



Changes in APRI and FIB-4 in HBeAg-negative treatment-naive chronic hepatitis B patients with significant liver histological lesions receiving 5-year entecavir therapy

Ruyu Liu¹ · Jiang Guo² · Yao Lu¹ · Lu Zhang¹ · Ge Shen¹ · Shuling Wu¹ · Mi Chang¹ · Leiping Hu¹ · Hongxiao Hao¹ · Minghui Li¹ · Yao Xie¹

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Abstract

According to guidelines, antiviral therapy for adults with immune-active chronic hepatitis B (CHB) should be adopted to decrease the risk of liver-related complications. Fibrosis assessment during antiviral treatment is a key step in antiviral therapy evaluation. Liver biopsy is the gold standard for assessing the degree of liver necroinflammation and fibrosis. However, because of its cost and the risk of life-threatening complications, performing a liver biopsy more than once after long-term effective treatment is difficult. In this study, we aimed to evaluate changes in liver fibrosis during 5 years of entecavir (ETV) treatment using noninvasive fibrosis markers in hepatitis B e-antigen (HBeAg)-negative treatment-naive CHB patients who require antiviral therapy. A total of 303 HBeAg-negative treatment-naive patients were enrolled in this study. Liver biopsy was performed before initiation of antiviral therapy. The diagnosis of CHB was made according to Chinese guidelines for the management of CHB. Patients requiring antiviral therapy (liver fibrosis stage \geq F2, METAVIR scoring system) were treated with ETV for at least 5 years. These patients were followed up at 6-month intervals. A clinical and virological evaluation was performed at baseline and again at 12, 24, 36, 48, and 60 months during ETV treatment. Aspartate Aminotransferase to Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) index were used to assess dynamic changes in liver fibrosis in HBeAg-negative CHB patients after 1, 2, 3, 4, and 5 years of ETV treatment. All enrolled patients underwent liver biopsy at baseline. Using the METAVIR fibrosis stages, there were 107, 125, 54, and 17 patients in F1, F2, F3, and F4 stages, respectively. The APRI and FIB-4 indexes enabled the correct identification of patients with severe fibrosis (METAVIR F3–F4), with an area under the receiver operating characteristic curve of 0.77 (95% confidence interval [CI] 0.72–0.83) and 0.76 (95% CI 0.70–0.82), respectively. The APRI values decreased significantly in F2 and F3 patients after 1 year of ETV therapy ($P < 0.01$). However, for F4 patients, APRI values decreased significantly at year 3 ($P < 0.05$). The FIB-4 values of F2, F3, and F4 patients who received ETV treatment were significantly decreased after 1, 3, and 5 years of ETV therapy, respectively ($P < 0.05$). APRI and FIB-4 values decreased significantly during 5-year ETV treatment in HBeAg-negative CHB patients, indicating that these noninvasive fibrosis tests might be useful for monitoring improvement in liver fibrosis and assessing treatment efficacy during long-term ETV treatment.

Keywords Hepatitis B virus · Chronic hepatitis B · Liver fibrosis · Entecavir · APRI · FIB-4

Ruyu Liu and Jiang Guo have been contributed equally to this work.

✉ Ruyu Liu
liuruyu28@163.com

Extended author information available on the last page of the article

Introduction

It is estimated that the global prevalence of hepatitis B surface antigen (HBsAg) is 3.9%, corresponding to 291,992,000 infections [1], and in China, the prevalence is 7.20% [1]. Chronic hepatitis B virus (HBV) infection can lead to chronic liver inflammation and subsequent fibrosis [2]. Studies show that every year, about 786,000 people will die of chronic HBV infection-related diseases, including liver cirrhosis and hepatocellular carcinoma (HCC) [3]. In

a long-term longitudinal study, progression to cirrhosis was found to be positively associated with baseline HBV-DNA level in treatment-naïve patients with chronic hepatitis B (CHB) [2]. The risk of cirrhosis was independently increased with increasing HBV-DNA levels [4]. A high viral load has been reported to induce the progression of fibrosis and cirrhosis in CHB patients [5]. One study also showed that the cumulative incidence of HCC increased from 1.3% in patients with HBV-DNA < 300 copies/mL to 15% in patients with HBV-DNA $\geq 10^6$ copies/mL [6]. Reports showed that antiviral agents can be used to suppress HBV replication, retard disease progression, and improve disease outcomes in patients with CHB [7, 8]. Entecavir (ETV), along with tenofovir disoproxil fumarate and peginterferon, was the preferred antivirals [9, 10].

According to the guidelines for the management of CHB, the prognosis and treatment decisions of CHB patients depend on the degree of liver histological lesions [9]. Patients with significant liver histological lesions should be considered for immediate antiviral therapy [9]. Patients with significant liver histological lesions defined as inflammatory grade \geq A2 and/or fibrosis stage \geq F2 (METAVIR scoring systems) are at increased risk of developing cirrhosis and HCC [11]. During long-term antiviral therapy, it is important to monitor the dynamic changes in the severity of liver fibrosis. Liver biopsy is the best choice for assessing the degree of liver necroinflammation and fibrosis. However, it has some limitations, including high costs, bleeding risk, and patient discomfort [12]. In CHB patients who cannot or are reluctant to undergo liver biopsy, noninvasive fibrosis tests may be needed for prognosis and treatment decisions.

Several noninvasive fibrosis tests based on serum indices (such as Aspartate Aminotransferase to Platelet Ratio Index [APRI] and Fibrosis-4 [FIB-4]) are now available and increasingly used for evaluating the severity of liver fibrosis [9], and they reduce the need for liver biopsy. According to the World Health Organization's guidelines for the management of CHB, for the diagnosis of significant fibrosis (fibrosis stages \geq F2), the summary sensitivity of APRI was 78%, while the summary specificity of APRI was 92% [12]. For the diagnosis of cirrhosis, the summary sensitivity of APRI was 65%, while the summary specificity of APRI was 89% [12]. A cutoff value of FIB-4 ≥ 1.45 differentiated moderate fibrosis from severe fibrosis with a sensitivity of 71% and a specificity of 73% [13]. FIB-4 cutoffs of 1.6 and 3.6 provided 93% negative predictive value and 90% positive predictive value for detection of cirrhosis, respectively [14]. The use of noninvasive fibrosis tests could predict significant fibrosis and cirrhosis and help with the optimal selection of patients for anti-HBV therapy. However, few studies have evaluated the changes in APRI and FIB-4 values during long-term antiviral treatment in hepatitis B e-antigen (HBeAg)-negative CHB patients.

