**Name of journal:** ***World Journal of*** ***Gastroenterology***

**Manuscript NO: 34523**

**Manuscript Type: ORIGINAL ARTICLE**

***Retrospective Cohort Study***

**Diagnostic value of FIB-4, APRI and liver stiffness measurement in hepatitis B virus-infected patients with persistently normal alanine aminotransferase**

YW Tan *et al*.FIB-4, APRI and LSM in PNALT

You-Wen Tan, Xing-Bei Zhou, Yun Ye, Cong He, Guo-Hong Ge

**You-Wen Tan, Xing-Bei Zhou, Yun Ye, Cong He, Guo-Hong Ge,** Department of Hepatosis, The Third Hospital of Zhenjiang Affiliated Jiangsu University, Zhenjiang 212001, Jiangsu Province, China

**Author contributions**: Tan YW designed the research; Ye Y, He C, and Ge GH collected and analysed the data, and drafted the manuscript; Zhou XB performed the research; Ye Y, and He C interpreted the data and revised the statistical analysis; Tan YW revised the article; all authors have read and approved the final version to be published; Tan YW and Zhou XB contributed equally to this work.

**Institutional review board statement:** The study was reviewed and approved for publication by our Institutional Reviewer.

**Informed consent statement:** All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

**Conflict-of-interest statement:** All the Authors have no conflict of interest related to the manuscript.

**Data sharing statement:** The original anonymous dataset is available on request from the corresponding author at tyw915@sina.com

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Correspondence to: Dr. You-Wen Tan,** Department of Hepatosis, the Third Hospital of Zhenjiang Affiliated Jiangsu University, Daijiamen 300, Zhenjiang 212001, Jiangsu Province, China. tyw915@sina.com

**Telephone**: +86-511-88614915

**Fax**: +86-511-88970796

**Received:** May 2, 2017

**Peer-review started:** May 4, 2017

**First decision:** June 6, 2017

**Revised:** June 10, 2017

**Accepted:** July 12, 2017

**Article in press:**

**Published online:**

**Abstract**

***AIM***

To analyze the diagnostic value of FIB-4, aspartate aminotransferase-to-platelet ratio index (APRI), and liver stiffness measurement (LSM) in patients with hepatitis B virus infection who have persistently normal alanine transaminase (PNALT).

***METHODS***

We enrolled 245 patients with chronic hepatitis B: 95 in the PNALT group, 86 in the intermittently elevated ALT (PIALT1) group (ALT1-2ULN), and 64 in the PIALT2 group (ALT > 2ULN). All the patients received a percutaneous LB directed by ultrasonography. LSM, biochemical tests, and complete blood cell counts were performed.

***RESULTS***

The pathological examination revealed moderate inflammatory necrosis ratios of 16.81% (16/95), 32.56% (28/86), and 45.31% (28/64), and moderate liver fibrosis of 24.2% (23/95), 33.72% (29/86), and 43.75% (28/64) in the PNALT, PIALT1, and PIALT2 groups, respectively. The degrees of inflammation and liver fibrosis were significantly higher in the PIALT group than in the PNALT group (*P* < 0.05). No significant difference in area under the curve (AUC) difference was found between APRI and FIB-4 in the PNALT group. Therefore, significant differences among LSM, APRI, and FIB-4 were compared in the PNALT group (*P* all < 0.05). Therefore, in the PIALT1 and PIALT2 groups, no significant difference (*P* > 0.05) in the AUC difference was found regardless of APRI compared with FIB-4 or LSM compared with APRI or FIB-4 (*P* all > 0.05).

***CONCLUSION***

We evaluated three common noninvasive hepatic fibrosis techniques in different ALT groups. The results showed that APRI and FIB-4 were not the ideal noninvasive hepatic fibrosis model for PNALT patients. LSM was superior to APRI and FIB-4 in PNALT patients because of the influence of liver inflammation and necrosis.

**Key words:** FIB-4; APRI; Liver stiffness measurement; Hepatitis B virus; Normal; Alanine aminotransferase

**© The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** To analyze the diagnostic value of FIB-4, aspartate aminotransferase-to-platelet ratio index (APRI), and liver stiffness measurement (LSM) in patients with hepatitis B virus infection who have persistently normal alanine transaminase (PNALT). We enrolled 245 patients with chronic hepatitis B: 95 in the PNALT group, 86 in the intermittently elevated ALT (PIALT1) group (ALT1-2ULN), and 64 in the PIALT2 group (ALT > 2ULN). The results showed that APRI and FIB-4 was not the ideal noninvasive hepatic fibrosis model for PNALT patients. LSM was superior to APRI and FIB-4 in PNALT patients because of the influence of liver inflammation and necrosis.

