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Original Article

Serial Liver Stiffness Measurement and Serum Biomarkers Are Not Strong Predictors of the Regression of Fibrosis among Chronic Hepatitis B Patients Receiving Antiviral Therapy Based on Triple Liver Biopsies

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Background/Aims: Noninvasive indexes can be used to diagnose and stage liver fibrosis caused by chronic hepatitis B (CHB). We aimed to evaluate whether changes in the liver stiffness measurement (LSM) and serum biomarkers can predict liver fibrosis regression in CHB patients based on triple liver biopsies.

Methods: This multicenter cohort study was based on triple liver biopsies and lasted for 260 weeks. Liver fibrosis regression was defined as Ishak decreased ≥1 stage or predominantly regressive by P-I-R classification with stable Ishak stage. Twelve noninvasive models were validated externally and yielded area under the receiver operating characteristic curve (AUROC) values ≥0.700 for predicting significant fibrosis in the training set.

Results: A total of 175 CHB patients were included (median age: 38 years, 76.6% male). A total of 69.2% (117/169) and 79.6% (78/98) patients achieved liver fibrosis regression at week 78 and week 260, respectively. The mixed effects model revealed significant group×time interactions between the regression and non-regression groups for aminotransferase to platelet ratio index (APRI; p=0.041), new algorithm attributed to age, alanine aminotransferase, gamma-glutamyl transferase algorithm (p=0.022), and King's score (p=0.016) from baseline to week 78 as well as for APRI (p=0.046) from baseline to week 260. The AUROC values for model changes were all <0.750 for predicting liver fibrosis regression. Additionally, the changes in the LSM and most noninvasive models were significantly correlated with the changes of Ishak-histology activity index score.

Conclusions: Changes in the LSM and noninvasive models were not strong predictors of liver fibrosis regression after 78 weeks and 260 weeks of treatment among CHB patients. It is critical to develop a dynamic noninvasive model for assessing liver fibrosis regression (ClinicalTrials.gov identifier NCT02849132). **(Gut Liver, 2025;19:889-899)**

Key Words: Chronic hepatitis B; Liver cirrhosis/regression; Noninvasive diagnosis; Transient elastography; Dynamics

INTRODUCTION

Chronic hepatitis B (CHB) is a formidable health challenge in China, with an estimated prevalence of hepatitis B surface antigen at 5.6% in the general population.[1] According to the World Health Organization, CHB accounted for approximately 1.1 million deaths in 2022 worldwide, mainly attributable to complications such as cirrhosis and liver cancer.[2] Also, liver fibrosis is recognized as the critical factor accelerating the disease's progression. Recent studies have indicated that antiviral therapy led to regression in more than half of the patients.[3] Despite liver biopsy still being con-

sidered the gold standard for assessing liver fibrosis regression, serial biopsies are considered to be impractical.[4]

Although the newly updated World Health Organization guideline and China guideline for CHB prevention and treatment in 2022 recommended noninvasive methods such as aspartate aminotransferase to platelet ratio index (APRI), fibrosis index based on the 4 factor (FIB-4) for liver fibrosis detection, there is currently no consensus on a noninvasive approach for monitoring fibrosis regression.[2,5] Due to few studies evaluating biomarker changes in relation to histology in CHB patients after antiviral treatment, and the non-linear nature of extracellular matrix deposition and degradation, the American Association for the Study of Liver Diseases 2024 practice guideline cautions not recommend to use serial blood biomarker panels for predicting liver fibrosis regression.[6]

Previous studies showed the changes of APRI and FIB-4 could not identify liver fibrosis regression.[7,8] Other serum biomarkers in predicting liver fibrosis regression were not clear. As for liver stiffness measurement (LSM), which has been widely used in clinical practice to diagnose fibrosis and cirrhosis in these years, whether its dynamic changes could reflect liver fibrosis regression was controversial in studies. Some studies have shown no association between changes in LSM and regression, whereas others have indicated that changes in LSM can predict regression, albeit with varying threshold values.[9-12] Furthermore, existing studies have not assessed the effectiveness of LSM and noninvasive models in predicting liver fibrosis regression after long-term antiviral therapy, specifically over a period of 260 weeks. Therefore, in this multicenter cohort study, we assessed the dynamic changes of serial LSM and serum biomarkers in regression and non-regression groups to evaluate whether they could predict liver fibrosis regression in CHB patients at week 78 and week 260.