In this study, we enrolled 303 HBeAg-negative CHB patients. All patients underwent liver biopsy. A total of 196 of 303 patients with significant liver histological lesions at baseline were treated with ETV, and the dynamic changes in APRI and FIB-4 values were evaluated during 5-year ETV treatment. We aimed to investigate the influence of long-term antiviral treatment on noninvasive fibrosis tests and evaluate the roles of APRI and FIB-4 indexes in monitoring long-term prognoses in HBeAg-negative CHB patients.

Methods

Patients

A total of 303 HBeAg-negative treatment-naïve patients were enrolled in this retrospective study from September 2008 to May 2015 at Beijing Ditan Hospital, Beijing, China. All patients were positive for HBsAg for more than 6 months at treatment commencement. Liver biopsies were performed at the baseline of all patients. Patients whose liver fibrosis stages were determined as F2, F3, and F4 (METAVIR scoring system) received 0.5 mg of ETV daily. The exclusion criteria adopted were (1) coinfection with hepatitis C, D, and human immunodeficiency virus; (2) HCC development after ETV commencement during the study period; and (3) lack of compliance with ETV treatment for more than 3 months. Finally, 196 treatment-naïve HBeAg-negative patients were enrolled. In this study, virological response was defined as HBV-DNA level undetectable by quantitative polymerase chain reaction assay with a lower limit detection of 20 IU/mL during the 5-year ETV therapy. This study was approved by the Institutional Review Board of Beijing Ditan Hospital, and informed consent was waived.

Laboratory data

Biochemical tests including liver enzymes, total bilirubin (TBIL), and HBV-DNA were performed at baseline and after 1, 2, 3, 4, and 5 years of ETV treatment and were measured using standard laboratory procedures. The upper limits of normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were defined as 50 U/L and 40 U/L (male) and 40 U/L and 35 U/L (female), respectively. The upper limit of normal TBIL was defined as 18.8 μ mol/L. Serum HBV-DNA levels were checked by real-time polymerase chain reaction at our hospitals. The lowest detection limit of serum HBV-DNA among our hospitals was 20 IU/mL (COBAS TaqMan HBV Test v2.0, Roche Diagnostics, Branchburg, NJ, USA). Therefore, a serum HBV-DNA level of < 20 IU/mL was defined as the limit of detectability.

Liver histological assessment

Percutaneous liver biopsy was performed, and paraffin sections were made according to a previous study [15]. The biopsy samples were assessed by two independent pathologists who were blinded to the results of the noninvasive fibrosis tests. Discordant cases were reviewed by a third highly experienced pathologist. The METAVIR scoring system was adopted as the pathological diagnosis standard of liver inflammation and fibrosis. Liver fibrosis was divided into five stages: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis [16].

Noninvasive fibrosis models

APRI and FIB-4 models were used to quantify the degrees of liver fibrosis. These indices were calculated using the following formulae: APRI = [(AST/upper limit of normal)/platelet count ($10^9/L$)] \times 100; FIB-4 = age (years) \times AST [U/L]/(platelet count ($10^9/L$) \times (ALT [U/L])^{1/2}). These models of liver fibrosis were calculated at entry and after 1, 2, 3, 4, and 5 years of ETV treatment.

Statistical analyses

Results are presented as follows: normal distribution data as mean \pm SD, nonnormal distribution continuous data as median (interquartile range; IQR), and categorical variables as number (percentage). *t* test or Mann–Whitney test was performed between different groups. The FIB-4 results were compared with the METAVIR scores with areas under the receiver operating characteristic (ROC) curve. One-way analysis of variance (ANOVA) was used to

compare continuous variables at entry and after 1, 2, 3, 4, and 5 years of ETV treatment. Statistical significance was accepted for *P*-values of < 0.05 ($*P < 0.05$, $**P < 0.01$). The analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of patients

The baseline characteristics of 303 study subjects are shown in Table 1. A total of 201 (66.34%) patients were male, and 102 patients were women (33.66%). According to the METAVIR scoring system, the number patients in each METAVIR fibrosis stage was as follows: F1 = 107 (35.3%), F2 = 125 (41.3%), F3 = 54 (17.8%), and F4 = 17 (5.6%), respectively. Baseline median serum ALT (U/L), AST (U/L), mean platelet counts ($10^9/L$), mean serum TBIL ($\mu\text{mol/L}$), HBV-DNA, median APRI values and FIB-4 value levels in F1, F2, F3, and F4 groups are shown in Table 1. Among the entire sample, the median APRI value increased from 0.41 (IQR 0.3–0.59) in the F1 group to 0.7 (IQR 0.43–1.58) in the F4 group. The median FIB-4 value increased as a function of the fibrosis score, from 1.01 (IQR 0.73–1.30) in the F1 group to 1.97 (IQR 0.96–3.34) in the F4 group (Table 1). The differences were statistically significant ($P < 0.05$) only when groups with severe fibrosis (F3 or F4) were compared with groups with moderate fibrosis (F1) or intermediate fibrosis (F2) (Table 1). Indeed, because of the rather large overlapping of the results, the APRI and FIB-4 indexes could not significantly discriminate the F1 group from the F2 group ($P > 0.05$; Table 1).

Table 1 Baseline characteristics of HBeAg-negative, treatment-naive CHB patients

Characteristic	METAVIR stage			
	F1	F2	F3	F4
Age (year)	40.07 \pm 10.21	40.59 \pm 9.62	42.35 \pm 9.62	39.94 \pm 11.40
Patient numbers [<i>n</i> (%)]	107 (35.3%)	125 (41.3%)	54 (17.8%)	17 (5.6%)
Gender (male/female)	64/43	84/41	40/14	13/4
ALT [U/L (median)]	42.65 (22.1–57.45)	38.3 (28.25–52.05)	41.5 (28.55–62.75)	47.9 (30.7–79.3)
AST [U/L (median)]	27.85 (21.6–37.9)	28.6 (24.3–38.6)	31.8 (26.6–44.2)	30.4 (25.9–61.35)
TBIL [$\mu\text{mol/L}$ (mean \pm SD)]	14.16 \pm 5.67	15.38 \pm 7.69	16.32 \pm 5.92	18.77 \pm 14.9
Platelet count ($\times 10^9/L$) (mean \pm SD)	192.31 \pm 51.46	178.55 \pm 62.91	146.04 \pm 34.82	147.86 \pm 62.74
HBV-DNA (mean \pm SD) (log ₁₀ IU/mL)	3.67 \pm 1.59	3.98 \pm 1.73	4.31 \pm 1.81	3.11 \pm 1.82
APRI value	0.41 (0.3–0.59)	0.46 (0.31–0.60)	0.62 (0.42–1.05)**	0.70 (0.43–1.58)**
FIB-4 value	1.01 (0.73–1.30)	1.08 (0.77–1.56)	1.48 (1.13–1.97)**	1.97 (0.96–3.34)**

