

Original paper

Clinical usefulness of new noninvasive serum biomarkers for the assessment of liver fibrosis and steatosis in children with chronic hepatitis C

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Abstract

Aim of the study: Recently, novel serum markers modified by the body mass index z-score (BMI z-score) were proposed as a reliable noninvasive alternative for the detection of significant fibrosis and steatosis in children with chronic hepatitis C (CHC). The aim of this study was to evaluate the clinical usefulness of these biomarkers.

Material and methods: Thirty children aged 9.4 ± 3.7 years (14 males, 16 females) with CHC were included in this study. In all patients, histopathological evaluation of the liver fibrosis was performed using a 5-point METAVIR scoring system (≥ 2 points = significant fibrosis). Significant steatosis was diagnosed with $> 33\%$ of hepatocytes affected. The following noninvasive markers of liver disease were calculated: the modified aspartate transaminase (AST)-to-platelet ratio index (M-APRI: BMI z-score \times APRI), the modified Fibrosis-4 index (M-FIB-4: BMI z-score \times FIB-4), and a novel marker, B-AST (BMI z-score \times AST). The clinically useful cut-offs for each marker were selected as simple round numbers, indicating significant fibrosis and steatosis.

Results: Significant fibrosis was detected in 7/30 (23%) cases, and significant steatosis was observed in 4 (13%) patients. Comparison with the histopathological evaluation revealed that B-AST < 0 excluded significant fibrosis, and < 100 excluded all patients with significant steatosis. For the M-APRI, < 0 excluded significant fibrosis, and < 0.5 excluded significant steatosis. For the M-FIB-4, < 0 excluded significant fibrosis and < 0.2 excluded significant steatosis.

Conclusions: Negative values of all three markers that included the BMI z-score excluded all patients with both significant fibrosis and significant steatosis.

Key words: chronic hepatitis C, fibrosis, steatosis, biomarkers.

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Introduction

According to the recent recommendations of the European Association for the Study of the Liver (EASL), in patients with chronic hepatitis C (CHC), liver biopsy may be replaced by noninvasive methods to assess liver disease severity prior to antiretroviral treatment [1]. The ability to determine fibrosis has been evaluated for many of the noninvasive methods, including imaging studies (elastography) and serum

biomarkers [2-4]. However, data on the diagnostic performance of these noninvasive tests in children with CHC are lacking, and none of these methods has been fully validated in children to date [2, 3, 5].

In our recent study, we demonstrated that novel serum biomarkers modified by including the body mass index z-score (BMI z-score) in their formulas show a better diagnostic performance in detecting significant fibrosis and significant steatosis compared to standard tests (aspartate transaminase-to-platelet ratio

index, APRI, and the Fibrosis-4 index, FIB-4) [6]. We also proposed and evaluated a novel simple biomarker, B-AST, based on the BMI z-score and aspartate aminotransferase (AST) only (B-AST = AST \times BMI z-score). Its diagnostic performance was excellent in predicting significant fibrosis and significant steatosis in pediatric patients with CHC [6].

In the present study we aimed to continue the research in this field and to analyze the clinical usefulness of the novel serum biomarkers including the BMI z-score for detection of significant fibrosis and steatosis in children with CHC in comparison with the results of the histopathological evaluation as a gold standard.

Material and methods

In this prospective clinicopathological study, we included consecutive pediatric treatment-naïve patients with CHC who underwent a liver biopsy in our tertiary health care department between 2010 and 2014. Liver biopsy was performed as part of the qualification procedure for antiviral treatment. Simultaneously with the liver biopsy, the following three noninvasive serum biomarkers were calculated, modifying the previously evaluated markers [7, 8]:

1. Modified APRI (M-APRI) = BMI z-score \times [AST (IU/l)/AST ULN (IU/l)/platelet count ($10^9/l$)] \times 100;
2. Modified FIB-4 (M-FIB-4) = BMI z-score \times [age (years) \times AST (IU/l)]/[platelet count ($10^9/l$) \times $\sqrt{\text{ALT} \left(\frac{\text{IU}}{\text{l}} \right)}$];
3. B-AST = BMI z-score \times AST (IU/l).

The biomarker determinations were performed using commercially available laboratory kits (XT-1800i, Sysmex for platelets; Vitros 5600, Ortho-Clinical Diagnostics, Johnson & Johnson for biochemical parameters). The diagnostic performance for all three biomarkers was calculated using area under the ROC curve (AUROC) analysis, and these data were presented in our previous study [6].