MATERIALS AND METHODS

1. Patients

A total of 175 treatment-naive hepatitis B patients with baseline liver biopsy proven Ishak ≥3 were enrolled from June 2013 to September 2015. Crucial inclusion criteria were as follows: age between 18 and 65 years; hepatitis B virus (HBV) DNA levels >20,000 IU/mL for hepatitis B e-antigen (HBeAg)-positive patients or >2,000 IU/mL for HBeAg-negative patients in non-cirrhosis patients, and HBV DNA levels >2,000 IU/mL for HBeAg-positive patients or >200 IU/mL for HBeAg-negative patients in cirrhosis patients. Each enrolled patient consented to undergo pre-treatment and post-treatment liver biopsy at week 78 and/or at week 260. The main exclusion criteria include decompensated cirrhosis (ascites, variceal bleeding or hepatic encephalopathy); hepatitis C, human immunodeficiency virus coinfection or other comorbid liver diseases; Hepatocellular carcinoma; malignant tumor of other organs; any severe diseases of other organs; psychological or neurological diseases; pregnant or lactating women.

Demographic data were collected at baseline, while serum biomarkers and LSM were collected at 26-week intervals from baseline to week 260. This study was conducted in compliance with the Declaration of Helsinki. Informed consent of all the enrolled patients and approval by the Ethics Committee of Beijing Friendship Hospital, Capital Medical University (approval number BJFH-EC/2013-029 and BJFH-EC/2016-P2-021-01) were obtained. The study protocol has been registered at ClinicalTrials.gov (NCT02849132).

2. Noninvasive models and LSM

The inclusion criteria for models were as follows: (1) the models were developed based on CHB patients, or the models developed based on chronic hepatitis C patients required further validation in a cohort of CHB patients; (2) liver biopsy was set as the gold standard; (3) the variables of the models were measured in our cohort; or (4) models had external validation, and the area under the receiver operating characteristic curve (AUROC) in the training set was ≥0.700.

Finally, a total of 12 noninvasive models with external validation and AUROC of at least 0.700 for predicting significant fibrosis in the training set were analyzed in our study (Supplementary Tables 1 and 2). The included noninvasive models were Hui,[13] Mehdi's,[14] gamma-glutamyl transferase (GGT) to cholinesterase and platelet ratio (GCPR),[15] a new algorithm attributed to age, alanine aminotransferase, GGT (AAG),[16] a novel score for predicting fibrosis stage in chronic viral hepatitis (ATA),[17] a noninvasive model composed of ALT, age, platelet, LSM (AAPL),[18] the age-male-albumin-bilirubin-platelets-LSM score (aMAP-LSM),[19] age and platelet count score (AP),[20] APRI,[21] fibrosis index (FI),[22] FIB-4,[23] King's,[24] which comprised demographic information (age, sex, and body mass index [BMI]), liver biochemical test (albumin, platelet, alanine aminotransferase, aspartate aminotransferase, GGT, alkaline phosphatase, total bilirubin, international normalized ratio, prothrombin time, cholinesterase), virological test (HBV DNA), and LSM. With the exception of five models based on chronic hepatitis C or human immunodeficiency virus coinfection patients, which are AP, APRI, FI, FIB-4, and King's, all other models are based on CHB patients. The AUROC values of the above fibrosis diagnosis models ranged from 0.71 to 0.92, and ATA model had the highest AUROC value.

LSM was conducted using transient elastography (FibroScan; Echosens, Paris, France), in accordance with the established standard procedures. An LSM examination was deemed reliable if it yielded 10 valid measurements with a success rate of ≥75% and an interquartile range/median of ≤30%.

3. Histological assessment

Liver biopsies were conducted percutaneously under ultrasonographic guidance for all patients. Specimens were stained with hematoxylin and eosin, Masson's trichrome, and reticulin. Fibrosis stage and inflammation scores evaluated by the Ishak modified histology activity index grading and staging system were collected.[25] Liver biopsy cases were evaluated by two pathologists (Hui Liu and Jing Chang) independently, and a third senior pathologist (Tailing Wang) reevaluated the specimens for cases with inconsistent scores. Three pathologists were all blinded to the clinical information of the patients.

Furthermore, liver specimens were further assessed by P-I-R classification (Neil D. Theise and Yameng Sun), which involves three classifications, predominantly progressive (P), indeterminate (I) and predominantly regressive (R), for dynamic assessment of liver fibrosis regression during antiviral treatment.[26] Predominantly regressive refers to a condition in which more than 50% of the septa consist of thin, densely compact stroma with few or no inflammatory cells. Predominantly progressive means over 50% of the septa consist of broad, loosely aggregated collagen fibers, accompanied by a large number of inflammatory cells. Indeterminate means an equal mix of both types of septa.

Then, fibrosis regression was defined as (1) Ishak fibrosis score decreased ≥1 stage after treatment and (2) predominantly regressive by P-I-R score with unchanged Ishak fibrosis stage.