**Compared with the F1 or F2 group, $P < 0.01$

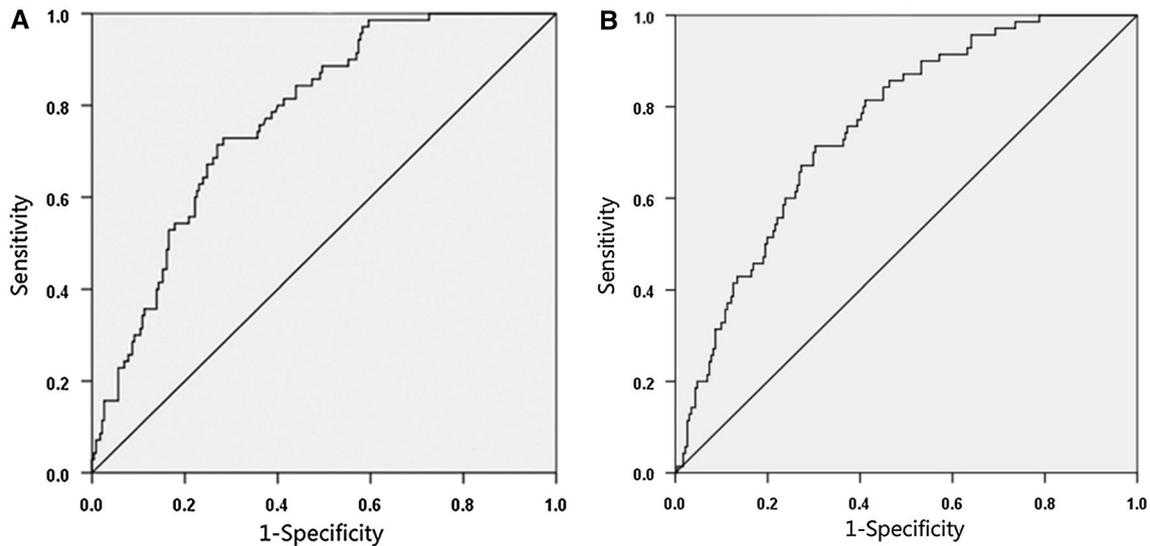


Fig. 1 ROC of the APRI index for the diagnosis of severe fibrosis (F3–F4, **a**) and ROC of the FIB-4 index for the diagnosis of severe fibrosis (F3–F4, **b**)

Table 2 Suggested negative predictive value of APRI for the prediction of severe fibrosis

APRI index	Liver biopsy (METAVIR)		Total
	F1–F2	F3–F4	
<0.544	90.5% (<i>n</i> =171)	9.5% (<i>n</i> =18)	189
≥0.544	54.4% (<i>n</i> =62)	45.6% (<i>n</i> =52)	114
Total	233	70	303

Table 3 Suggested positive predictive value of APRI for the prediction of severe fibrosis

APRI index	Liver biopsy (METAVIR)		Total
	F3–F4	F1–F2	
≥1.30	94.4% (<i>n</i> =17)	5.6% (<i>n</i> =1)	18
<1.30	18.6% (<i>n</i> =53)	81.4% (<i>n</i> =232)	285
Total	70	233	303

Comparison of APRI and FIB-4 indexes and liver biopsy results

The APRI index enabled the effective identification of patients with severe fibrosis (METAVIR F3–F4) with an area under the ROC curve of 0.77 (95% confidence interval [CI], 0.72–0.83; Fig. 1a). An APRI value lower than 0.544 had a negative predictive value of 90.5% to exclude any severe fibrosis (F3–F4), with a sensitivity of 72.9% and a specificity of 71.7% (Table 2). An APRI value ≥ 1.30 had a positive predictive value of 94.4% to predict severe fibrosis (F3–F4), with a sensitivity of 22.9% and a specificity of 94.3% (Table 3). The FIB-4 index enabled the effective identification of patients with severe fibrosis with an area under the ROC curve of 0.76 (95% CI 0.70–0.82; Fig. 1b). A FIB-4 index lower than 1.33 had a negative predictive value of 89.3% to exclude any severe fibrosis (F3–F4), with a sensitivity of 70% and a specificity of 70.1% (Table 4). A FIB-4 index ≥ 2.04 had a positive predictive value of 91.3% to predict severe fibrosis (F3–F4), with a sensitivity of 31.4% and a specificity of 91.3% (Table 5).

Table 4 Suggested negative predictive value of FIB-4 for the prediction of severe fibrosis

FIB-4 index	Liver biopsy (METAVIR)		Total
	F1–F2	F3–F4	
<1.33	89.3% (<i>n</i> =167)	10.7% (<i>n</i> =20)	187
≥1.33	56.9% (<i>n</i> =66)	43.1% (<i>n</i> =50)	116
Total	233	70	303

Table 5 Suggested positive predictive value of FIB-4 for the prediction of severe fibrosis

FIB-4 index	Liver biopsy (METAVIR)		Total
	F3–F4	F1–F2	
≥2.04	91.3% (<i>n</i> =21)	8.7% (<i>n</i> =2)	23
<2.04	17.5% (<i>n</i> =49)	82.5% (<i>n</i> =231)	280
Total	70	233	303

Biochemical improvements after 1, 2, 3, 4, and 5 years of ETV treatment

Patients with fibrosis stage \geq F2 were treated with ETV. During 5 years of ETV therapy, ALT and AST decreased significantly ($P < 0.05$; Figs. 2 and 3). The ALT normalization rates after 1, 2, 3, 4, and 5 years of ETV therapy were 80.1% (157/196), 85.7% (168/196), 89.8% (176/196), 93.9% (184/196), and 94.4% (185/196), respectively (Table 6). The AST normalization rates at years 1, 2, 3, 4, and 5 were 85.7% (168/196), 93.4% (183/196), 95.4% (187/196), 97.4% (191/196), and 99% (194/196), respectively (Table 6). TBIL decreased significantly at year 3 in the F3 group (Fig. 4, $P < 0.05$). In the F2 and F4 groups, TBIL showed a decreasing trend. However, compared with baseline, there were no significant differences (Fig. 4). Along with the duration of antiviral therapy, platelet counts showed a decreasing trend in CHB patients in the F2, F3, and F4 groups, but there were no significant differences (Fig. 5, $P > 0.05$).

Virological improvement after 1, 2, 3, 4, and 5 years of ETV treatment

Patients with fibrosis stage \geq F2 were treated with ETV. The undetectable HBV-DNA rates were 75.5% (148/196), 84.2% (165/196), 91.3% (179/196), 96.9% (190/196), and 98.5% (193/196) after 1, 2, 3, 4, and 5 years of ETV treatment, respectively (Table 6). At year 5, the HBV-DNA levels of three patients with nonvirological response were 5.97, 2.45, and 2.64 IU/mL(log₁₀ IU/mL), respectively. The HBV-DNA levels in the patients with virological response were all undetectable (< 20 IU/mL).

