59	0.10	85.2	0.804 (0.68-0.93)
RPR+Fibroscan	100.0	93.3	14.93	0.00	95.5	0.836 (0.73-0.94)

APRI: aspirate aminotransferase to platelet ratio index; FIB-4: fibrosis index based on the 4 factors; RPR: red blood cell distribution width to platelets ratio; DA: diagnostic accuracy; AUROC: area under receiver operating characteristic curve.

Fibroscan were 0.696, 0.708, 0.736 and 0.756, respectively.

Optimal combination algorithms

After individual evaluations of the four non-invasive methods, we further explored paired combination of these methods. AUROC for predicting significant fibrosis were 0.779, 0.804, 0.836, respectively (**Table 3**). RPR+Fibroscan had the highest AUROC (**Figure 2**), meanwhile the combination improved the performance to a better level than that achieved by RPR or Fibroscan alone (ROCs: 0.836 vs 0.736 or 0.756). RPR+Fibroscan was the best performing algorithm with sensitivity, specificity, LR+, LR-, accuracy of 100%, 93.3%, 14.94, 0, 95.5%, respectively. However the consistency of RPR and Fibroscan was mediocre, with only 37.9% of the patients. Next, we performed McNemar's test to compare the agreement between RPR+Fibroscan and LB. There was an almost perfect agreement (Kappa = 0.899, $P < 0.001$) between RPR+Fibroscan and LB.

RPR and Fibroscan agreed on the diagnosis of the stage of fibrosis in 22 patients (37.9%). There was only 1 discordant case who were classified as ≥ S2 by RPR-4+Fibroscan but < S2 by LB. Among the remaining 36 patients, Fibroscan agreed with LB results in 14 cases and RPR agreed with LB in 22 cases. The best

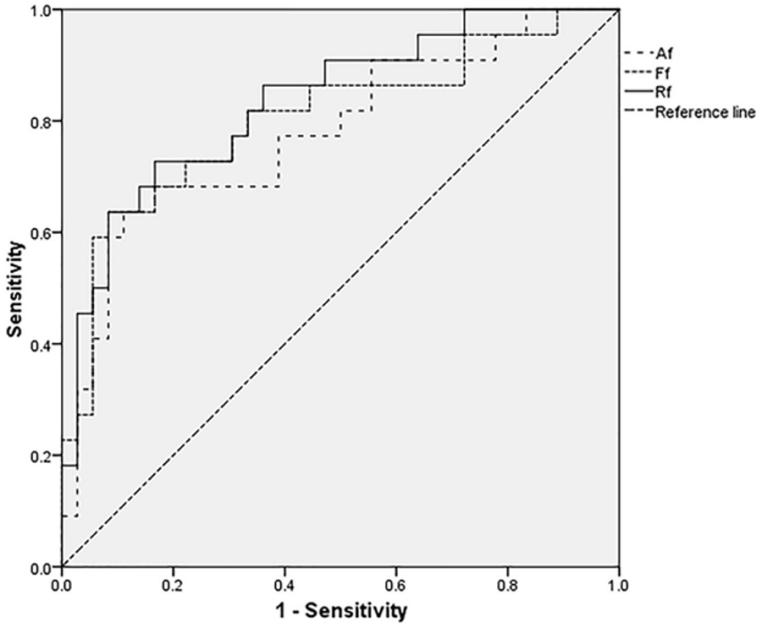

Figure 2. AUROC curves for fibrosis scores (APRI+Fibroscan, FIB-4+Fibroscan, RPR+Fibroscan) at different stages of fibrosis: S0-1 vs S ≥ 2, with an area under the receiver operating characteristic curve (AUROC) of 0.779, 0.804, 0.836, respectively. Af, APRI+Fibroscan; Ff, FIB-4+Fibroscan; Rf, RPR+Fibroscan.

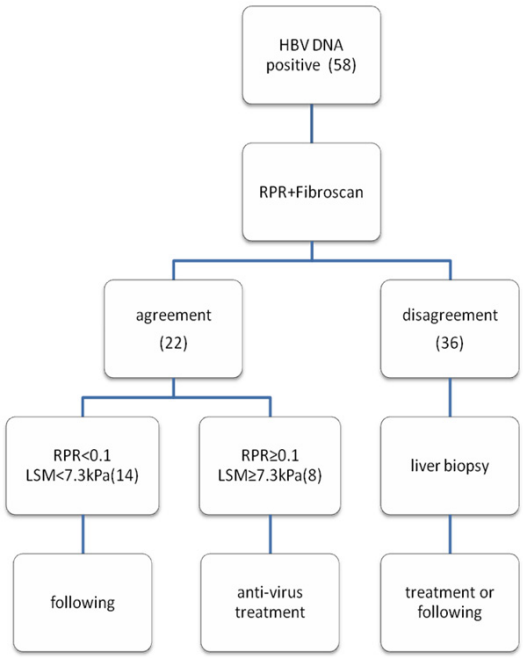

Figure 3. Proposed algorithms for patient management using the combination of RPR and Fibroscan for diagnosing liver fibrosis stages.

diagnostic algorithm for these six combinations is reported in **Figure 3**.