4. Statistical analysis

Data were analyzed using SPSS 27.0 (IBM Corp., Armonk, NY, USA) and R 4.4.1 (package 'lme4'; R Foundation for Statistical Computing, Vienna, Austria). Quantitative variables were expressed as medians with interquartile ranges, which were compared with the Mann-Whitney test. Categorical variables were demonstrated with numbers, percentages and were compared using the chi-square test. The group differences of these noninvasive models in dynamic changes (group×time interactions) between regression and non-regression groups were analyzed by mixed effects model. Noninvasive models that had significant dynamic changes were further analyzed to determine the association between the changes in them and fibrosis regression by logistic analysis. Then, noninvasive models significantly associated with fibrosis regression calculated AUROCs.

To further explore the relationship between inflammation and the noninvasive models, the correlation between changes of noninvasive models as well as the comprised variables, and changes of Ishak-histology activity index (HAI) grading and staging system score were assessed by Spearman correlation analysis. All p-values reported were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

1. Patient clinical and histological characteristics

A total of 175 treatment-naive CHB patients with baseline liver biopsy proven Ishak fibrosis stage ≥3 were included. Among them, 77 patients (44%) underwent post-treatment liver biopsy only at week 78, six patients (3.4%) had post-treatment liver biopsy only at week 260, and 92 patients (52.6%) had liver biopsies at three time points: baseline, week 78, and week 260. In total, 169 patients underwent two liver biopsies at baseline and week 78, while 98 patients had two liver biopsies at baseline and week 260 (Fig. 1). The mean age of the participants was 38 years, with the majority being male (n=134, 76.6%). The clinical and histological characteristics of all enrolled patients were summarized in Table 1. At baseline, approximately 54.3% of patients (95/171) had with BMI ≥23 kg/m^2, 22.8% (40/175) patients with hepatic steatosis ≥5% based on liver biopsy, and 2.9% of patients (5/174) had diabetes mellitus. Patients received entecavir-based treatment from baseline to week 260, including 47.9% (n=81) receiving pegylated interferon (IFN)-α add on treatment from week 26 to week 78. The serum levels of alanine aminotransferase, aspartate aminotransferase, HBV DNA, and LSM decreased significantly from baseline to week 260 ($p<0.001$).

The proportion of patients with cirrhosis decreased from 26.3% (46/175) at baseline to 22.5% (38/169) at week 78 and 15.3% (15/98) at week 260. The proportion of patients with Ishak fibrosis stage 0-2, which was 0% at baseline, increased to 16.6% (28/169) at week 78 and 28.5% (28/98) at week 260. Furthermore, the proportion of patients with Ishak-HAI score ≥10 at baseline was 18% (31/173), but decreased to 0% at week 78 and week 26. In contrast, the proportion of patients with Ishak-HAI score 0-3 was 6.9% (12/173) at baseline and increased to 38.1% (64/168) at week 78 and 85.7% (84/98) at week 260 (Table 1).

Fibrosis regression rate based on Ishak fibrosis stage and P-I-R classification after 78 weeks and 260 weeks of antiviral therapy were shown in Supplementary Fig. 1. Among patients with pre- and post-treatment liver biopsies at baseline and week 78, 69.2% (117/169) attained liver

