Title: Combined acoustic radiation force impulse, aminotransferase to platelet ratio index and Forns index assessment for hepatic fibrosis grading in hepatitis B

Author(s): Dong, CF; Xiao, J; Shan, LB; Li, HY; Xiong, YJ; Yang, GL; Liu, J; Yao, SM; Li, SX; Le, XH; Yuan, J; Zhou, BP; Tipoe, GL; Liu, YX

Citation: World Journal of Hepatology, 2016, v. 8 n. 14, p. 616-624

Issued Date: 2016

URL: http://hdl.handle.net/10722/227111

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Combined acoustic radiation force impulse, aspartate aminotransferase to platelet ratio index and Forns index assessment for hepatic fibrosis grading in hepatitis B

Chang-Feng Dong, Jia Xiao, Ling-Bo Shan, Han-Ying Li, Yong-Jia Xiong, Gui-Lin Yang, Jing Liu, Si-Min Yao, Sha-Xi Li, Xiao-Hua Le, Jing Yuan, Bo-Ping Zhou, George L Tipoe, Ying-Xia Liu

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

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Correspondence to: George L Tipoe, MD, PhD, School of Biomedical Sciences, LKS Faculty of Medicine, the University of Hong Kong, Pokfulam, Hong Kong, China. tgeorge@hku.hk

Received: January 8, 2016
Peer-review started: January 8, 2016
First decision: February 26, 2016
Revised: March 8, 2016
Accepted: April 20, 2016
Article in press: April 22, 2016
Published online: May 18, 2016

Abstract

AIM: To investigate the combined diagnostic accuracy of acoustic radiation force impulse (ARFI), aspartate aminotransferase to platelet ratio index (APRI) and Forns index for a non-invasive assessment of liver fibrosis in patients with chronic hepatitis B (CHB).

METHODS: In this prospective study, 206 patients had CHB with liver fibrosis stages F0-F4 classified by METAVIR and 40 were healthy volunteers were...
measured by ARFI, APRI and Forns index separately or combined as indicated.

RESULTS: ARFI, APRI or Forns index demonstrated a significant correlation with the histological stage (all \( P < 0.001 \)). According to the AUROC of ARFI and APRI for evaluating fibrotic stages more than F2, ARFI showed an enhanced diagnostic accuracy than APRI (\( P < 0.05 \)). The combined measurement of ARFI and APRI exhibited better accuracy than ARFI alone when evaluating \( \geq F2 \) fibrotic stage (\( Z = 2.77, P = 0.006 \)). Combination of ARFI, APRI and Forns index did not obviously improve the diagnostic accuracy compared to the combination of ARFI and APRI (\( Z = 0.958, P = 0.338 \)).

CONCLUSION: ARFI + APRI showed enhanced diagnostic accuracy than ARFI or APRI alone for significant liver fibrosis and ARFI + APRI + Forns index shows the same effect with ARFI + APRI.

Key words: Acoustic radiation force impulse; Aspartate aminotransferase to platelet ratio index; Forns index; Hepatitis B virus; Non-invasive diagnosis

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Core tip: Chronic hepatitis B (CHB) is a major health problem in a lot of countries all over the world, particularly in China. An accurate staging of liver fibrosis is critical for prognosticating this disease. However, although it is still the golden standard, liver biopsy is hindered by its inherent drawbacks in clinical applications. In this study, we demonstrated that non-invasive methods, including acoustic radiation force impulse (ARFI), aspartate aminotransferase to platelet ratio index (APRI) and Forns index showed significant correlations with the histological staging results from liver biopsy. The combined measurement of ARFI and APRI had the best diagnostic accuracy, which provided an ideal and convenient non-invasive diagnostic method for the detection of hepatic fibrosis of CHB patients in clinical practice.

INTRODUCTION

Chronic liver injury, such as chronic hepatitis B (CHB), may cause inflammation and necrosis of hepatocytes, leading to hepatic fibrosis. It is a long-term pathological change with certain possibility (about 20%) of progressing to liver cirrhosis\(^1\). Unlike cirrhosis, hepatic fibrosis is reversible at its early stage when proper clinical therapeutic interventions are applied\(^2\). Therefore, an accurate staging of liver fibrosis is critical for prognosticating this disease. To date, the gold standard for staging hepatic fibrosis is still the liver biopsy, which cannot be routinely performed because of its inherent limitations, such as pain, bleeding, inaccurate staging from sampling error, and variability of biopsy interpretation\(^3\). During the past decades, considerable efforts have been invested in developing non-invasive methods of assessments, which may provide accurate evaluation of liver fibrosis comparable to liver biopsy. Indeed, these non-invasive methods have several advantages such as high safety margin, simple, convenient, reproducible, and inexpensive.