Chronic hepatitis C was diagnosed in children with at least 6-month history of hepatitis based on positive anti-HCV testing and was confirmed with nucleic acid testing: positive HCV RNA real-time polymerase chain reaction analysis (RT-PCR method; Amplicor, Roche; Cobas TaqMan, Roche). Both biochemical and serological tests were performed using commercially available laboratory kits (Vitros 5600, Ortho-Clinical Diagnostics, Johnson & Johnson).

BMI z-scores were calculated using the World Health Organization (WHO) Child Growth Standards

and Growth Reference data with the WHO anthropometric calculator, AnthroPlus v.1.0.4.

Histopathological evaluation was performed using the METAVIR scoring system. Fibrosis was scored on a 5-point scale (F0 = no fibrosis; F \geq 2 = significant fibrosis; F4 = cirrhosis) [9]. Steatosis was considered significant in cases in which $>$ 33% of hepatocytes were affected.

Statistical analysis

All statistical analyses were performed using the licensed MedCalc Statistical Software, ver. 17.4 (MedCalc, Ostend, Belgium). A two-sided *p*-value $<$ 0.05 was considered significant. Data were tested for normal distribution using the Kolmogorov-Smirnov test and were expressed as the mean \pm standard deviation or median (interquartile range, IQR), respectively.

To evaluate the possible clinical usefulness of the three biomarkers, scatterplots of each marker according to the presence of significant fibrosis and significant steatosis were constructed. Based on the obtained results, clinically useful cut-offs for each marker were selected as simple round numbers, indicating significant fibrosis and steatosis, respectively.

Ethical statement

The investigation was concordant with the principles outlined in the Declaration of Helsinki and its amendments. Written informed consent was collected from all the patients and/or their parents/guardians before their inclusion in the study.

Results

Patients

Thirty children (14 boys and 16 girls) aged 9.4 ± 3.7 years were included in the study group, characterized in detail in our previous paper [6]. The mean duration of hepatitis C virus (HCV) infection was 8.2 ± 3.1 years. Most of the children (22/30, 73%) were infected vertically by an infected mother. The majority of patients (21/30, 70%) presented with HCV genotype 1 (19/30, 63% with HCV genotype 1b, and 2/30, 7% with 1a), whereas 5/30 (17%) had genotype 3 and 4/30 (13%) genotype 4. The mean BMI z-score value was 0.39 ± 1.04 . The median levels of ALT and AST were 60.5 (36-79) and 50 (37-68) IU/l, respectively. The mean platelet count was $308.2 \pm 90.5 \times 10^9/l$. The median HCV viral load was 5.95×10^5 (2.25×10^5 – 1.53×10^6) IU/ml.

Histopathological evaluation of fibrosis and steatosis

On histopathological evaluation, 22/30 (73%) patients presented with fibrosis, including 7/30 (23%) with significant fibrosis (all 7 children with METAVIR F = 2; no child had a METAVIR F score of 3 or 4). In 8/30 (27%) patients, liver steatosis was observed, and in 4 (13%) of them, the steatosis was significant (> 33% of hepatocytes affected).

Noninvasive markers of liver fibrosis and steatosis

Mean values (mean ± standard deviation, SD) of the analyzed biomarkers were as follows: 0.28 ± 0.69 for

M-APRI, 0.09 ± 0.28 for M-FIB-4, and 31.71 ± 69.87 for B-AST.

Clinical usefulness of new noninvasive markers

Based on the comparison with the histopathological evaluation, we suggest the following clinically useful values for the novel simple marker B-AST: B-AST < 0 excludes significant fibrosis, and < 100 excludes all patients with significant steatosis (Fig. 1). Consequently, patients with negative B-AST values have neither significant fibrosis nor significant steatosis. For the M-APRI, < 0 excludes significant fibrosis, and < 0.5 excludes significant steatosis (Fig. 2). Thus, negative M-APRI values