Tan YW, Zhou XB, Ye Y, He C, Ge GH. Diagnostic value of FIB-4, APRI and liver stiffness measurement in hepatitis B virus-infected patients with persistently normal alanine aminotransferase. *World J Gastroenterol* 2017; In press

**INTRODUCTION**

Approximately a third of the world’s population has serological evidence of past or present hepatitis B virus (HBV) infection, and 350–400 million people are known to be chronic HBV surface antigen (HBsAg) carriers. The disease spectrum and natural history of chronic HBV infection are diverse and varied, ranging from an inactive carrier state to a progressive chronic hepatitis B (CHB) infection, which may progress to cirrhosis and hepatocellular carcinoma (HCC)[[1](#_ENREF_1),[2](#_ENREF_2)]. Chronic HBV infection is a dynamic process, and its natural history was schematically divided into ﬁve phases by the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines (2012) as follows[[1](#_ENREF_1)]: (1) the “immune tolerant” phase; (2) the “immune reactive HBeAg-positive phase”; (3) the “inactive HBV carrier state”; (4) “HBeAg-negative CHB” phase; and (5) the “HBeAg-negative CHB” or “HBsAg-negative” phase.

Although serum levels of alanine transaminase (ALT), an enzyme released from hepatocytes during liver injury, should reflect the degree of liver damage[[3](#_ENREF_3)], not all patients with chronic HBV infection have persistently elevated ALT levels. Patients in the immune-tolerant phase and inactive carriers have persistently normal ALT (PNALT) levels[[4](#_ENREF_4),[5](#_ENREF_5)], while a proportion of patients with HBeAg-negative CHB may have intermittently normal ALT levels. Histological injury in patients with normal ALT levels has also been reported[[5-10](#_ENREF_5)]. Furthermore, some large cohort studies have shown that patients with CHB who have normal serum ALT levels were also at risk for the development of cirrhosis and HCC[[11](#_ENREF_11),[12](#_ENREF_12)]. Moreover, liver biopsy (LB) is also the current gold standard for assessing hepatic inﬂammation and ﬁbrosis in patients with chronic HBV who have PNALT[[7](#_ENREF_7)]. The invasiveness of liver puncture, the limitation of the specimen, and the causes of poor patient compliance have restricted the application of liver biopsy. The limitation of LB have led to the development of noninvasive models such as FIB-4[[13](#_ENREF_13)] and aspartate aminotransferase (AST)-to-platelet ratio index (APRI)[[14](#_ENREF_14)] for evaluating fibrosis in patients with chronic HBV infection. Liver stiffness measurement (LSM) by using transient elastography (FibroScan) has been widely used in the diagnosis of chronic liver fibrosis[[15](#_ENREF_15),[16](#_ENREF_16)]. However, the diagnostic values of FIB-4, APRI, and LSM in patients with HBV infection with PNALT is not clear.

In this study, we aimed to comprehensively evaluate the characteristics of histological abnormalities in a large population of Chinese CHB patients with PNALT. Moreover, we aimed to analyze the diagnostic values of FIB-4, APRI, and LSM in patients with HBV who have PNALT.

**MATERIALS AND METHODS**

***Ethics Statement***

The study was approved by the medical ethics committee of The Third Hospital of Zhenjiang Affiliated Jiangsu University (No. 2013011), and written informed consent was obtained from each patient prior to participation. The study was conducted in accordance with the Declaration of Helsinki.

***Patients***

This was a retrospective cohort study of patients with CHB diagnosed between January 2011 and June 2016 at the Liver Clinic and Department of Hepatosis, The Third Hospital of Zhenjiang Affiliated Jiangsu University. The patients were examined every 3 to 6 mo, or more often if clinically indicated. At each visit, liver biochemistry and HBV serology, including HBsAg, HBeAg, anti-HBe, and HBV DNA levels, and genotype, were evaluated. The inclusion criteria were as follows[[17](#_ENREF_17)]: (1) HBsAg-positive for at least the previous 6 months; (2) HBV DNA level of > 1000 copies/mL; and (3) patients with PNALT levels who had at least three ALT values taken in the year prior to baseline liver biopsy, with all values > 40 IU/L and remaining so until the start of treatment or the last follow-up if not treated. Patients were categorized as having PIALT levels if they had at least three ALT values taken, and at least one measurement of > 40 IU/L in the year prior to the baseline biopsy, or any time until the start of treatment or the last follow-up if not treated (intermittently elevated)[[6-8](#_ENREF_6),[10](#_ENREF_10),[18](#_ENREF_18),[19](#_ENREF_19)]. The exclusion criteria were as follows: (1) hepatitis A, C, or D, or human immunodeficiency virus coinfection; (2) evidence of liver disease with another etiology; (3) use of hepatotoxic drugs or regular consumption of alcohol; (4) received previous antiviral (HBV) therapy or any liver functional protection therapy to alleviate hepatic inflammation; and (5) less than three normal ALT values taken prior to the biopsy. The clinical data from these participants were given new numbers and anonymized before analysis. All data were provided separately as Supporting Information.

***Biochemical and serologic tests***

Biochemical tests and complete blood cell counts were performed by using routine automated analyzers. The normal upper limit of ALT level was 40 IU/L. HBsAg, HBeAg, and anti-HBe levels were assayed with commercially available enzyme-linked immunosorbent assay (ELISA) kits. HBV DNA level was measured by using real-time polymerase chain reaction (PCR), with a lower detection limit of 1000 copies/mL (DaAn Gene Co, China).