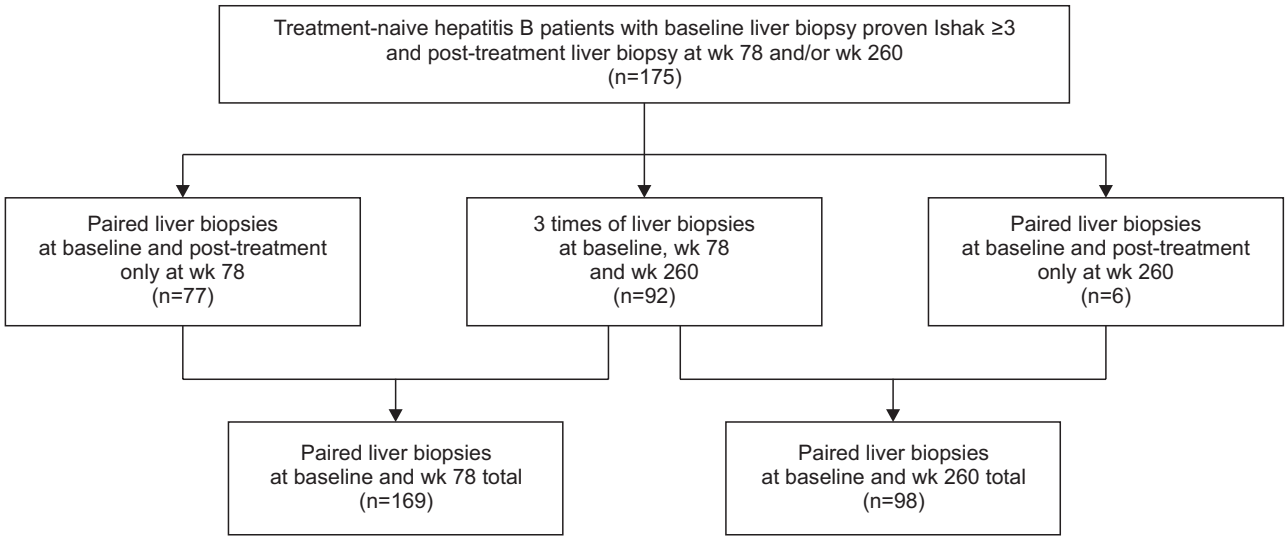

Fig. 1. Flowchart. A total of 175 patients with baseline Ishak fibrosis scores ≥3 were enrolled. A total of 169 and 98 patients who underwent paired liver biopsies at week 78 and week 260 after antiviral treatment, respectively, were included in the final analysis.

Table 1. Patient Characteristics at Baseline, Week 78, and Week 260

Characteristic	Baseline (n=175)	Week 78 (n=169)	Week 260 (n=98)	p-value
Age, yr	38±10	-	-	-
Male sex	134 (76.6)	-	-	-
BMI, kg/m^2	23.5 (21.3–25.7)	-	-	-
BMI ≥23 kg/m^2 (n=171)	95 (54.3)	-	-	-
Hepatic steatosis ≥5%	40 (22.8)	-	-	-
Diabetes mellitus (n=174)	5 (2.9)	-	-	-
Drinking history	36 (21.1)	-	-	-
HBV DNA, log IU/mL	6.3 (4.7–7.4)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	<0.001
PLT, 10^9/L	150.0 (115.0–185.0)	149.5 (116.8–191.8)	187.0 (152.0–221.0)	<0.001
ALT, U/L	72.0 (38.5–123.0)	26.0 (19.0–34.5)	22.0 (14.7–27.4)	<0.001
AST, U/L	49.7 (34.0–79.1)	25.0 (20.0–30.4)	20.0 (17.9–25.0)	<0.001
ALP, U/L	81.0 (67.8–101.1)	69.5 (57.0–85.5)	73.5 (60.0–89.5)	<0.001
GGT, U/L	51.0 (28.8–88.3)	27.0 (18.0–38.2)	20.0 (14.0–31.0)	<0.001
ALB, g/L	42.0 (39.0–46.0)	45.0 (42.6–47.3)	47.0 (44.8–49.0)	<0.001
TB, μmol/L	15.0 (11.8–20.0)	13.0 (9.9–16.8)	15.1 (11.3–18.7)	0.001
INR	1.09 (1.03–1.14)	1.00 (0.96–1.05)	1.01 (0.96–1.04)	<0.001
LSM, kPa	12.5 (8.6–18.5)	7.1 (5.7–9.4)	5.9 (4.6–7.9)	<0.001
Ishak-HAI score*				<0.001
0–3	12 (6.9)	64 (38.1)	84 (85.7)	
4–6	67 (38.7)	101 (60.1)	14 (14.3)	
7–9	63 (36.4)	3 (1.8)	0	
≥10	31 (18.0)	0	0	
Ishak fibrosis stage				<0.001
0–1	0	5 (3.0)	6 (6.1)	
2	0	23 (13.6)	22 (22.4)	
3	69 (39.4)	72 (42.6)	25 (35.7)	
4	60 (34.3)	31 (18.3)	20 (20.4)	
5	32 (18.3)	33 (19.5)	11 (11.2)	
6	14 (8.0)	5 (3.0)	4 (4.1)	

Data are presented as mean±SD, number (%), or median (interquartile range).
BMI, body mass index; HBV, hepatitis B virus; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; ALB, albumin; TB, total bilirubin; INR, international normalized ratio; LSM, liver stiffness measurements; HAI, histology activity index.
*Data missing: baseline (n=2), week 78 (n=1).

fibrosis regression. For patients with paired liver biopsy at baseline and week 260, the proportion of patients who achieved fibrosis regression was 79.6% (78/98).

Among patients who underwent liver biopsies both at baseline and at week 78, 10.1% (17/169) attained significant liver fibrosis regression (Ishak stage decreased ≥2 stage), and 34.3% (58/169) attained mild liver fibrosis regression (Ishak stage decreased=1). For patients with paired pre- and post-treatment liver biopsies (baseline/week 260), the proportion of patients who achieved significant fibrosis regression and mild fibrosis regression were 24.4% (24/98) and 37.8% (37/98) (Supplementary Fig. 2).