Acoustic radiation force impulse (ARFI) is a new quantitative assessment method of estimating tissue stiffness through measurement of shear wave velocity (SWV, measured in m/s). Its quantitative representation is named as virtual touch tissue quantification, which gives an objective numerical evaluation of the tissue stiffness\(^4\-6\). ARFI imaging offers a quantitative assessment of the hepatic parenchyma elasticity to non-invasively grade and stage hepatic fibrosis. It has been used to diagnose hepatic fibrosis of patients with CHB\(^7\), hepatitis C\(^8\), cirrhosis\(^9\) and non-alcoholic fatty liver disease (NAFLD)\(^10\). In addition, ARFI is often performed with serum liver functions tests [e.g., alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, and albumin] to generate better prediction and evaluation of liver fibrosis\(^11\). Among these, AST platelet ratio (APRI) is a serum hepatic function test which has been proposed as a non-invasive tool for the assessment of liver fibrosis in CHB\(^12\) or chronic hepatitis C\(^13\). Another important serum test is Forns index method, which uses simple obtained parameters including age, gamma-glutamyltransferase (GGT), cholesterol, and platelet count (PLT), but it requires a relatively complicated calculation\(^14\). One of the advantages of APRI and Forns index over the other non-invasive tests is that they are based on readily available blood tests and simple to use. Although these strategies have been widely applied in the past decade for hepatitis C evaluation\(^15\-16\), their accuracy for CHB grading are still not comparable with liver biopsy. Therefore, a combined use of these non-invasive methods may be another promising and practical diagnostic application in CHB patients. In the current study, we aimed to compare the accuracy among ARFI, APRI, Forns index and their combinations for non-invasive diagnosis grading and prognosis of human CHB-induced hepatic fibrosis.

MATERIALS AND METHODS

Subjects of study

This prospective study was approved by the ethical committee of Shenzhen Third People’s Hospital. All study procedures and methods were in accordance with...
the approved guidelines. All patients in this study were fully informed about the research protocol including the data handling and the privacy of personal data. After this procedure, patients signed the written consent. A total of 246 subjects were consecutively enrolled in this study, including 206 CHB subjects and 40 healthy subjects. These 206 CHB cases were selected from 245 CHB patients diagnosed by liver biopsy in Shenzhen Third People’s Hospital, from May 2011 to December 2014. Of the 206 CHB patients, there were 39 female cases (18.9%) and 167 male cases (81.1%). Inclusion criteria are: (1) patients must be 18-65 years old; (2) with hepatitis B surface antigen positive for more than 6 mo; (3) without receiving antiviral treatment before this study; (4) ALT and AST were < 2 × upper limit of normal (ULN) in the past 6 mo; (5) 18.5 < body mass index (BMI) < 31.0; (6) length of liver biopsy tissue ≥ 15 mm and contains at least 10 portal peridal areas; (7) hemoglobin > 90 g/L, prothrombin time 11-15.1 s; (8) activated partial thromboplastin time and thrombin time were at a normal range; and (9) cardiac and renal functions were normal. Negative for the following: Human immuno-deficiency virus, hepatitis A virus, hepatitis C virus (HCV), hepatitis D virus, hepatitis E virus super-infection or co-infection, auto-immune liver diseases, alcoholic steatosis, NAFLD, hepatocellular carcinoma (HCC), ascites, as well as jaundice. Of the 245 eligible CHB patients, 39 were excluded because of the following: NAFLD (n = 10), received antiviral treatment before this study (n = 8), jaundice (n = 5), alcoholic steatosis (n = 6), HCV infection (n = 2), auto-immune liver disease (n = 1), with age < 18 (n = 4), with age > 65 (n = 1), and declined to participate (n = 2). Healthy group consisted of 40 volunteers, with 30 males and 10 females, aged range from 20-53 years old, with mean age of 39.8 ± 11.45 years and no hepatitis B virus (HBV) or HCV infection, no hypertension, diabetes, fatty liver and other apparent diseases. The BMI of healthy subjects were between 18.5 and 31.0. Other parameters were similar to the CHB patients. All CHB patients were examined by ARFI one day before or on the day of liver biopsy. All the subjects had blood or sera drawn for the detection of platelet and fibrotic serological markers.