***Genotype determination by multiplex PCR***

Genotyping was performed by using multiplex PCR with specific primers for each genotype (A–F) of HBV[[20](#_ENREF_20)].

***Liver biopsy and histological assessment***

Liver biopsies were obtained by using a 16-G core aspiration needle, a biopsy length of at least 1.5 cm, and six portal tracts or more. Biopsies were fixed, paraffin-embedded, and stained with hematoxylin and eosin for morphological evaluation and Masson’s trichrome stain for the assessment of fibrosis. The pathologist who reviewed all biopsy specimens was blinded to the biochemical and virological results of the patients, the amount of necrosis and inflammation, and the degree of fibrosis according to the Knodell scoring system[[21](#_ENREF_21)]. Knodell necroinflammatory scores were classified into four categories as follows: minimal (0–3), mild (4–6), moderate (7–9), and severe (10–14) CHB[[22](#_ENREF_22)]. Minimal and mild were considered as insignificant necroinflammatory scores, while moderate and severe were considered as significant necroinflammatory scores. The Knodell fibrosis scores were classified into four categories as follows: minimal (0), mild (1), moderate (3), and severe (4) fibrosis. Minimal and mild were considered as insignificant fibrosis scores, while moderate and severe were considered as significant fibrosis scores.

***Liver stiffness measurement***

LSM was assessed by using transient elastography (FibroScan502, Echosens, Paris, France) with the 3.5-MHz standard probe by the same operator (experience, > 10000 measurements) who was blinded to the other parameters of the patients, as previously described. The examination was performed with the patient lying down in the dorsal decubitus position, with the right arm in maximal abduction. The tip of the probe transducer was placed on the skin, between the ribs at the level of the right lobe of the liver. The results were expressed in kPa, and each LSM corresponds to the median of 10 validated measurements.

***Statistical analysis***

Results are presented as median (range) or mean ± SD as appropriate. Data on demographic and clinical features of the CHB patients were analyzed by using Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc, Chicago, IL, United States). Statistical analyses were performed by using chi-square and Fisher exact tests for categorical variables. The Student *t* test or one-way analysis of variance was used for group comparisons of parametric quantitative data. The equations for the two noninvasive markers analyzed were as follows: FIB-4 = (Age × AST)/(PLT × ALT1/2)

APRI = (AST/ULN) × 100/PLT. The receiver-operating characteristic (ROC) curve was used to calculate the cutoff values of FIB-4, APRI, and LSM. The ROC analysis was performed by using the software 21 MedCalc version 10.4.7.0 (MedCalc, Mariakerke, Belgium). All *P* values were two-sided.

**RESULTS**

***Clinical and pathological characteristics of patients in CHB groups with different levels of ALT***

Table 1 shows that among 245 cases of CHB, 95 were in the PNALT group; 86, in the PIALT1 group (ALT1-2ULN); and 64, in the PIALT2 group (ALT > 2ULN). Body mass index (BMI), platelet count (PLT), prothrombin activity (PTA), ALT and AST (aspiration aminotransferase), serum albumin, E antigen status (positive or negative), HBsAg level, and HBV DNA expression level (≥ 3, < 5, and ≥ 5) were analyzed. We found that the differences in age, ALT, AST, PLT, and other factors were statistically significant (*P* < 0.05) between the PNALT and PIALT groups. No significant differences were found in E antigen status, HBsAg level, and HBV DNA virological indicators. The pathological examination revealed moderate inflammatory necrosis ratios of 16.81% (16/95), 32.56% (28/86), and 45.31% (28/64), and moderate liver fibrosis of 24.2% (23/95), 33.72% (29/86), and 43.75% (28/64) in the PNALT, PIALT1, and PIALT2 groups, respectively. The degrees of inflammation and liver fibrosis in the PIALT group were significantly higher than those in the PNALT group (*P* < 0.05).

***Diagnostic value of APRI in the three groups of CHB***

We considered non-significant hepatic fibrosis and significant hepatic fibrosis as categorical variables and APRI as a variable to test the AUC of APRI in the PNALT, PIALT1, and PIALT2 groups and in all the patients. The AUC of APRI was 0.518 in the PNALT group (95%CI: 0.414–0.622; specificity, 43.1%; sensitivity, 69.6%; cutoff value, 0.202; *P* = 0.7852), 0.659 in the PNALT1 group (95%CI: 0.548–0.757; specificity, 34.5%; sensitivity, 82.4%; cutoff value, 0.524; *P* = 0.011), 0.735 in the PNALT2 group (95%CI: 0.609–0.837; specificity, 83.7%; sensitivity, 85.7%; cutoff value, 1.26; *P* < 0.001), and 0.65 for all the patients (95%CI: 0.587–0.710; specificity, 88.5%; sensitivity, 54.4%; cutoff value, 1.15; *P* < 0.001). APRI showed a high diagnostic value for hepatic fibrosis in the ALT abnormalities of CHB in comparison with the normalities of CHB (Figure 1).