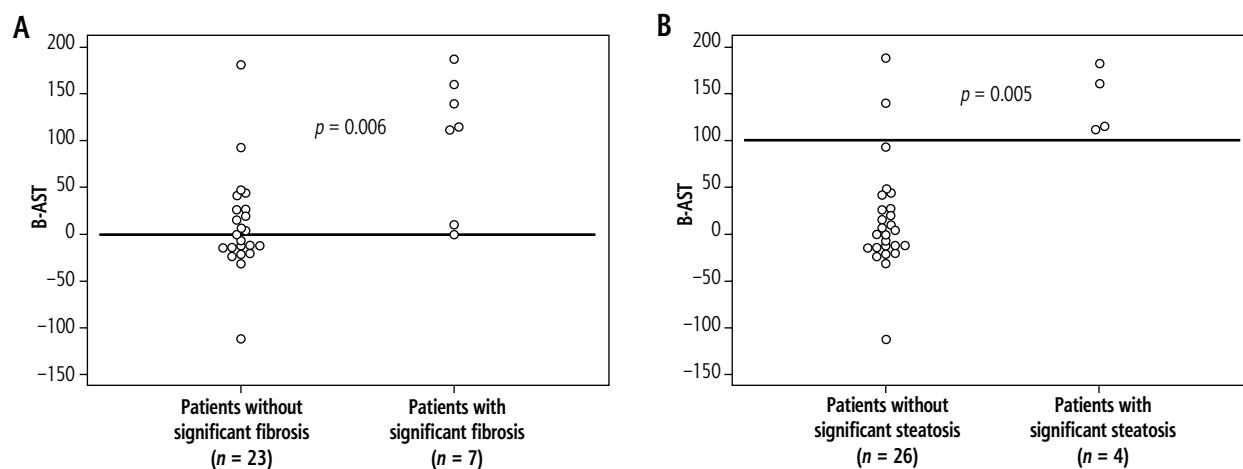


Fig. 1. Scatterplots of B-AST according to the presence of significant fibrosis (A) and significant steatosis (B). Solid lines = B-AST cut-offs for significant fibrosis (A) and significant steatosis (B). In A, none of the patients with B-AST below 0 (solid line) had significant fibrosis. In B, none of the patients with B-AST below 100 (solid line) had significant steatosis. Thus, with B-AST values below 0, all patients with both significant fibrosis and significant steatosis could be excluded

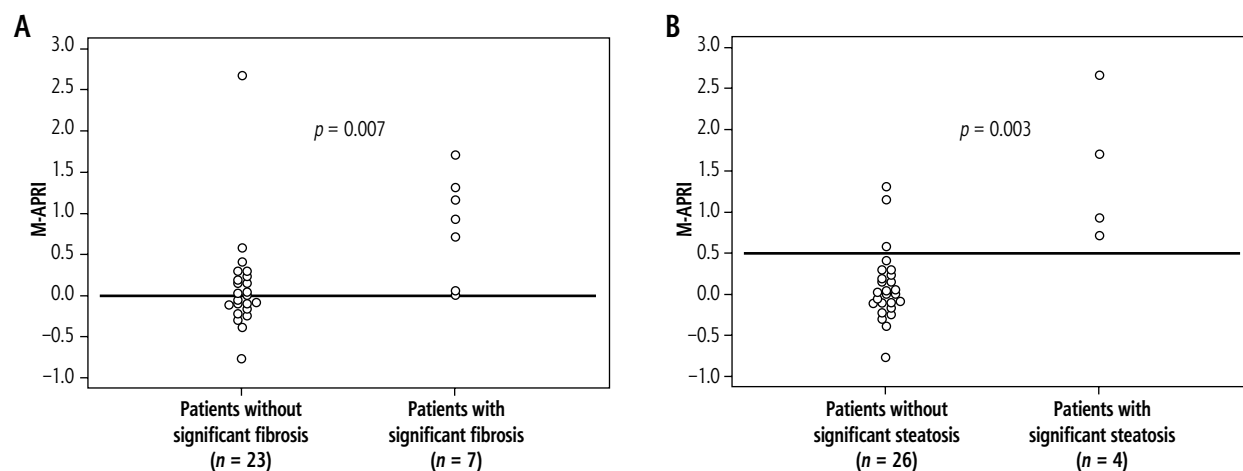


Fig. 2. Scatterplots of M-APRI according to the presence of significant fibrosis (A) and significant steatosis (B). Solid lines = M-APRI cut-offs for significant fibrosis (A) and significant steatosis (B). In A, none of the patients with M-APRI values below 0 (solid line) had significant fibrosis. In B, none of the patients with M-APRI values below 0.5 (solid line) had significant steatosis. Thus, with M-APRI values below 0, all patients with both significant fibrosis and significant steatosis could be excluded