Liver biopsy and pathological staging
Liver biopsy tissue specimens were collected by needle puncture (MN1613, Bard Biopsy Systems, Tempe, AZ) under the Color Doppler Ultrasound guidance in a separate clinic setting for diagnostic purposes. The liver specimen was 15-20 mm in length, including at least 10 portal vein areas. Then it was embedded by paraffin and stained by Sirius Red (Sigma-Aldrich, St. Louis, MO). Liver fibrosis stage was assessed by the METAVIR scoring system (F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis and a few septa; F3 = numerous fibrosis without cirrhosis; and F4 = cirrhosis[17]). The METAVIR scoring system was previously used in other reports on CHB[18,19]. Two independent pathologists were responsible for the staging of all samples without additional information about the specimens they checked.

ARFI
The detection of ARFI in the liver was performed under fasting conditions using Siemens Acuson S2000 with probe detector 4C1, frequency 2.0-4.0 MHz (Siemens Healthcare, Erlangen, Germany) according to routine instructions. ARFI was mainly conducted by a radiologist (Dong CF) with assistant from another physician and a nurse. Dong CF has 11-year experience in clinical radiology and 4-year experience in ARFI diagnosis. Form of the liver capsule and the echogenicity of hepatic parenchyma were recorded. Detection of SWV (m/s) of hepatic segments s5, s6, s7 and s8 was repeated for 3 times and the mean values were calculated. Thus, 12 measurements of hepatic segments s5, s6, s7, s8 were recorded. Our pilot study in healthy volunteers showed that when compared with conventional ARFI protocol (mean value from 10 measurements), the current protocol exhibited similar results with smaller standard deviation (1.08 ± 0.21 m/s vs 1.11 ± 0.12 m/s; t = 0.6794, P > 0.05). This is consistent with a report that showed the reproducibility of measurements in the right lobe was higher[20]. Images and data of ARFI were saved for analysis.

Blood markers for APRI and Forns index evaluations
AST was determined in the same laboratory prior to the liver biopsy using Siemens ADVIA 2400 Chemistry system (Siemens Healthcare). Enzymatic activity was measured at 37 ℃, according to International Federation of Clinical Chemistry standards. Platelet count was assessed by an automatic blood cell analyzer (XE-5000 Automated Hematology System, Sysmex, Lincolnshire, IL). The ULN range of AST was considered as 40 U/L. APRI = AST/(ULN)/PLT(106/L) × 100.
Forns index = 7.811 - 3.131 × Ln(PLT) + 0.781 × Ln(GGT) + 3.467 × Ln(age) - 0.014 × (cholesterol)

Combined assessments of ARFI + APRI/ARFI + Forns index
A logistic regression analysis model for hepatic fibrosis ≥ F2 has been established by using the ENTER method.

Statistical analysis
Continuous normal distribution data were represented with means ± SD. Categorical normal distribution data were represented with median ± quartile (M ± Q). Kruskal-Wallis test was used to analyze the differences among these different groups. When there was a statistical significance (P < 0.05), a post-hoc Bonferroni test was applied to analyze data between two groups. P < 0.05 was considered to be statistically significant using a SPSS 13.0, IBM, Armonk, NY. The box plot was used to record the mean and degree of variation. MedCalc software (Ostend, Belgium) was used to draw receiver operating characteristic curve (ROC) and calculate cut-off value, sensitivity, specificity, positive predictive
Table 1  Results of basic information and acoustic radiation force impulse/aspartate aminotransferase to platelet ratio index/Forns index of all examinees