***Diagnostic value of FIB-4 in the three groups of CHB***

We considered non-significant and significant hepatic fibroses as categorical variables and FIB-4 as a variable to test the AUC of FIB-4 in the PNALT, PIALT1, and PIALT2 groups and in all the patients. The AUC of FIB-4 was 0.597 in the PNALT group (95%CI: 0.492– 0.697; specificity, 68.1%; sensitivity, 52.2%; cutoff value, 0.698; *P* = 0.152), 0.642 in the PNALT1 group (95%CI: 0.531–0.742; specificity, 67.9%; sensitivity, 56.9%; cutoff value, 1.174; *P* = 0.021), 0.667 in the PNALT2 group (95%CI: 0.538–0.780; specificity, 52.8%; sensitivity, 78.7%; cutoff value, 1.46; *P* = 0.015), and 0.659 for all the patients (95%CI: 0.596–0.718; specificity, 66.8%; sensitivity, 74.2%; cutoff value, 0.96; *P* < 0.001). FIB-4 showed a high diagnostic value for hepatic fibrosis in the ALT abnormalities of CHB in comparison with the normalities of CHB (Figure 2).

***Diagnostic value of LSM by FibroScan in the three groups of CHB***

We considered non-significant and significant hepatic fibroses as a categorical variable and LSM as a variable to test the AUC of LSM in the PNALT, PIALT1, and PIALT2 groups and in all the patients. The AUC of LSM was 0.769 in the PNALT group (95%CI: 0.009–0.879; 95%CI: 0.709–0.850; specificity, 79.2%; sensitivity, 70.1%; cutoff value, 7.3; *P* < 0.001), 0.8 in the PNALT1 group (95%CI: 0.700–0.879; specificity, 72.4%; sensitivity, 82.7%; cutoff value, 7.5; *P* < 0.001), 0.708 in the PNALT2 group (95%CI: 0.581–0.815; specificity, 72.7%; sensitivity, 67.9%; cutoff value, 8.5; *P* = 0.017), and 0.763 for all the patients (95%CI: 0.596–0.718; specificity, 78.2%; sensitivity, 70.1%; cutoff value, 7.5; *P* < 0.001). LSM showed a high diagnostic value for the three groups of CHB, and even the sensitivity and specificity of the diagnostic value in the PNALT2 showed a downward trend (Figure 5).

***Comparison of the diagnostic value of the three noninvasive liver fibrosis markers in CHB***

The diagnostic values of the three noninvasive markers using APRI, FIB-4, and FibroScan were measured in the PNALT, PIALT1, and PIALT2 and in all the patients. We considered non-significant and significant hepatic fibroses as categorical variables and the three noninvasive liver fibrosis markers or technology as a variable. Table 2 shows no significant difference in the AUC difference between APRI and FIB-4 in the PNALT group; therefore, significant differences were found among LSM, APRI, and FIB-4 in the PNALT group (*P* all < 0.05). Similarly, in the PIALT1 and PIALT2 groups, no significant difference (*P* > 0.05) was found in AUC difference regardless of APRI in comparison with FIB-4 or LSM in comparison with APRI or FIB-4 (*P* all >0.05). From the overall patients, a significant difference in area difference was only found between LSM and APRI, and the difference between the APRI and FIB-4 curves was not statistically significant (*P* > 0.05). No significant difference was found in the APRI comparison between FIB-4 and LSM (*P* > 0.05; Figure 6).

**DISCUSSION**

Hepatic fibrosis, in fact, is a compensatory repair process associated with inflammation and necrosis of the liver. Therefore, ultimately from 25% to 40% of liver fibrosis will progress to cirrhosis and even develop into liver cancer. Early liver fibrosis can be reversed after correct treatment; thus, early diagnosis of liver fibrosis will be beneficial to the treatment of CHB.

Liver biopsy is still the gold standard assessment tool for liver fibrosis in CHB, regardless that it is invasive, expensive, and associated with risk of complications and poor patient compliance, and of subjective differences in pathologists. The accuracy of the pathological diagnosis of liver fibrosis can only be approximately 90% and even reported to be < 80%[[23](#_ENREF_23)]. Therefore, noninvasive diagnostic markers of liver fibrosis have been developed, such as serum markers and models, imaging, transient liver hardness, and other noninvasive technology. Although these noninvasive diagnostic methods have their own advantages and disadvantages, they have not completely replaced the possibilities offered by liver biopsy. However, new technologies and methods have become greatly improved. Among the noninvasive tests developed are FIB-4, APRI, and LSM; previous analyses have shown these noninvasive markers and technology to be strong predictors of liver fibrosis.

A multicenter, retrospective study reported that the AUC of APRI were 0.72, 0.812, and 0.707 in F2, F3, and F4 CHB patients, respectively[[24](#_ENREF_24)]. The AUC of APRI was 0.65, 0.659, and 0.735 in our PNALT, PIALT1, and PIALT2 CHB patients, respectively. The results showed that the diagnostic value of APRI in the CHB patients with elevated ALT levels was better than that in the CHB patients with normal ALT. APRI is the ratio of AST to PLT, and elevated AST levels has a higher APRI value and thus is more likely to distinguish patient groups with different AST levels.