Improvements in APRI value after 1, 2, 3, 4, and 5 years of ETV treatment

Improvements in APRI values during ETV treatment are shown in Table 6. Compared with baseline, the APRI value decreased significantly after 1 year of ETV treatment in F2 and F3 groups (Fig. 6; $P < 0.01$). For the F4 group, the median APRI values showed a decreasing trend during ETV

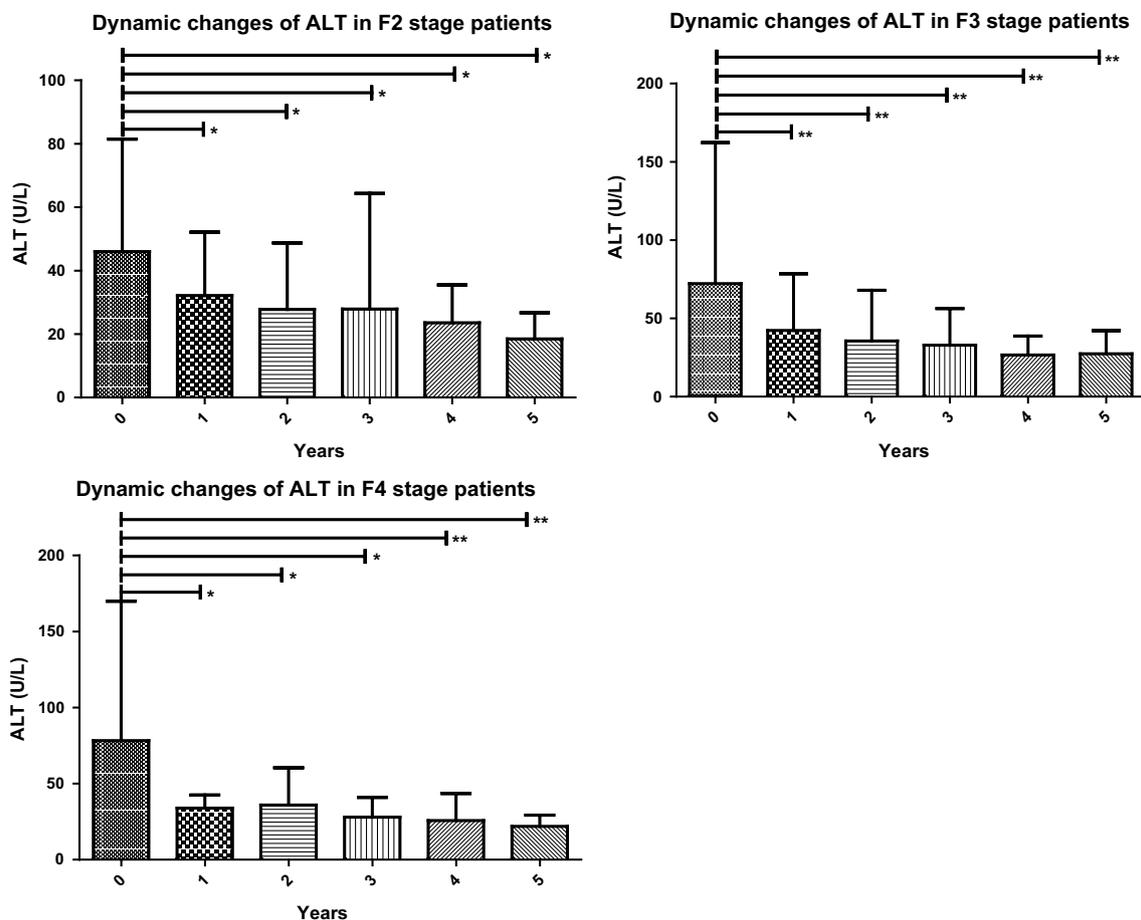


Fig. 2 Dynamic changes of ALT in patients of different fibrosis stages. ALT decreased significantly after 1, 2, 3, 4, and 5 years of ETV therapy

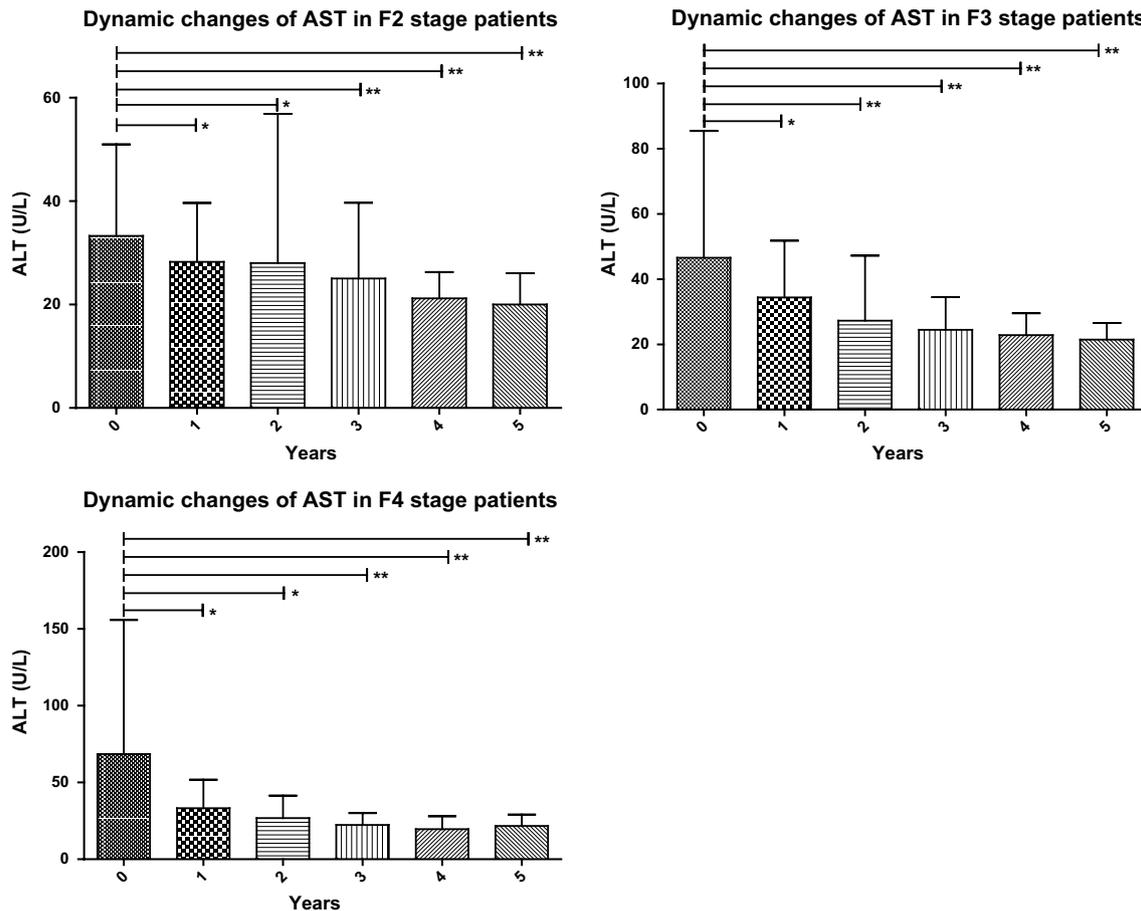


Fig. 3 Dynamic changes of AST in patients of different fibrosis stages. AST decreased significantly after 1, 2, 3, 4, and 5 years of ETV therapy