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Gender (male/female)</th>
<th>BMI</th>
<th>ARFI</th>
<th>APRI</th>
<th>Forns index</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 (n = 40)</td>
<td>39.8 ± 11.45</td>
<td>30/10</td>
<td>22.91 ± 2.31</td>
<td>1.09 (1.01, 1.21)</td>
<td>0.19 (0.14, 0.28)</td>
<td>5.58 ± 1.33</td>
</tr>
<tr>
<td>F1 (n = 41)</td>
<td>33.07 ± 7.97</td>
<td>33/8</td>
<td>22.37 ± 2.24</td>
<td>1.19 (1.15, 1.26)</td>
<td>0.34 (0.26, 0.44)</td>
<td>5.60 ± 1.19</td>
</tr>
<tr>
<td>F2 (n = 52)</td>
<td>38.27 ± 7.66</td>
<td>43/9</td>
<td>22.26 ± 2.41</td>
<td>1.31 (1.21, 1.45)</td>
<td>0.42 (0.32, 0.64)</td>
<td>6.73 ± 1.09</td>
</tr>
<tr>
<td>F3 (n = 59)</td>
<td>39.83 ± 8.73</td>
<td>47/12</td>
<td>22.44 ± 2.57</td>
<td>1.52 (1.35, 1.64)</td>
<td>0.45 (0.32, 0.86)</td>
<td>7.58 ± 1.53</td>
</tr>
<tr>
<td>F4 (n = 54)</td>
<td>43.85 ± 10.81</td>
<td>44/10</td>
<td>22.35 ± 2.47</td>
<td>1.92 (1.74, 2.14)</td>
<td>0.80 (0.51, 1.68)</td>
<td>9.45 ± 2.60</td>
</tr>
</tbody>
</table>

For age and Forns index, data were represented in mean ± SD. For ARFI and APRI data, results were exhibited in median ± quartile. *Means significant change against the F0 group; †Means significant change against the F1 group; ‡Means significant change against the F2 group; §Means significant change against the F3 group. For gender, ARFI and APRI comparisons, size of test α = n/n = 0.005; for age and Forns index comparison, size of test α = 0.05. ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index; BMI: Body mass index.

Figure 1  Box plots show correlation between noninvasive tests and histological stages from liver biopsy. Top and bottom of boxes represent first and third quartiles, respectively. Length of box represents interquartile range within which 50% of values are located. Line through each box represents median. Error bars mark the minimum and maximum values (range). Small circles represent the outliers. Triangles represent the extreme value, which is > 3 × interquartile range. ARFI: Acoustic radiation force impulse; APRI: Aspartate transaminase to platelet ratio index.

Values, negative predictive values, AUROC of ARFI and APRI for every liver fibrotic stage. The ROC curve of two variables combination (ARFI + APRI and ARFI + Forns index) and three variables combination (ARFI + APRI + Forns index) for significant hepatic fibrosis (≥ F2) was also analyzed. When AUROC > 0.5, the closer of AUROC to 1, the better diagnostic outcome it provided. Comparison of AUROC among these parameters and their combination was analyzed by the Delong test.\(^{21}\)

RESULTS

Results of basic information, ARFI, APRI, and Forns index

Basic information (e.g., age and gender) and assessment results of ARFI, APRI, and Forns index of all subjects were shown in Table 1. The average ages of subjects with significant or serious fibrosis (F2, F3 and F4) were significantly higher than subjects with mild fibrosis (F1) (P = 0.009 for F2 vs F3, P < 0.001 for F2 vs F4, and P < 0.001 for F3 vs F4). Also, male patients showed higher incidence of hepatic fibrosis (from F1 to F4) than female patients. The differences of ARFI results among F0, F1, F2, F3 and F4 groups were significant (P < 0.05). For Forns index, except for F0 and F1 group, the differences among other groups were significant (P < 0.05). Results of APRI indicated that only F4 showed significant change from other groups (F0, F1, F2 and F3) (all P < 0.001), while the F1, F2, and F3 groups showed significantly higher values than the F0 group (all P < 0.001) (Table 1).

Correlations between ARFI, APRI, Forns index and hepatic pathology

The median, quartile, minimum value, maximum value and outlier of ARFI, APRI and Forns index were shown in box type image (Figure 1). There was a high correlation between the staging of ARFI/APRI/Forns index and the hepatic histology, with correlation coefficient 0.845 (P < 0.001), 0.641 (P < 0.001) and 0.644 (P < 0.001), respectively (Table 2). In ENTER model, Y axis was the result from liver biopsy and the X axis was the results from ARFI + APRI or ARFI + Forns Index combined assessments. The equation for ARFI + APRI was y = -13.27 + 9.11 ARFI + 5.03 APRI, while the equation for ARFI + Forns index was y = -15.08 + 8.67 ARFI + 0.70 Forns index.