In a study of 388 cases of cirrhosis of varied severity assessed using APRI and FIB-4, the AUC were 0.68 (95%CI: 0.63–0.74) and 0.73 (95%CI: 0.68-0.78)[[25](#_ENREF_25)], respectively. In our study, the AUC of FIB-4 was 0.597 for PNALT, 0.642 for PNALT1, and 0.667 for PNALT2. The diagnostic value of FIB-4 in the CHB patients with elevated ALT levels was better than that in the CHB patients with normal ALT levels and APRI.

We detected LSM by FibroScan in the CHB patients, with an AUC of 0.769 for PNALT, 0.8 for PIALT1, 0.708 for PIALT2, and 0.763 for all the patients. LSM has a good diagnostic value for the three groups of CHB patients. Furthermore, the diagnostic value in the high ALT group was not as good as that in the PNALT group, and even the sensitivity and specificity of the diagnostic fibrosis value in the PNALT2 group had a downward trend. In a test report for FibroScan in China[[26](#_ENREF_26)], the AUC were 0.916 and 0.971 for the diagnosis of stage ≥2 liver fibrosis (F ≥ 2, FO–l *vs* F2–4) and cirrhosis (F = 4, F0–3 *vs* F4), and the sensitivity and accuracy of AUC in the ALT level ≥ 2 ULN group were significantly lower than those in the other lower-level ALT groups. The reason is that LSM is susceptible to liver inflammation[[27](#_ENREF_27),[28](#_ENREF_28)] and cholestasis[[29](#_ENREF_29),[30](#_ENREF_30)].

We compared three noninvasive methods for the diagnosis of hepatic fibrosis. The results showed that the AUC differences among LSM, APRI, and FIB-4 were statistically significant in the PNALT group (*P* all < 0.05) but not in the PIALT1 and PIALT2 groups (*P* all > 0.05), regardless of the comparison of between APRI and FIB-4, and between LSM and APRI or FIB-4 (*P* all > 0.05). For the all the patients, we found that the difference was statistically significant only between LSM and APRI (*P* < 0.05).

In a previous report from Korea, the diagnostic value of LSM for hepatic fibrosis was compared with that of APRI. The results suggest that LSM is superior to APRI in 916 patients with CHB (AUC: 0.774 *vs* 0.72 for ≥ F2, 0.849 *vs* 0.812 for ≥F3, and 0.902 *vs* 0.707 for F4; all *P* < 0.05)[[24](#_ENREF_24)]. Another report revealed that LSM was better than APRI and FIB-4 when the LSM cutoff value was >13.6 kPa for the diagnosis of portal hypertension in cirrhosis patients[[31](#_ENREF_31)].

In conclusion, we evaluated three common noninvasive hepatic fibrosis techniques in different ALT groups. The results showed that APRI and FIB-4 were not the ideal noninvasive hepatic fibrosis models in the PNALT patients and that APRI and FIB-4 established according to the common blood biochemical indicators were more suitable for active CHB. LSM determined liver hardness for assessment of the degree of liver fibrosis. Therefore, LSM was superior to APRI and FIB-4 in the patients with PNALT because of the influence of liver inflammation and necrosis.

**COMMENTS**

***Background***

Although serum levels of alanine transaminase (ALT), an enzyme released from hepatocytes during liver injury, should reflect the degree of liver damage, Furthermore, some large cohort studies have shown that patients with chronic hepatitis B (CHB) who have normal serum ALT levels were also at risk for the development of cirrhosis and hepatocellular carcinoma. Moreover, liver biopsy is also the current gold standard for assessing hepatic inﬂammation and ﬁbrosis in patients with chronic hepatitis B virus (HBV) who have persistently normal alanine transaminase (PNALT). The invasiveness of liver puncture, the limitation of the specimen, and the causes of poor patient compliance has restricted the application of liver biopsy.

***Research frontiers***

The limitation of liver biopsy have led to the development of noninvasive models such as FIB-4 and aspartate aminotransferase-to-platelet ratio index (APRI) for evaluating fibrosis in patients with chronic HBV infection. Liver stiffness measurement (LSM) by using transient elastography (FibroScan) has been widely used in the diagnosis of chronic liver fibrosis. However, the diagnostic values of FIB-4, APRI, and LSM in patients with HBV infection with PNALT is not clear.

***Innovations and breakthroughs***

The authors evaluated three common noninvasive hepatic fibrosis techniques in different ALT groups. The results showed that APRI and FIB-4 was not the ideal noninvasive hepatic fibrosis model for PNALT patients. LSM was superior to APRI and FIB-4 in PNALT patients because of the influence of liver inflammation and necrosis.

***Applications***

Noninvasive models such as FIB-4, APRI and Liver stiffness measurement for evaluating fibrosis in patients with chronic HBV infection

***Terminology***

PNALT means hepatitis B virus infection who have persistently normal alanine transaminase.

***Peer-review***

The manuscript is well written and the numerical simulations are well performed.