Discussion

In this study, we evaluated clinical values of four non-invasive methods, APRI, FIB-4, RPR and Fibroscan as well as paired combinations in a cohort of 58 CHB patients with persistent normal liver function. We found that a combination of RPR+Fibroscan can detect significant fibrosis with a good accuracy of 95.5%, as confirmed by liver biopsy.

High prevalence of histologically significant necroinflammation or fibrosis in HBV patients with PNALT has been reported [2, 3]. Considering ALT level cannot reflect significant fibrosis correctly, we rely on additional non-invasive markers and LB to detect fibrosis. The diagnostic performance of the four non-invasive methods tested in this study was compared based on AUROC and diagnosis accuracy. RPR showed a better diagnostic accuracy among the three serum biomarkers. The best AUROC of these serum biomarkers was 0.736, diagnostic accuracy was 74.1%, which was lower than the values reported by previous studies [15, 16, 22]. The differences can be explained by varying degrees of fibrosis since AUROC is highly influenced by the prevalence of fibrosis stage. The percentage of S0-1 detected in our study was higher compared to other studies. Furthermore, the diagnostic performance of serum biomarkers in PNALT patients was not as good as in individuals with elevated ALT levels [23-26]. To our knowledge, this study represents the first validation of RPR diagnostic value in HBV patients with normal liver function, and RPR showed the best diagnostic performance among other serum biomarkers.

We explored different non-invasive detection combinations of serum markers and Fibroscan to reduce liver biopsy in CHB patients. Among the four non-invasive methods and paired combinations, the combination of Fibroscan and RPR revealed the best diagnostic performance for significant fibrosis. This combination scan

covered 37.9% of the cohort patients, 95.5% of patients with S ≥ 2 were detected by Fibroscan+RPR, as well as histology. RPR consists of two common hematological parameters and is easy to calculate. Fibroscan presents no risk compared to liver biopsy. Thus, this non-invasive method has potential for wide clinical applications.

To increase the diagnostic accuracy, many combinations of non-invasive methods have been proposed. The resultant diagnostic accuracies ranged from 84 to 94%, and reduced LB by 48 to 77% in patients with significant fibrosis [27-31]. Since elevated ALT influences the accuracy of serum biomarkers and Fibroscan [25, 26, 32, 33], only patients with normal liver function were selected in this study. We expect that our results could be validated in large cohorts. Furthermore, this study is the first to report that RPR evaluated in patients with normal liver function and used in a combination with another non-invasive method, resulting in impressive accuracy.

There are a few limitations in this study. The enrolled patients in a retrospective study may have been subjected to a biased selection. And the number of patients was small because only HBV-infected patients with normal liver function were included. The main findings need to be verified in large cohorts.

In conclusion, we evaluated the diagnostic performance of four non-invasive methods in HBV patients with normal liver function, and found similar diagnostic performances among these four. A combination of RPR and Fibroscan can improve the performance of each individual non-invasive method, and help predict significant fibrosis in 40% of patients without LB.

Disclosure of conflict of interest

None.

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References

[1] Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS and Kao JH. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016; 10: 1-98.

[2] Lai M, Hyatt BJ, Nasser I, Curry M and Afdhal NH. The clinical significance of persistently normal ALT in chronic hepatitis B infection. J Hepatol 2007; 47: 760-767.

[3] Gui HL, Wang H, Yang YH, Wu YW, Zhou HJ, Guo SM, Lin LY, Wang L, Cai W, Chen R, Guo Q, Zhou XQ, Bao SS and Xie Q. Significant histopathology in Chinese chronic hepatitis B patients with persistently high-normal alanine aminotransferase. J Viral Hepat 2010; 17 Suppl 1: 44-50.

[4] Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar Schall R, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013; 381: 468-475.

[5] Liaw YF. Impact of therapy on the long-term outcome of chronic hepatitis B. Clin Liver Dis 2013; 17: 412-423.

[6] Bedossa P, Dargere D and Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003; 38: 1449-1457.

[7] Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002; 97: 2614-2618.

[8] Maharaj B, Maharaj RJ, Leary WP, Cooppan RM, Naran AD, Pirie D, Pudifin DJ. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. Lancet 1986; 1: 523-525.

[9] Afdhal NH. Diagnosing fibrosis in hepatitis C: is the pendulum swinging from biopsy to blood tests? Hepatology 2003; 37: 972-974.

[10] Patel K, Bedossa P and Castera L. Diagnosis of liver fibrosis: present and future. Semin Liver Dis 2015; 35: 166-183.

[11] Sharma S, Khalili K and Nguyen GC. Non-invasive diagnosis of advanced fibrosis and cirrhosis. World J Gastroenterol 2014; 20: 16820-16830.