Determination of the cut-off values of hepatic fibrosis staging

There were significantly different interval ranges between different liver fibrotic stages and the corresponding ARFI and APRI results. In order to determine the cut-off value of each fibrotic stage, we applied ROC to analyze the data from both ARFI and APRI (Figure 2). From the result, it showed that the diagnostic performance of ARFI for predicting stages more than F2, F3 and F4 was 91% (95%CI: AUROC = 0.87-0.95, P < 0.05), 94% (95%CI:
AUROC = 0.90-0.96, P < 0.05), 96% (95%CI: AUROC = 0.93-0.98, P < 0.05), and the best cut-off value of F2, F3 and F4 was 1.29, 1.43 and 1.62 m/s. However, APRI measurement showed decreased accuracy of diagnosing significant fibrosis when compared with ARFI (Table 3).

**Combined assessment of ARFI + APRI/ARFI + Forns index/ARFI + APRI + Forns index for hepatic fibrosis ≥ F2**

Firstly we established a logistic regression analysis model for hepatic fibrosis ≥ F2 in which the Y axis was the result from liver biopsy and the X axis was the results from combined ARFI + APRI/ARFI + Forns index assessment (Table 4). From the AUROC results of Table 5, when evaluating patients with hepatic fibrosis ≥ F2, there was a significant change between the AUROCs of ARFI + APRI and ARFI alone (0.940 and 0.913, respectively; Z = 2.77, P = 0.006), also between ARFI + Forns index and ARFI alone (0.933 and 0.913, respectively; Z = 2.091, P = 0.037), ARFI + APRI + Forns index and ARFI alone (0.944 and 0.913, respectively; Z = 2.893, P = 0.004), indicating an enhanced screening ability of the combined assessment than ARFI alone. However, the change between ARFI + APRI and ARFI + APRI + Forns index was not significant (0.940 and 0.944, respectively; Z = 0.958, P = 0.338), suggesting that Forns index cannot further improve the diagnostic accuracy for staging hepatic fibrosis ≥ F2 when using a combined method of ARFI + APRI (Figure 3).

**DISCUSSION**

To date, the gold standard for the diagnosis of liver fibrosis remains to be liver biopsy. In most circumstances, patients find it difficult to accept liver biopsy due...
to its complications. From 2009, with the introduction of ARFI, the clinical research on non-invasive assessment of fibrosis rapidly progressed. As an advanced imaging technology, ARFI is capable of providing biomechanical information on the tissue stiffness and elasticity using conventional ultrasound scanning of anatomical location and structure\(^{22,23}\). However, its utility, particularly in combination with other non-invasive methods in hepatitis B, has not been adequately evaluated.

In the current study, CHB patients with different stages of liver fibrosis were diagnosed by ARFI, APRI, Forns index and their combined assessments. Our results demonstrated that the mean SWV value from ARFI was highly correlated with the staging of liver fibrosis classified by liver biopsy (METAVIR classification). This result indicated that biomechanical properties (e.g., hepatic elasticity and stiffness) had progressed from liver fibrosis to cirrhosis during the development of CHB, which was consistent with the pathological progression of hepatocyte degeneration, necrosis, inflammation reaction, hepatocyte regeneration, formation of connective tissue fiber intervals, and liver lobule structural failure during the course of liver fibrosis of HBV infection\(^{24}\).

With the progression of liver fibrosis from F2 to F4, the effectiveness of ARFI on the diagnosis of liver fibrosis also increased. That is, when the value of SWV was lower than 1.29 m/s (clinically F0 and F1 patients), hepatic fibrosis could be unlikely significant. SWV higher than 1.43 m/s could be likely considered as an indication for serious liver fibrosis (F3, sensitivity 82.3% and specificity 89.5%), and SWV > 1.62 m/s could be diagnosed as early cirrhosis (F4, sensitivity 90.7% and specificity 92.2%). In addition, when they were used