**REFERENCES**

1 **Yan H**, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J, Li W. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife* 2012; **1**: e00049 [PMID: 23150796 DOI: 10.7554/eLife.00049]

2 **McMahon BJ**. The natural history of chronic hepatitis B virus infection. *Semin Liver Dis* 2004; **24** Suppl 1: 17-21 [PMID: 15192797 DOI: 10.1055/s-2004-828674]

3 **Kim WR**, Flamm SL, Di Bisceglie AM, Bodenheimer HC; Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology* 2008; **47**: 1363-1370 [PMID: 18366115 DOI: 10.1002/hep.22109]

4 **Nunnari G**, Pinzone MR, Cacopardo B. Lack of clinical and histological progression of chronic hepatitis C in individuals with true persistently normal ALT: the result of a 17-year follow-up. *J Viral Hepat* 2013; **20**: e131-e137 [PMID: 23490382 DOI: 10.1111/jvh.12029]

5 **Lai M**, Hyatt BJ, Nasser I, Curry M, Afdhal NH. The clinical significance of persistently normal ALT in chronic hepatitis B infection. *J Hepatol* 2007; **47**: 760-767 [PMID: 17928090 DOI: 10.1016/j.jhep.2007.07.022]

6 **Wang H**, Xue L, Yan R, Zhou Y, Wang MS, Cheng MJ, Hai-Jun Huang. Comparison of histologic characteristics of Chinese chronic hepatitis B patients with persistently normal or mildly elevated ALT. *PLoS One* 2013; **8**: e80585 [PMID: 24260428 DOI: 10.1371/journal.pone.0080585]

7 **Papatheodoridis GV**, Manolakopoulos S, Liaw YF, Lok A. Follow-up and indications for liver biopsy in HBeAg-negative chronic hepatitis B virus infection with persistently normal ALT: a systematic review. *J Hepatol* 2012; **57**: 196-202 [PMID: 22450396 DOI: 10.1016/j.jhep.2011.11.030]

8 **Kumar M**, Sarin SK, Hissar S, Pande C, Sakhuja P, Sharma BC, Chauhan R, Bose S. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. *Gastroenterology* 2008; **134**: 1376-1384 [PMID: 18471514 DOI: 10.1053/j.gastro.2008.02.075]

9 **Lin CL**, Liao LY, Liu CJ, Yu MW, Chen PJ, Lai MY, Chen DS, Kao JH. Hepatitis B viral factors in HBeAg-negative carriers with persistently normal serum alanine aminotransferase levels. *Hepatology* 2007; **45**: 1193-1198 [PMID: 17464993 DOI: 10.1002/hep.21585]

10 **Dai CY**, Chuang WL, Huang JF, Yu ML. Hepatitis B e antigen-negative patients with persistently normal alanine aminotransferase levels and hepatitis B virus DNA &gt; 2000 IU/mL. *Hepatology* 2009; **49**: 704-5; author reply 705-6 [PMID: 19177587 DOI: 10.1002/hep.22723]

11 **Yuen MF**, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, Wong BC, Lai KC, Lai CL. Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. *Gut* 2005; **54**: 1610-1614 [PMID: 15871997 DOI: 10.1136/gut.2005.065136]

12 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218 DOI: 10.1001/jama.295.1.65]

13 **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 [PMID: 16729309 DOI: 10.1002/hep.21178]

14 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

15 **Huang R**, Jiang N, Yang R, Geng X, Lin J, Xu G, Liu D, Chen J, Zhou G, Wang S, Luo T, Wu J, Liu X, Xu K, Yang X. Fibroscan improves the diagnosis sensitivity of liver fibrosis in patients with chronic hepatitis B. *Exp Ther Med* 2016; **11**: 1673-1677 [PMID: 27168788 DOI: 10.3892/etm.2016.3135]

16 **Mikolasevic I**, Orlic L, Franjic N, Hauser G, Stimac D, Milic S. Transient elastography (FibroScan(®)) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand? *World J Gastroenterol* 2016; **22**: 7236-7251 [PMID: 27621571 DOI: 10.3748/wjg.v22.i32.7236]

17 **Liao B**, Wang Z, Lin S, Xu Y, Yi J, Xu M, Huang Z, Zhou Y, Zhang F, Hou J. Significant fibrosis is not rare in Chinese chronic hepatitis B patients with persistent normal ALT. *PLoS One* 2013; **8**: e78672 [PMID: 24205292 DOI: 10.1371/journal.pone.0078672]

18 **Wang H**, Xue L, Yan R, Zhou Y, Wang MS, Cheng MJ, Huang HJ. Comparison of FIB-4 and APRI in Chinese HBV-infected patients with persistently normal ALT and mildly elevated ALT. *J Viral Hepat* 2013; **20**: e3-10 [PMID: 23490387 DOI: 10.1111/jvh.12010]

19 **Arora S**, O'Brien C, Zeuzem S, Shiffman ML, Diago M, Tran A, Pockros PJ, Reindollar RW, Gane E, Patel K, Wintfeld N, Green J. Treatment of chronic hepatitis C patients with persistently normal alanine aminotransferase levels with the combination of peginterferon alpha-2a (40 kDa) plus ribavirin: impact on health-related quality of life. *J Gastroenterol Hepatol* 2006; **21**: 406-412 [PMID: 16509866 DOI: 10.1111/j.1440-1746.2005.04059.x]