Table 6 Dynamic changes of ALT, AST, TBIL, HBV-DNA, APRI, and FIB-4 at 1–5 years of ETV therapy

Characteristic	Antiviral therapy duration (year)						
	0	1	2	3	4	5	
ALT normal/total [n (%)]	128/196 (65.3%)	157/196 (80.1%)	168/196 (85.7%)	176/196 (89.8%)	184/196 (93.9%)	185/196 (94.4%)	
AST normal/total [n (%)]	147/196 (75%)	168/196 (85.7%)	183/196 (93.4%)	187/196 (95.4%)	191/196 (97.4%)	194/196 (99%)	
TBIL normal/total [n (%)]	143/196 (73%)	168/196 (85.7%)	180/196 (91.8%)	179/196 (91.3%)	184/196 (93.9%)	188/196 (95.9%)	
HBV-DNA negative [n (%)]	0/196 (0%)	148/196 (75.5%)	165/196 (84.2%)	179/196 (91.3%)	190/196 (96.9%)	193/196 (98.5%)	
F2 group							
APRI value	0.46 (0.31–0.60)	0.37 (0.29–0.49)	0.35 (0.24–0.45)	0.30 (0.23–0.44)	0.26 (0.21–0.40)	0.25 (0.18–0.38)	
FIB-4 value	1.08 (0.77–1.56)	1.11 (0.85–1.44)	0.97 (0.78–1.37)	1.08 (0.84–1.40)	1.01 (0.71–1.28)	0.94 (0.77–1.29)	
F3 group							
APRI value	0.62 (0.42–1.05)	0.47 (0.33–0.70)	0.38 (0.24–0.49)	0.32 (0.26–0.47)	0.39 (0.25–0.54)	0.38 (0.24–0.43)	
FIB-4 value	1.48 (1.13–1.97)	1.42 (1.07–1.99)	1.32 (0.90–1.98)	1.21 (0.86–1.58)	1.12 (0.88–1.75)	0.93 (0.83–1.13)	
F4 group							
APRI value	0.70 (0.43–1.58)	0.58 (0.42–0.90)	0.48 (0.31–0.89)	0.51 (0.34–0.84)	0.34 (0.29–0.74)	0.26 (0.23–0.41)	
FIB-4 value	1.97 (0.96–3.34)	1.69 (0.92–2.25)	1.18 (0.93–1.97)	1.38 (1.05–1.95)	1.26 (0.95–1.61)	0.87 (0.79–1.33)	

therapy. Compared with baseline, APRI values significantly decreased after 3 years of ETV treatment (Fig. 6; $P < 0.05$). The number of patients whose APRI value < 0.544 had been

increasing while the number of patients whose $APRI \geq 1.30$ had been decreasing year by year during 5 years of ETV treatment (Table 7).

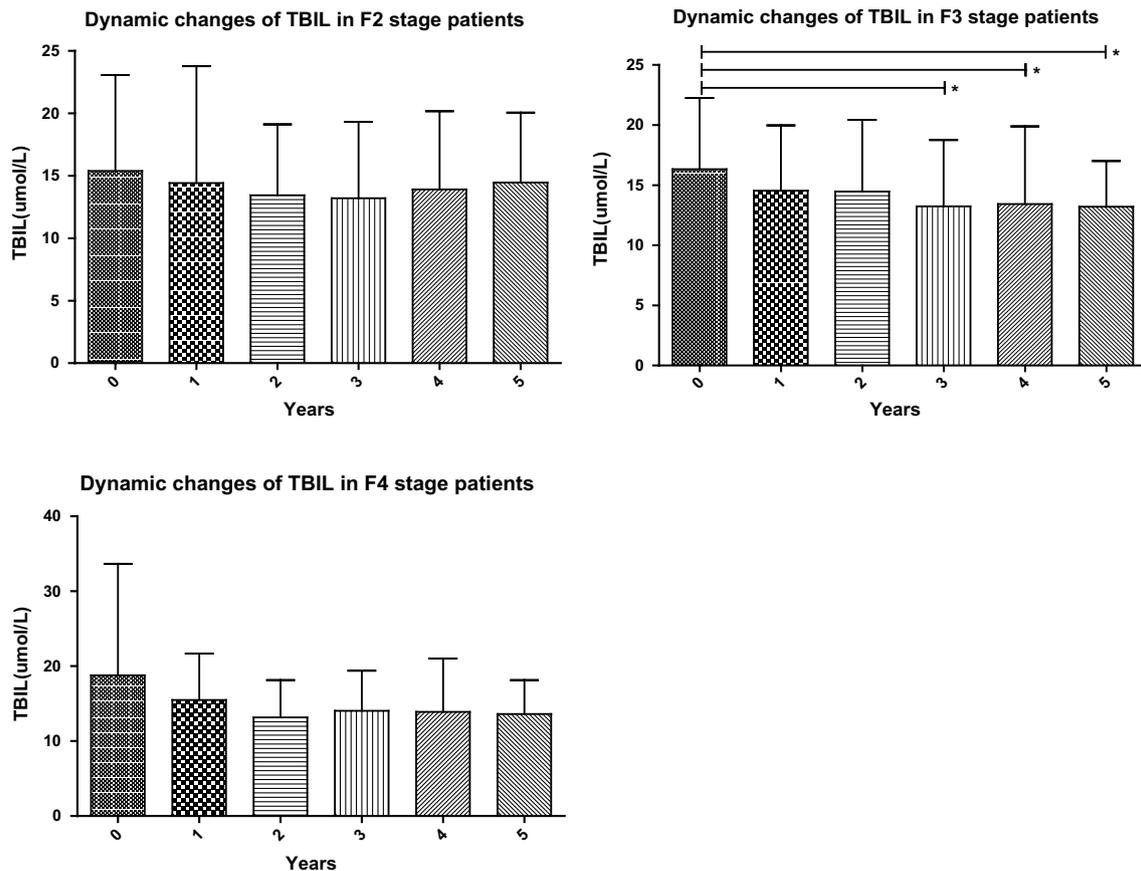


Fig. 4 Dynamic changes of TBIL in patients of different fibrosis stages. TBIL decreased significantly after 3 years of ETV therapy in the F3 group