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**Figure 2** Receiver operating characteristic curves for acoustic radiation force impulse and aspartate transaminase to platelet ratio index for diagnosis of hepatic fibrosis (F1-F4). ARFI: Acoustic radiation force impulse; APRI: Aspartate transaminase to platelet ratio index.
ARFI was the best way for the diagnosis of fibrosis ≥ F2; ARFI provides a dynamic technical support for non-invasive diagnosis of liver fibrosis. This result is in line with a report found that ARFI correlated well with liver biopsy and thus was a reliable ultrasound-based method for the assessment of advanced fibrosis induced by CHB

Currently it is difficult for non-invasive diagnostic methods to differentiate F0 and F1 fibrotic stages. However, in this study, we found that there was a significant change of ARFI readings between the F0 and F1 groups (Table 1). It is known that stage F2 possesses significant diagnostic value in determining the progression of liver disease and anti-viral therapy choice. At this stage, patients have more risk in developing complications such as portal hypertension, cirrhosis, and HCC than patients without significant liver fibrosis

If patients receive anti-viral therapy promptly during this period, it is possible to retard or even reverse the pathological progression of fibrosis. Thus, early accurate diagnosis and appropriate therapy to patients at F2 fibrosis evidently decreases the morbidity and mortality of patients with CHB

ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index.

ARFI, APRI and Forns index in CHB diagnosis

**Table 5** Comparing area under the receiver operating characteristic curve of acoustic radiation force impulse/acoustic radiation force impulse + aspartate aminotransferase to platelet ratio index/acoustic radiation force impulse + Forns index/acoustic radiation force impulse + aspartate aminotransferase to platelet ratio index + Forns index in patients with fibrosis stage ≥ F2

<table>
<thead>
<tr>
<th>Comparison</th>
<th>AUROC</th>
<th>Difference</th>
<th>95% CI</th>
<th>Z</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARFI</td>
<td>0.913</td>
<td>0.027</td>
<td>0.008</td>
<td>0.046</td>
<td>2.770</td>
</tr>
<tr>
<td>ARFI + APRI</td>
<td>0.940</td>
<td>0.007</td>
<td>0.001</td>
<td>0.034</td>
<td>2.770</td>
</tr>
<tr>
<td>ARFI + Forns index</td>
<td>0.933</td>
<td>0.031</td>
<td>0.010</td>
<td>0.053</td>
<td>2.893</td>
</tr>
<tr>
<td>ARFI + APRI + Forns index</td>
<td>0.944</td>
<td>0.004</td>
<td>-0.009</td>
<td>0.018</td>
<td>0.958</td>
</tr>
<tr>
<td>ARFI + APRI + Forns index</td>
<td>0.944</td>
<td>0.005</td>
<td>-0.009</td>
<td>0.018</td>
<td>0.958</td>
</tr>
<tr>
<td>ARFI + APRI + Forns index + Forns index</td>
<td>0.933</td>
<td>0.011</td>
<td>-0.001</td>
<td>0.023</td>
<td>1.789</td>
</tr>
</tbody>
</table>

AUROC: Area under the receiver operating characteristic curve; ARFI: Acoustic radiation force impulse; APRI: Aspartate aminotransferase to platelet ratio index.

![Figure 3](image-url)
they partially reflected the pro-inflammatory response and hepatic compensation. The most important finding of this study was that combined measurement of ARFI and APRI exhibited better accuracy than ARFI or APIR alone when evaluating ≥ F2 fibrosis stage. Combination of ARFI, APRI and Forns index did not further improve the diagnostic effect than the combination of ARFI and APRI.

In conclusion, ARFI, APRI and Forns index correlated well with the histological liver fibrosis stages in CHB patients. ARFI showed better accuracy than APRI when evaluating F2, F3 and F4 stages. Combined check with ARFI and APRI showed a significant enhancement of diagnostic accuracy than ARFI or APRI alone. ARFI + APRI exhibited similar enhancement of diagnostic accuracy of hepatic fibrosis with ARFI + APRI + Forns index when evaluating fibrotic stages more than F2 in CHB patients. This study provides an ideal and convenient non-invasive diagnostic method for the detection of hepatic fibrosis of CHB patients in clinical practice.

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j.dld.2007.10.011


P- Reviewer: Banerjee S, Malnick SDH, Pai CG S- Editor: Qi Y L- Editor: A E- Editor: Liu SQ