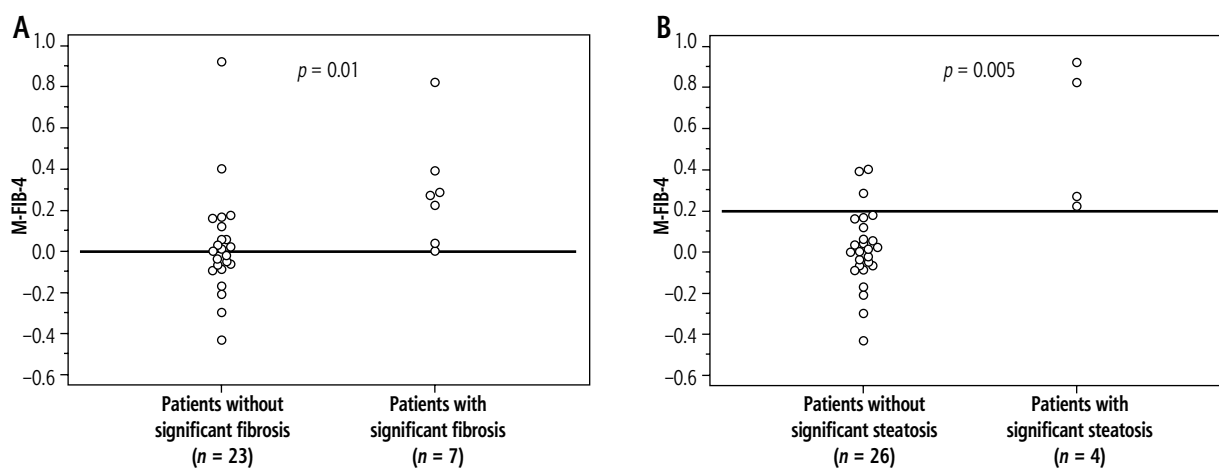


Fig. 3. Scatterplots of M-FIB-4 according to the presence of significant fibrosis (**A**) and significant steatosis (**B**). Solid lines = M-FIB-4 cut-offs for significant fibrosis (**A**) and significant steatosis (**B**). In **A**, none of the patients with M-FIB-4 values below 0 (solid line) had significant fibrosis. In **B**, none of the patients with M-FIB-4 values below 0.2 (solid line) had significant steatosis. Thus, with M-FIB-4 values below 0, all patients with both significant fibrosis and significant steatosis could be excluded

exclude all patients with significant fibrosis and steatosis. For the M-FIB-4, < 0 excludes significant fibrosis and < 0.2 excludes significant steatosis (Fig. 3). Consequently, patients with M-FIB-4 values < 0 have neither significant fibrosis nor steatosis. Thus, negative values of all three markers that included the BMI z-score exclude all patients with both significant fibrosis and significant steatosis.

Discussion

Determination of liver fibrosis before implementation of antiviral therapy in patients with CHC is essential for evaluation of the urgency of treatment and duration of therapy, and may indicate the need for intensive monitoring of the patient [10]. Due to the limitations of the liver biopsy procedure, several noninvasive serum markers of liver fibrosis have been developed recently; however, their diagnostic performance in pediatric patients with CHC is limited [5, 6, 11].

The results of our recent study indicate that biomarkers modified by including the BMI z-score in the formulas of previously used markers, APRI and FIB-4, perform better for predicting severe fibrosis and steatosis. In addition, the novel, simple, inexpensive, and easily available biomarker B-AST, with a cut-off of 92.8, showed 71% sensitivity and 95% specificity for detecting significant fibrosis [6]. Its sensitivity for predicting severe steatosis was 100% and specificity 92% [6].

In the present study, we proposed simple round cut-offs for all three novel biomarkers, which would allow all patients with significant fibrosis and steatosis to be excluded. Since negative B-AST values exclude all patients with both significant fibrosis and steatosis,

one may speculate that liver biopsy could be avoided in all patients who presented with a B-AST value < 0 . In our present study, 11/30 (37%) patients had a negative B-AST value and consequently would not be considered as candidates for liver biopsy.

The main limitation of this study is the small number of patients included in the study group. However, nowadays liver biopsy is rarely performed in children. Therefore, an analysis of the diagnostic performance of the new noninvasive methods in comparison with the histopathological assessment as a gold standard is rarely possible. Thus, the results of this study should be considered unique, although they need to be confirmed in larger cohorts of patients.

In conclusion, based on the results of this study, negative values of all three novel biomarkers based on the BMI z-score allow one to exclude all patients with significant fibrosis and steatosis. Thus, all children with CHC presenting with B-AST < 0 could avoid the liver biopsy procedure.

Disclosure

Authors report no conflict of interest.

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