2. Dynamic changes of LSM and noninvasive models in fibrosis regression and non-regression patients

The group comparisons of dynamic changes in LSM and 12 noninvasive models from baseline to week 78/week 260 were shown in Figs 2 and 3, respectively. From baseline to week 78, for patients with paired liver biopsies, significant group×time interactions were observed between the regression and non-regression groups for the APRI (p=0.041), AAG (p=0.022), and King's (p=0.016) (Fig. 3). The values of LSM, Mehdi's model and AAPL consistently declined after treatment both in regression and non-regression groups. In contrast, APRI, FIB-4, AP, Hui, GCPR, ATA, FI, and King's models, which include platelet, initially declining from baseline to week 26, then rose from week 26 to week 52, before declined again from week 52 to week 78, primarily due to thrombocytopenia induced by IFN-α treatment.

From baseline to week 260, for patients with paired liver biopsies, significant group×time interaction was observed between the regression and non-regression groups in APRI (p=0.046) (Fig. 4). The LSM, aMAP-LSM advanced fibrosis and AAPL exhibited a sustained decline in both regression and non-regression groups. The trajectories of other noninvasive models in both the regression and non-regression groups were nearly overlapping from baseline to week 260.

3. The association between the changes of noninvasive models and liver fibrosis regression

Among LSM and 12 noninvasive models, three models with significant dynamic changes in two groups were further analyzed to determine association with regression, which were AAG, King's, and APRI. The change/change rate of AAG (odds ratio [OR]=0.852, p=0.016 and OR=0.991, p=0.026), and the change of King's (OR=0.979, p=0.021) were associated with liver fibrosis regression at week 78. The change rate of APRI (OR=0.974, p=0.010) was associated with liver fibrosis regression at week 260 (Table 2).

4. The predictive performance of noninvasive models

For these three models, the AUROCs of the changes/changes rate for AAG (AUROC, 0.618; 95% confidence interval [CI], 0.525 to 0.712 and AUROC, 0.615; 95% CI, 0.517 to 0.712), and King's (AUROC, 0.631; 95% CI, 0.536 to 0.726) to predict liver fibrosis regression at week 78 were less than 0.750. The AUROC of the change of APRI (AUROC, 0.705; 95% CI, 0.586 to 0.826) to predict liver fibrosis regression at week 260 was also less than 0.750 (Fig. 4). After adjusting metabolic factors with BMI ≥23 kg/m^2 and hepatic steatosis ≥5%, the AUROCs of changes/change rate of those models were not excellent with all AUROCs <0.750. In the subgroup analysis, no statistically significant differences were found in the AUROC between the groups (p>0.05) (Supplementary Table 3).

We also evaluated the performance of different threshold values in the changes of LSM or platelet, which were proposed in several previous studies, for predicting liver fibrosis regression were shown in Supplementary Table 4. Their performance was not particularly remarkable.

5. The correlation between dynamic changes of noninvasive models and inflammation based on Ishak-HAI score

We analyzed the correlation between dynamic changes in LSM and 12 noninvasive models and Ishak-HAI scores from baseline to weeks 78 and 260 (Supplementary Table 5). From baseline to week 78, the dynamics of most noninvasive models were positively correlated with the Ishak-HAI score changes (p<0.05), except for the aMAP-LSM advanced fibrosis (r=0.175, p=0.056) and cirrhosis scores (r=0.090, p=0.317). The change in AAG showed the strongest correlation (r=0.504, p<0.001), while the change in AP had the weakest correlation (r=0.189, p=0.019).

From baseline to week 260, similar correlations were observed (p<0.05), except for the aMAP-LSM cirrhosis score (r=0.208, p=0.064) and AP score (r=0.111, p=0.292). Notably, the change in AAPL (r=0.537, p<0.001) showed a strong association, while the aMAP-LSM advanced fibrosis score exhibited the weakest correlation (r=0.292, p=0.009).