Improvements in FIB-4 value after 1, 2, 3, 4, and 5 years of ETV treatment

Improvements in FIB-4 values are shown in Table 6 during ETV therapy. Compared with baseline, the FIB-4 value decreased significantly after 1 year of ETV treatment in F2 group (Fig. 7; $P < 0.05$). Median FIB-4 values of the F3 group after 1, 2, 3, 4, and 5 years of ETV treatment are also shown in Table 4. It decreased significantly at 3 years of ETV treatment compared with baseline (Fig. 7; $P < 0.05$), while for the F4 group, compared with baseline, the FIB-4 value decreased significantly after 5 years of antiviral therapy (Fig. 7; $P < 0.05$). The number of patients whose FIB-4 value < 1.33 had been increasing while the number of patients whose FIB-4 value ≥ 2.04 had been decreasing year by year during 5 years of ETV therapy (Table 7).

Discussion

Liver fibrosis is correlated with the risk of developing cirrhosis and liver-related complications [17]. Assessing the severity of liver fibrosis will help determine the treatment decision and prognosis in patients with CHB. Liver biopsy has traditionally been considered the reference method for evaluation of liver fibrosis, but it provides only a snapshot and not an insight into the dynamic changes during the process of fibrogenesis (progression, static, or regression) [15]. In addition, liver biopsy is a costly and invasive procedure carrying a risk of life-threatening complications; this limits the use of liver biopsy for mass screening and follow-up evaluation. Noninvasive fibrosis tests, using either FibroScan or serum fibrosis models, are adequate for the diagnosis of significant fibrosis/cirrhosis in patients with CHB and can

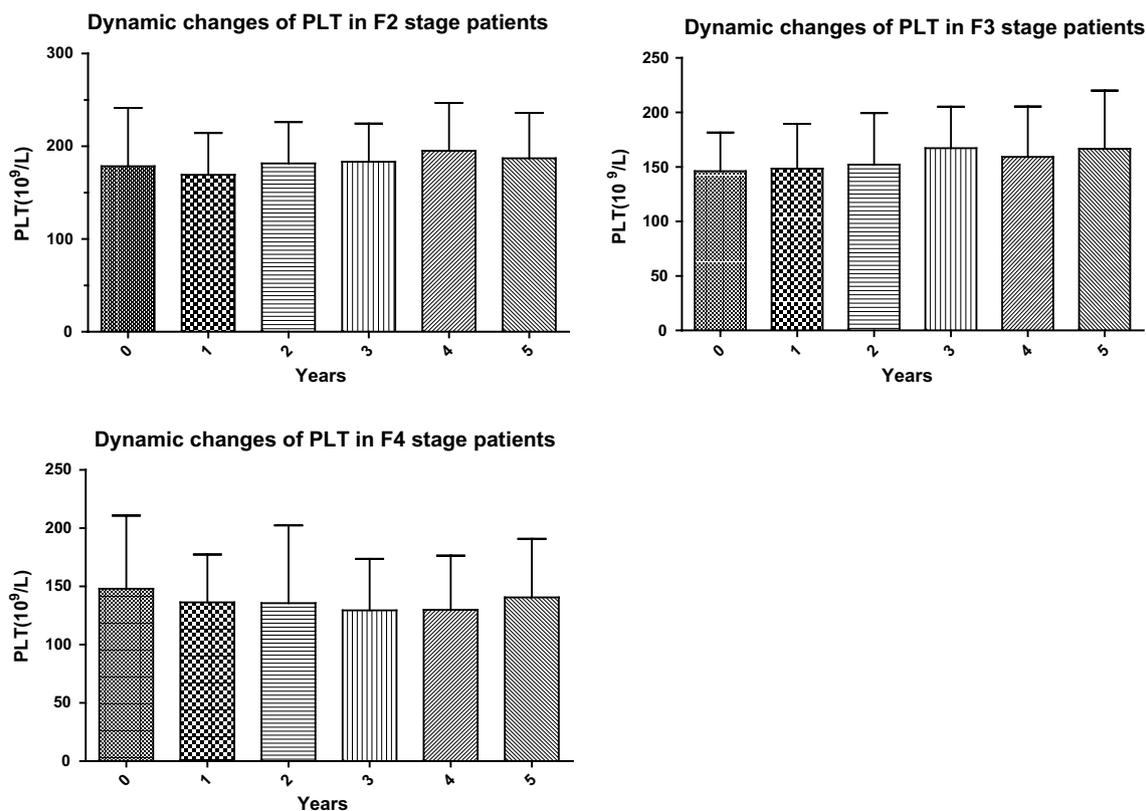


Fig. 5 Dynamic changes of platelet count in patients of different fibrosis stages. There was no difference during 5 years of ETV therapy

be used to prioritize patients for antiviral therapy based on the severity of liver fibrosis [9]. However, few studies have evaluated the value of noninvasive fibrosis tests in monitoring treatment response and disease progression during long-term antiviral therapy.

Several studies have reported that long-term suppression of HBV replication using nucleos(t)ide analogues (NUCs) can improve liver fibrosis in CHB patients [17–21]. Monitoring the fibrotic burden in CHB patients receiving NUCs treatment is clinically relevant in identifying subpopulations with different clinical prognoses. Previous studies have investigated the influence of antiviral treatment on liver stiffness measurement (LSM) values using FibroScan [22–24]. These studies showed that 1-year antiviral therapy was associated with the decrease in LSM values; however, data on changes in liver fibrosis during long-term antiviral therapy are still lacking. Recently, Li et al. reported that LSM, APRI, and FIB-4 values decreased significantly after 3 years of ETV treatment in 104 CHB patients with significant liver histological lesions. This study mentioned that the changes in APRI and FIB-4 values in HBeAg-negative CHB patients during long-term antiviral therapy still need to be studied. In this study, we found that APRI and FIB-4 values decreased significantly after 5 years of ETV treatment in 196 HBeAg-negative CHB patients.

Our present study reported the 5-year efficacy of ETV in treatment-naïve HBeAg-negative CHB patients in the real-world Chinese scenario. Compared with liver biopsy results, our data showed the APRI and FIB-4 indexes enabled the correct identification of patients with severe fibrosis (METAVIR F3–F4) with an area under the ROC curve of 0.77 (95% CI 0.72–0.83) and 0.76 (95% CI 0.70–0.82), respectively. These results were consistent with previous studies [13, 25]. These data showed that the changes in APRI and FIB-4 values probably could reflect liver histological improvements during ETV treatment.