[12] Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, Bruguera M, Sanchez-Tapias JM and Rodes J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002; 36: 986-992.

[13] Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS and Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003; 38: 518-526.

[14] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006; 43: 1317-1325.

[15] Teshale E, Lu M, Rupp LB, Holmberg SD, Moorman AC, Spradling P, Vijayadeva V, Boscarino JA, Schmidt MA, Gordon SC; CHeCS Investigators. APRI and FIB-4 are good predictors of the stage of liver fibrosis in chronic hepatitis B: the Chronic Hepatitis Cohort Study (CHeCS). J Viral Hepat 2014; 21: 917-920.

[16] Mallet V, Dhalluin-Venier V, Roussin C, Bourliere M, Pettinelli ME, Giry C, Vallet-Pichard A, Fontaine H and Pol S. The accuracy of the FIB-4 index for the diagnosis of mild fibrosis in chronic hepatitis B. Aliment Pharmacol Ther 2009; 29: 409-415.

[17] Taefi A, Huang CC, Kolli K, Ebrahimi S and Patel M. Red cell distribution width to platelet ratio, a useful indicator of liver fibrosis in chronic hepatitis patients. Hepatol Int 2015; 9: 454-460.

[18] Chen B, Ye B, Zhang J, Ying L and Chen Y. RDW to platelet ratio: a novel noninvasive index for predicting hepatic fibrosis and cirrhosis in chronic hepatitis B. PLoS One 2013; 8: e68780.

[19] Marcellin P, Levy S and Erlinger S. Therapy of hepatitis C: patients with normal aminotransferase levels. Hepatology 1997; 26 Suppl 1: 133S-136S.

[20] Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991; 13: 372-374.

[21] Jia J, Hou J, Ding H, Chen G, Xie Q, Wang Y, Zeng M, Zhao J, Wang T, Hu X and Schuppan D. Transient elastography compared to serum markers to predict liver fibrosis in a cohort of Chinese patients with chronic hepatitis B. J Gastroenterol Hepatol 2015; 30: 756-762.

[22] Sebastiani G, Vario A, Guido M and Alberti A. Sequential algorithms combining non-invasive markers and biopsy for the assessment of liver fibrosis in chronic hepatitis B. World J Gastroenterol 2007; 13: 525-531.

[23] Amorim TG, Staub GJ, Lazzarotto C, Silva AP, Manes J, Ferronato Mda G, Shiozawa MB, Narciso-Schiavon JL, Dantas-Correa EB, Schiavon Lde L. Validation and comparison of simple noninvasive models for the prediction of liver fibrosis in chronic hepatitis C. J Viral Hepat 2006; 13: 659-670.

[24] Colletta C, Smirne C, Fabris C, Toniutto P, Rapetti R, Minisini R and Pirisi M. Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. Hepatology 2005; 42: 838-845.

[25] Liu CH, Lin JW, Tsai FC, Yang PM, Lai MY, Chen JH, Kao JH and Chen DS. Noninvasive tests for the prediction of significant hepatic fibrosis in hepatitis C virus carriers with persistently normal alanine aminotransferases. Liver Int 2006; 26: 1087-1094.

[26] Sebastiani G, Vario A, Guido M and Alberti A. Performance of noninvasive markers for liver fibrosis is reduced in chronic hepatitis C with normal transaminases. J Viral Hepat 2008; 15: 212-218.

[27] Wong GL, Wong VW, Choi PC, Chan AW and Chan HL. Development of a non-invasive algorithm with transient elastography (Fibroscan) and serum test formula for advanced liver fibrosis in chronic hepatitis B. Aliment Pharmacol Ther 2010; 31: 1095-1103.

[28] Sebastiani G, Vario A, Guido M, Noventa F, Plebani M, Pistis R, Ferrari A and Alberti A. Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. J Hepatol 2006; 44: 686-693.

[29] Leroy V, Hilleret MN, Sturm N, Trocme C, Renversez JC, Faure P, Morel F and Zarski JP. Prospective comparison of six non-invasive scores for the diagnosis of liver fibrosis in chronic hepatitis C. J Hepatol 2007; 46: 775-782.

[30] Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P and de Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005; 128: 343-350.

[31] Castéra L, Sebastiani G, Bail BL, de Lédinghen V, Couzigou P and Alberti A. Prospective comparison of two algorithms combining non-invasive methods for staging liver fibrosis in chronic hepatitis C. J Hepatol 2010; 52: 191-198.

[32] Salkic NN, Cickusic E, Jovanovic P, Denjagic MB, Iljazovic-Topcic S, Bevanda M and Ahmetagic S. Online combination algorithm for non-invasive assessment of chronic hepatitis B related liver fibrosis and cirrhosis in resource-limited settings. Eur J Intern Med 2015; 26: 628-634.

[33] Chan HL, Wong GL, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ and Wong VW. Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B. J Viral Hepat 2009; 16: 36-44.