6. The correlation between dynamic changes of variables in noninvasive models and liver fibrosis regression or liver inflammation improvements

We further analyzed the correlation between dynamic changes in variables of the noninvasive models and regression or liver inflammation improvement. From baseline to week 78, changes in platelet, aspartate aminotransferase, GGT, alkaline phosphatase, and total bilirubin were sig-

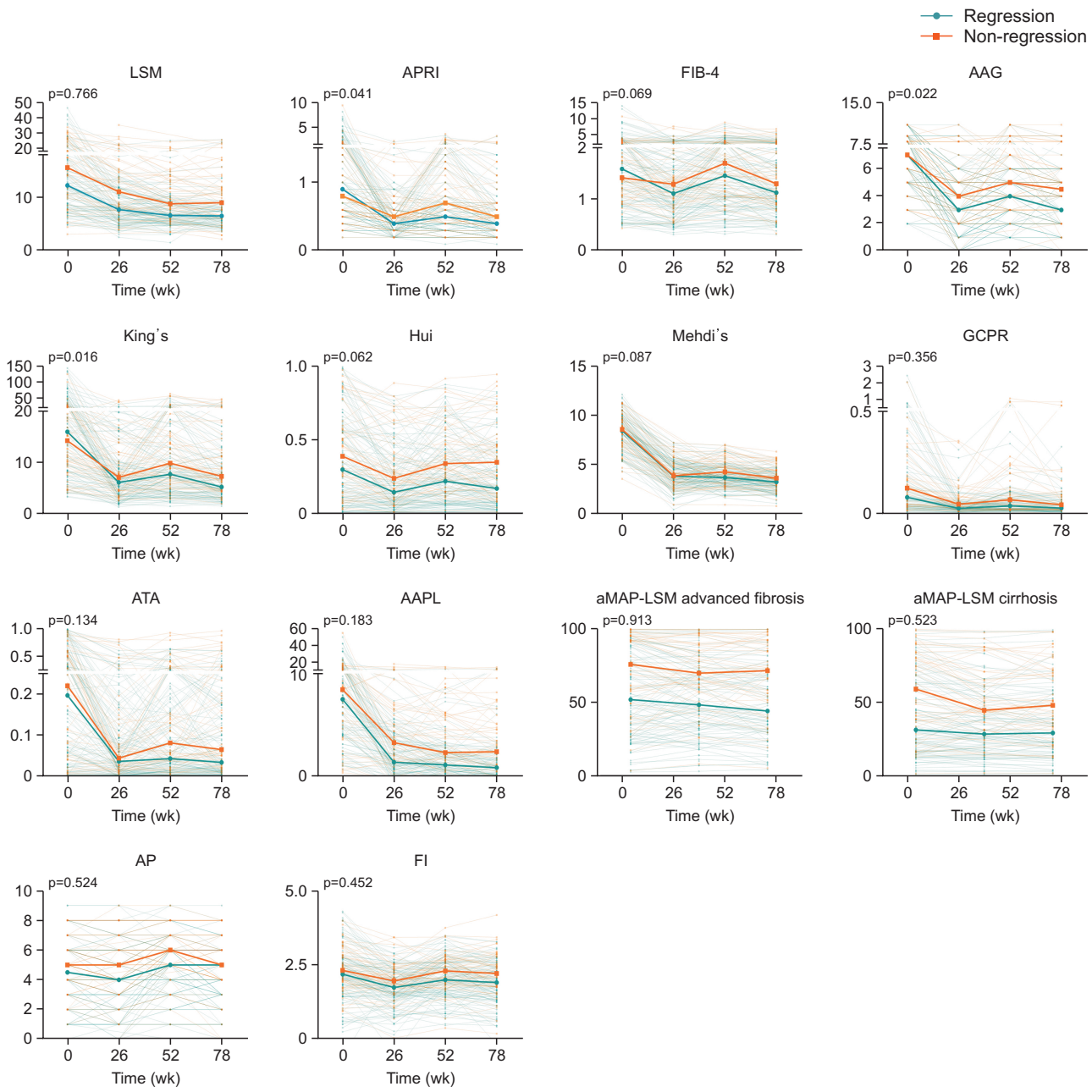

Fig. 2. Changes in the 12 noninvasive models and the LSM among chronic hepatitis B patients before and after 78 weeks of antiviral treatment. Changes in the LSM and noninvasive models, including the APRI, FIB-4, aMAP-LSM advanced fibrosis score, aMAP-LSM cirrhosis score, AP, Hui, Mehdi's, GCPR, AAG, ATA, AAPL, FI, King's, from baseline to week 78 for patients who attained liver fibrosis regression (green) or non-regression (orange) after 78 weeks of therapy. LSM, liver stiffness measurement; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis index based on the 4 factor; AAG, a new algorithm attributed to age, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT); GCPR, GGT to cholinesterase and platelet ratio; ATA, a novel score for predicting fibrosis stage in chronic viral hepatitis; AAPL, a noninvasive model composed of ALT, age, platelet, LSM; aMAP-LSM, age-male-albumin-bilirubin-platelets LSM score; AP, age and platelet count score; FI, fibrosis index.

nificantly correlated with liver fibrosis regression ($p<0.05$), and all variables, except total bilirubin, were significantly correlated with Ishak-HAI score changes ($p<0.05$). The change in GGT at week 52 showed the strongest positive correlation with inflammation ($r=0.547$, $p<0.001$), while the change in albumin at week 78 showed the strongest negative correlation ($r=-0.324$, $p<0.001$) (Supplementary Fig. 2).