20 **Kirschberg O**, Schüttler C, Repp R, Schaefer S. A multiplex-PCR to identify hepatitis B virus--enotypes A-F. *J Clin Virol* 2004; **29**: 39-43 [PMID: 14675868]

21 **Knodell RG**, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431-435 [PMID: 7308988]

22 **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 [PMID: 23234725 DOI: 10.1016/S0140-6736(12)61425-1]

23 **Jin SY**. [Role of liver biopsy in the assessment of hepatic fibrosis--its utility and limitations]. *Korean J Hepatol* 2007; **13**: 138-145 [PMID: 17585187]

24 **Seo YS**, Kim MY, Kim SU, Hyun BS, Jang JY, Lee JW, Lee JI, Suh SJ, Park SY, Park H, Jung EU, Kim BS, Kim IH, Lee TH, Um SH, Han KH, Kim SG, Paik SK, Choi JY, Jeong SW, Jin YJ, Lee KS, Yim HJ, Tak WY, Hwang SG, Lee YJ, Lee CH, Kim DG, Kang YW, Kim YS; Korean Transient Elastography Study Group. Accuracy of transient elastography in assessing liver fibrosis in chronic viral hepatitis: A multicentre, retrospective study. *Liver Int* 2015; **35**: 2246-2255 [PMID: 25682719 DOI: 10.1111/liv.12808]

25 **Martin J**, Khatri G, Gopal P, Singal AG. Accuracy of ultrasound and noninvasive markers of fibrosis to identify patients with cirrhosis. *Dig Dis Sci* 2015; **60**: 1841-1847 [PMID: 25586089 DOI: 10.1007/s10620-015-3531-1]

26 **Chen XB**, Zhu X, Chen LY, Chen EQ, Tang H. [Accuracy of FibroScan for the diagnosis of liver fibrosis influenced by serum alanine aminotransferase levels in patients with chronic hepatitis B]. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**: 286-290 [PMID: 21586228 DOI: 10.3760/cma.j.issn.1007-3418.2011.04.013]

27 **Liang XE**, Chen YP, Zhang Q, Dai L, Zhu YF, Hou JL. Dynamic evaluation of liver stiffness measurement to improve diagnostic accuracy of liver cirrhosis in patients with chronic hepatitis B acute exacerbation. *J Viral Hepat* 2011; **18**: 884-891 [PMID: 21062388 DOI: 10.1111/j.1365-2893.2010.01389.x]

28 **Wong GL**, Wong VW, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ, Chan HL. Increased liver stiffness measurement by transient elastography in severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol* 2009; **24**: 1002-1007 [PMID: 19457152 DOI: 10.1111/j.1440-1746.2009.05779.x]

29 **Harata M**, Hashimoto S, Kawabe N, Nitta Y, Murao M, Nakano T, Arima Y, Shimazaki H, Ishikawa T, Okumura A, Ichino N, Osakabe K, Nishikawa T, Yoshioka K. Liver stiffness in extrahepatic cholestasis correlates positively with bilirubin and negatively with alanine aminotransferase. *Hepatol Res* 2011; **41**: 423-429 [PMID: 21435129 DOI: 10.1111/j.1872-034X.2011.00797.x]

30 **Millonig G**, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Büchler MW, Seitz HK, Mueller S. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology* 2008; **48**: 1718-1723 [PMID: 18836992 DOI: 10.1002/hep.22577]

31 **Zhang W**, Wang L, Wang L, Li G, Huang A, Yin P, Yang Z, Ling C, Wang L. Liver stiffness measurement, better than APRI, Fibroindex, Fib-4, and NBI gastroscopy, predicts portal hypertension in patients with cirrhosis. *Cell Biochem Biophys* 2015; **71**: 865-873 [PMID: 25417057 DOI: 10.1007/s12013-014-0275-z]