In our study, the APRI and FIB-4 values began to decrease significantly after at least 1 year of therapy. These results were consistent with previous studies [17]. Li et al. reported that after 3 years of ETV therapy, the APRI and FIB-4 values significantly decreased in the F2–F4 group and the F4 group according to METAVIR fibrosis stages [17]. This study enrolled 104 CHB patients, 76 of whom were HBeAg-positive and 28 of whom were HBeAg-negative. In our study, the APRI and FIB-4 values of the F2 group decreased significantly at 1 year of antiviral therapy ($P < 0.01$, $P < 0.05$, respectively). For patients with F3, the APRI value decreased significantly at 1 year of ETV therapy ($P < 0.01$). Compared with the APRI value, the FIB-4 value significantly decreased 2 years later in the F3

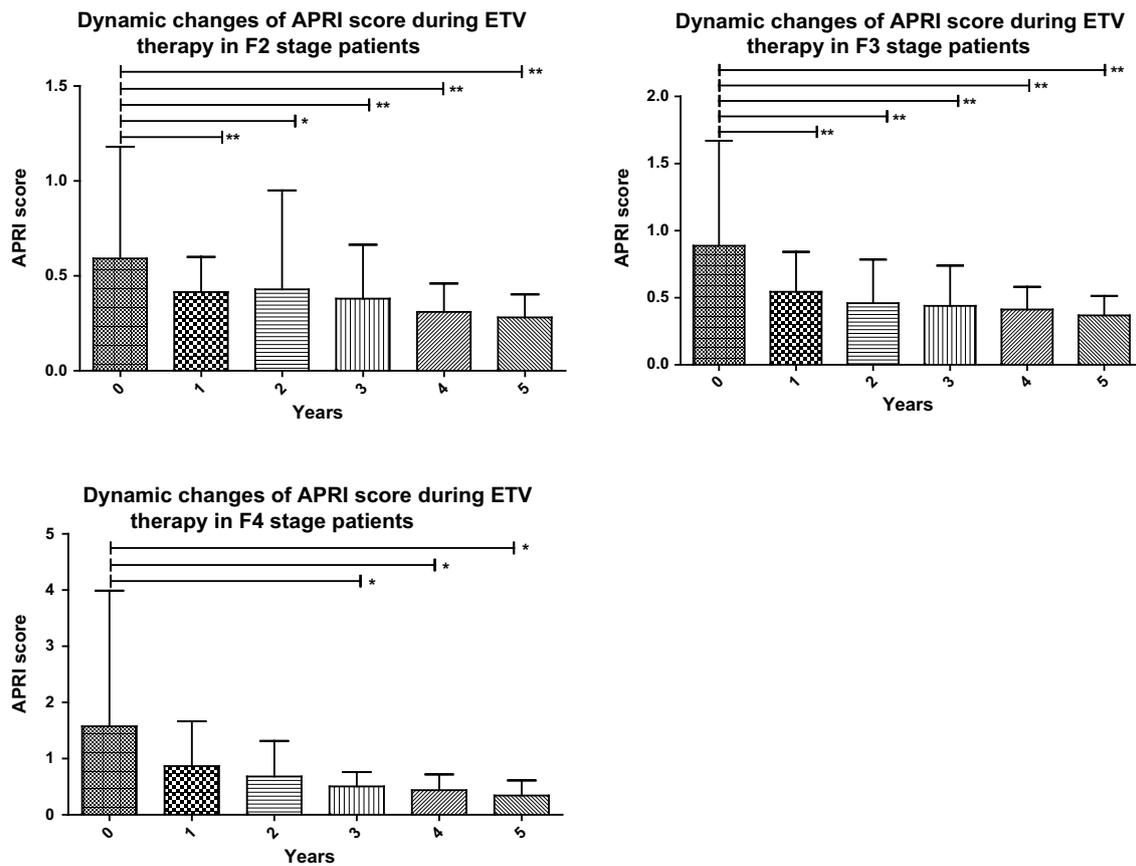


Fig. 6 Dynamic changes of APRI values during ETV therapy in patients of different fibrosis stages. APRI values decreased significantly after 1 year of ETV treatment in the F2 and F3 stage patients.

In F4 stage patients, the APRI value decreased significantly after 3 years of ETV therapy

Table 7 Dynamic changes of the proportion of patients whose APRI and FIB-4 values below negative predictive values or more than positive predictive values at 1 to 5 years of ETV therapy

Characteristics	Antiviral therapy duration (year)					
	0	1	2	3	4	5
APRI < 0.544 [F2–F4 groups, n (%)]	111 (56.6%)	145 (74%)	160 (81.6%)	167 (85.2%)	171 (87.2%)	176 (89.8%)
APRI ≥ 1.30 [F2–F4 groups, n (%)]	18 (9.2%)	14(7.1%)	11 (5.6%)	9 (4.6%)	5 (2.6%)	4 (2.0%)
FIB-4 < 1.33 [F2–F4 groups, n (%)]	104 (53.1%)	110 (56.1%)	127 (64.8%)	130 (66.3%)	143 (73%)	150 (76.5%)
FIB-4 ≥ 2.04 [F2–F4 groups, n (%)]	23 (11.7%)	17 (8.7%)	13 (6.6%)	10 (5.1%)	7 (3.6%)	5 (2.6%)

group ($P < 0.05$). For the F4 group, APRI and FIB-4 values decreased significantly at year 3 ($P < 0.05$) and year 5 ($P < 0.05$), respectively. Compared with Li’s report, our results showed that FIB-4 values decreased significantly at year 5. Sample size and patient type might have led to this difference. There were only 17 F4 stage patients in our study. Furthermore, the enrolled patients were all HBsAg-negative.

In our study, the undetectable HBV-DNA rates were 75.5, 84.2, 91.3, 96.9, and 98.5% after 1, 2, 3, 4, and 5 years of ETV treatment, respectively. A recent European field

practice study of patients treated with ETV ($n = 744$) showed that the 5-year cumulative probability of a viral response (HBV-DNA < 80 IU/mL) was 97%, and the patients’ ethnicity was classified as Caucasian, Asian (including China, Hong Kong, and Thailand), or other (including sub-Saharan Africans) [26]. Most recently, a Japanese study reported that after 1 to 5 years of ETV treatment, serum HBV-DNA levels were undetectable in 81, 89, 91, 94, and 96% of patients, respectively [27]. Our results were similar to those of previous reports [2, 26, 27].