From baseline to week 260, changes in alanine aminotransferase, aspartate aminotransferase, GGT, alkaline phosphatase, and total bilirubin were significantly correlated with liver fibrosis regression ($p<0.05$), and all variables correlated

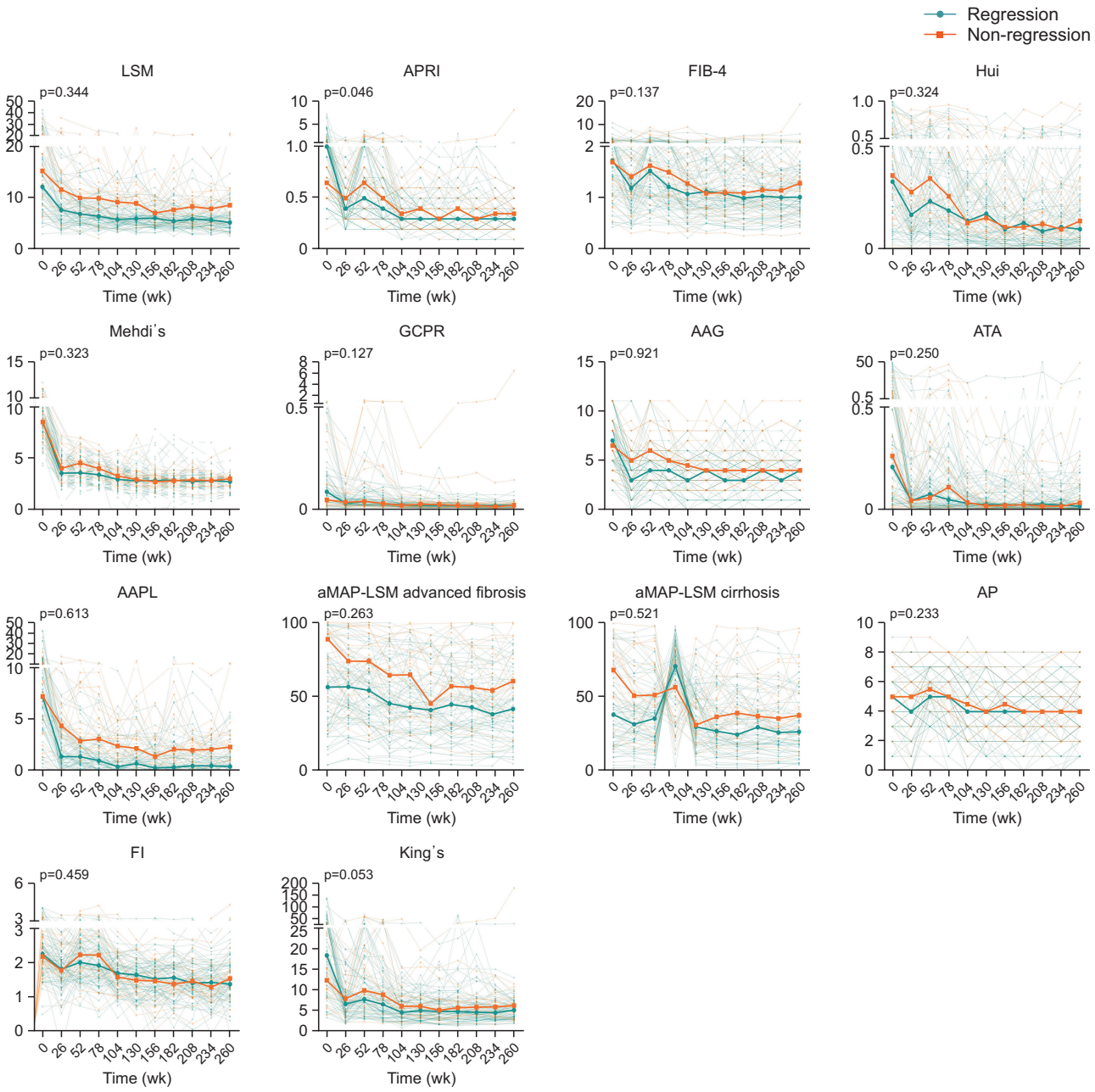

Fig. 3. Changes of the 12 noninvasive models and the LSM among chronic hepatitis B patients before and after 260 weeks of antiviral treatment. The changes in the LSM and noninvasive models, including the APRI, FIB-4, aMAP-LSM advanced fibrosis score, aMAP-LSM cirrhosis score, AP, Hui, Mehdi's, GCPR, AAG, ATA, AAPL, FI, King's, from baseline to week 260 for patients who attained liver fibrosis regression (green) or non-regression (orange) after 260 weeks of therapy. LSM, liver stiffness measurement; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis index based on the 4 factor; AAG, a new algorithm attributed to age, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT); GCPR, GCT to cholinesterase and platelet ratio; ATA, a novel score for predicting fibrosis stage in chronic viral hepatitis; AAPL, a noninvasive model composed of ALT, age, platelet, LSM; aMAP-LSM, age-male-albumin-bilirubin-platelets LSM score; AP, age and platelet count score; FI, fibrosis index.

with Ishak-HAI score changes ($p<0.05$). The change in GGT at week 182 showed the strongest positive correlation with inflammation ($r=0.619$, $p<0.001$), while the change in cholinesterase at week 208 had the strongest negative correlation ($r=-0.468$, $p<0.001$) (Supplementary Fig. 3).