**P-Reviewer:** Nakao T **S-Editor:** Qi Y **L-Editor: E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**Table 1 Demographic and clinical characteristics of chronic hepatitis B patients**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Characteristics** | **PNALT (*n* = 95)** | **PIALT (ALT 1-2 × ULN, *n* = 86)** | **PIALT (ALT ≥ 2 × ULN, *n* = 64)** | **Statistics** | ***P* value** |
|  |
| Age (yr) | 34.5 ± 11.2 | 34.2 ± 12.5 | 36.5 ± 13.5 | 5.423 | **0.0211** |
| Sex |  |  |  |  |  |
| Male | 70 (73.7) | 58 (67.4) | 46 (71.9) | 0.885 | 0.6422 |
| Female | 25(26.3) | 28 (32.6) | 18 (28.1) |
| BMI | 23.4 ± 2.65 | 24.0 ± 3.6 | 24.25 ± 3.37 | 1.231 | 0.5431 |
| PLT (× 109/L) | 200.1 ± 60.3 | 196.8 ± 65.4 | 186.5 ± 74.5 | 6.364 | **0.0181** |
| PTA (%) | 99.6 ± 6.7 | 99.6 ± 8.7 | 102.3 ± 10.3 | 0.674 | 0.7741 |
| ALB (g/L) | 41.3 ± 3.4 | 41.6 ± 3.7 | 42.4 ± 4.1 | 1.536 | 0.5431 |
| ALT (U/L) | 21.4 ± 4.3 | 56.2 ± 19.4 | 113.6 ± 55.3 | 25.754 | **< 0.0011** |
| AST (U/L) | 22.6 ±6.8 | 55.4 ± 16.6 | 124.5 ± 57.6 | 31.644 | **< 0.0011** |
| APRI | 0.32 ± 0.14 | 0.61 ± 0.44 | 1.25 ± 0.62 | 284.92 | **< 0.0011** |
| FIB-4 | 0.72 ± 0.36 | 1.12 ± 0.53 | 1.67 ± 0.84 | 56.37 | **< 0.0011** |
| FibroScan(KPa) | 5.33± 2.45 | 7.36 ± 3.14 | 10.22 ± 5.53 | 46.34 | **< 0.0011** |
| HBsAg (LgIU⁄L) | 4.41 ± 0.73 | 4.53 ± 0.88 | 4.38 ± 0.64 | 0.743 | 0.437 |
| HBV DNA (Lgcopies/mL) | 6.78 ± 2.13 | 6.53 ± 2.43 | 6.42 ± 2.54 | 0.864 | 0.2541 |
| ≥ 3,< 5 | 15 (15.8) | 11(12.8) | 10 (15.6) | 0.384 | 0.8252 |
| ≥ 5 | 80 (84.2) | 75 (87.2) | 54 (84.4) |
| E antigen |  |  |  |  |  |
| Positive | 44 (44.4) | 31 (36) | 29 (45.3) | 1.78 | 0.4112 |
| Negative | 55 (55.6) | 55(64) | 35 (54.7) |
| Necroinflammatory scores | 2.21 ± 2.14 | 3.47±3.64 | 4.74 ± 3.65 | 23.43 | **0.001** |
| minimal | 35 (32.4) | 20 (23.3) | 8 (12.5) | 18.69 | **0.005** |
| mild | 44 (38) | 38 (44.2) | 27 (42.4) |
| moderate | 15(15.8) | 26 (30.2) | 22 (34.4) |
| severe | 1(0.9) | 2 (2.3) | 7 (10.9) |
| fibrosis scores | 1.53 ± 0.46 | 2.38 ± 1.27 | 2.85 ± 1.75 | 15.237 | **0.0051** |
| minimal | 32 (33.7) | 18 (20.9) | 13 (20.3) | 13.275 | **0.0352** |
| mild | 40(42.1) | 40 (51.2) | 23 (35.9) |
| moderate | 21 (22.1) | 24 (27.9) | 21 (32.8) |
| severe | 2(2.1) | 5 (5.8) | 7 (10.9) |

1One-way analysis; 2Pearson χ2. APRI = [AST (U/L)/ULN × 100/PLT(109/L)]; FIB-4 = [age (yr) × AST (U/L)]/[PLT (109/L) × (ALT U/L)1/2].

**Table 2 Pairwise comparison of receiver-operating characteristic curves**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **PNALT** | **PIALT1** | **PIALT2** | **All patients** |
| APRI - FIB\_4 | 　 | 　 | 　 | 　 |
| Difference between areas  | 0.0628 | 0.0169 | 0.068 | 0.00904 |
| Standard Error | 0.0557 | 0.0699 | 0.0738 | 0.0245 |
| 95%CI | -0.046 - 0.172 | -0.120 - 0.154 | -0.077 - 0.213 | -0.039 - 0.057 |
| *z*statistic | 1.129 | 0.242 | 0.921 | 0.369 |
| Significance level（*P*） | 0.2588 | 0.8087 | 0.3569 | 0.712 |
| APRI- LSM |  |  |  |  |
| Difference between areas  | 0.256 | 0.124 | 0.0263 | 0.111 |
| Standard Error | 0.104 | 0.0896 | 0.0882 | 0.0518 |
| 95%CI  | 0.0525 -0.459 | -0.0511 - 0.300 | -0.147- 0.199 | 0.00909- 0.212 |
| z statistic | 2.465 | 1.389 | 0.298 | 2.135 |
| Significance level (*P*) | **0.0137** | 0.1649 | 0.7655 | **0.0327** |
| FIB\_4 -LSM |  |  |  |  |
| Difference between areas  | 0.193 | 0.141 | 0.0417 | 0.102 |
| Standard Error | 0.106 | 0.0938 | 0.0957 | 0.0531 |
| 95%CI  | -0.014 - 0.400 | -0.0425- 0.325 | -0.146 - 0.229 | -0.00246 - 0.206 |
| z statistic | 1.829 | 1.507 | 0.435 | 1.914 |
| Significance level (*P*) | **0.0374** | 0.1318 | 0.663 | 0.0557 |



**Figure 1 Diagnostic value of aspartate aminotransferase-to-platelet ratio index in chronic groups of hepatitis B.**



**Figure 2 Diagnostic value of FIB-4 in chronic groups of hepatitis B.**



**Figure 3 Diagnostic value of liver stiffness measurement in chronic groups of hepatitis B.**



**Figure 4 Comparison of the diagnostic value of three noninvasive liver fibrosis markers in chronic hepatitis B.**