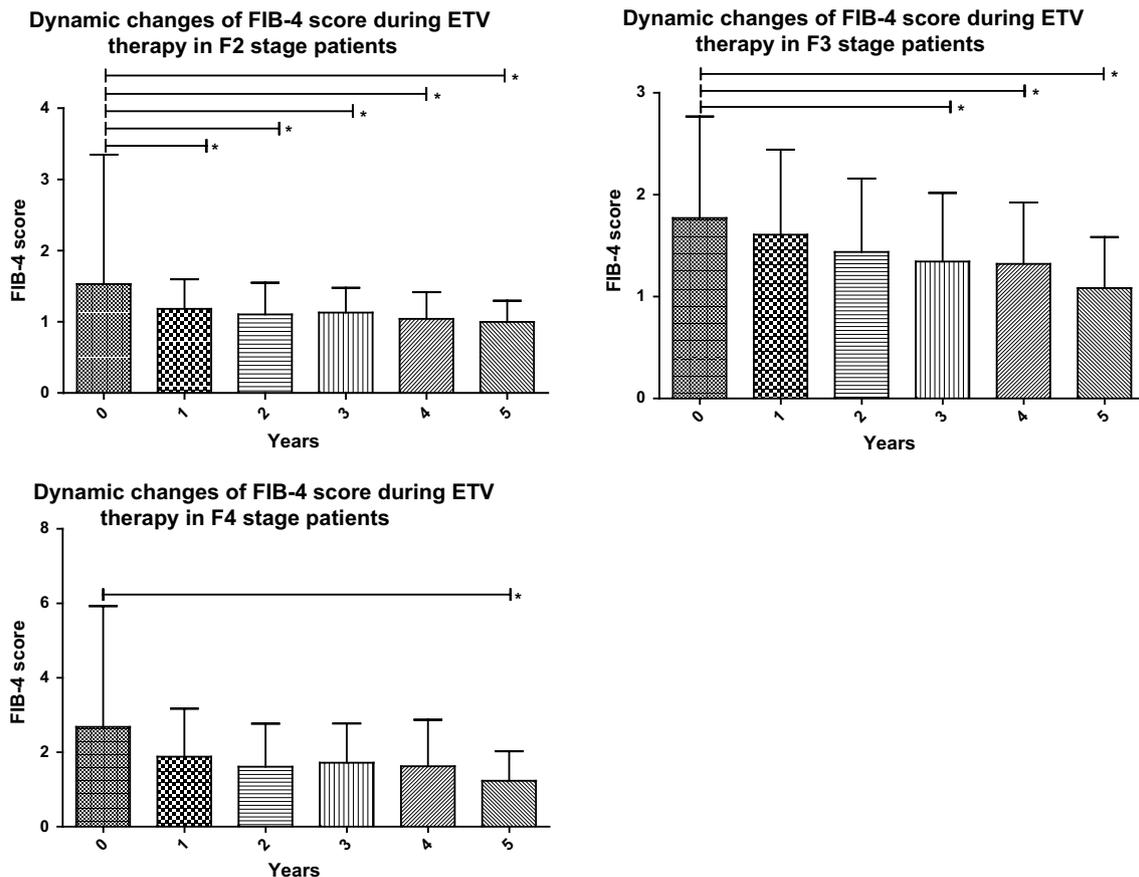


Fig. 7 Dynamic changes of FIB-4 values during ETV therapy in patients of different fibrosis stages. FIB-4 values decreased significantly after 1, 3, and 5 years of ETV treatment in F2, F3, and F4 stage patients

Our data showed that ALT normalization rates after 1, 2, 3, 4, and 5 years of ETV therapy were 80.1, 85.7, 89.8, 93.9, and 94.4%, respectively. A previous study reported that ALT normalization occurred in 66, 72, 75, 78, and 77% of treatment-naïve CHB patients at 1–5 years of ETV treatment [27]. Our normalization rates of ALT were higher than this report. The reason for this might be that the enrolled patients in these two studies were different. Compared with CHB and cirrhosis patients in the previous study, our patients were HBeAg-negative. Our data also showed that TBIL normalization rates after 1, 2, 3, 4, and 5 years of ETV therapy were 85.7% (168/196), 91.8% (180/196), 91.3% (179/196), 93.9% (184/196), and 95.9% (188/196), respectively. The dynamic changes were consistent with the ALT. The TBIL values decreased significantly in the F3 group after 3 years of ETV treatment. Compared with baseline, TBIL showed a decreasing trend in the F4 group, but there were no significant differences. The sample size of the F4 group ($n = 17$) might lead to these results.

This study also has limitations. First, patients in this study underwent liver biopsy only before ETV therapy, and paired biopsy data were not available after 5 years of

ETV therapy. Thus, we could not compare the changes in METAVIR fibrosis values after 5-year ETV treatment. However, in the real world, it is difficult to perform a second liver biopsy after long-term effective treatment due to cost and the risk of life-threatening complications; thus, a well-designed study with paired liver biopsy is required to confirm whether noninvasive fibrosis tests can be used to monitor the dynamic changes in the severity of liver fibrosis. Second, the sample size of the F4 stage CHB patients in our study was too small and should be enlarged to evaluate the efficacy of ETV treatment more precisely.

In conclusion, we found that APRI and FIB-4 values decreased significantly after 5-year ETV treatment in CHB patients with significant liver histological injury at baseline. Our results indicated that APRI and FIB-4 might be useful for monitoring regression of liver fibrosis and assessing treatment efficacy during long-term ETV treatment. The accurate rate of the negative and positive predictive values of APRI index was all higher than FIB-4 index. It indicated that APRI might be more accurate than FIB-4, while FIB-4 might be more useful because the patients' age is increasing during antiviral therapy,

because when we calculate FIB-4, the age was taken into account.

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Conflict of interest The authors declare no competing financial interests.

Ethical approval and informed consent All patients signed the informed consent before liver biopsy, and all clinical procedures were in accordance with the Declaration of Helsinki in 1983. The study protocol was permitted by the Institutional Review Board of Beijing Ditan Hospital.

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Affiliations

Ruyu Liu¹  · Jiang Guo² · Yao Lu¹ · Lu Zhang¹ · Ge Shen¹ · Shuling Wu¹ · Mi Chang¹ · Leiping Hu¹ · Hongxiao Hao¹ · Minghui Li¹ · Yao Xie¹

Minghui Li
wuhm2000@sina.com

Yao Xie
xieyao00120184@sina.com

² Tumor Interventional Department, Beijing Ditan Hospital, Capital Medical University, No. 8 Jingshun East Street, Chaoyang District, Beijing 100015, People's Republic of China

¹ Liver Diseases Center, Beijing Ditan Hospital, Capital Medical University, No. 8 Jingshun East Street, Chaoyang District, Beijing 100015, People's Republic of China