7. Subgroup analysis-excluding patients who received IFN-α treatment

Considering pegylated IFN-α treatment could induce thrombocytopenia, we analyzed noninvasive models excluding patients who received pegylated IFN-α treatment. The group comparisons of dynamic changes in LSM and

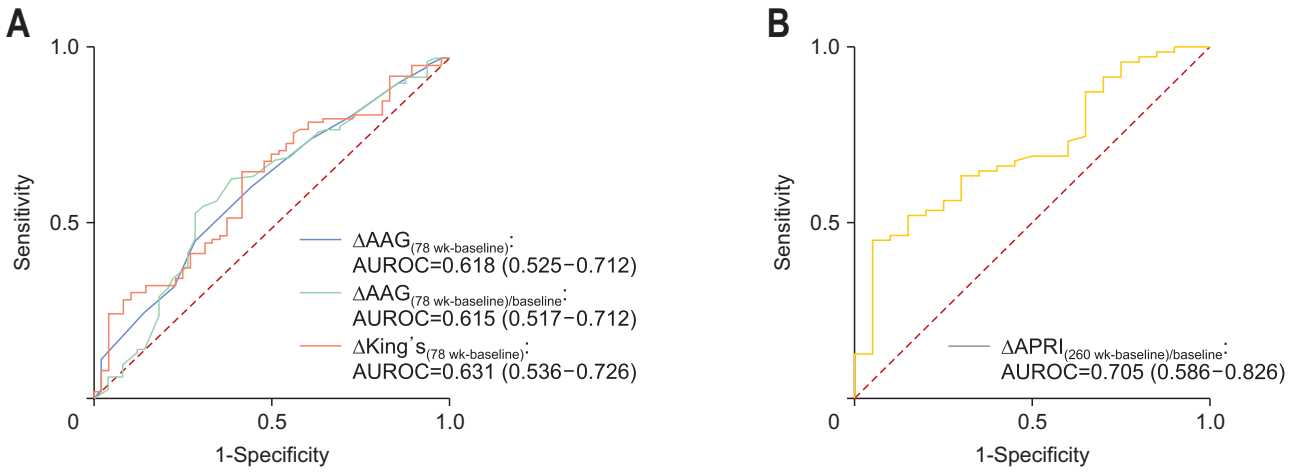

Fig. 4. The AUROC curves of the changes of noninvasive models to predict liver fibrosis regression. (A) The AUROC values of the change/change rate of AAG, and the change of King's to predict liver fibrosis regression at week 78. (B) The AUROC of the change rate of APRI to predict liver fibrosis regression at week 260. AUROC, area under the receiver operating characteristic curve; AAG, a new algorithm attributed to age, alanine aminotransferase, gamma-glutamyl transferase; APRI, aspartate aminotransferase to platelet ratio index.

Table 2. The Association between the Changes of Noninvasive Models and Liver Fibrosis Regression

Models	Liver fibrosis regression at wk 78		Liver fibrosis regression at wk 260	
	OR (95% CI)	p-value	OR (95% CI)	p-value
APRI				
Δ(78 wk-baseline)	0.757 (0.569–1.008)	0.056	-	-
Δ(78 wk-baseline)/baseline	0.996 (0.991–1.000)	0.073	-	-
Δ(260 wk-baseline)	-	-	0.321 (0.102–1.007)	0.051
Δ(260 wk-baseline)/baseline	-	-	0.974 (0.955–0.994)	0.010
AAG				
Δ(78 wk-baseline)	0.852 (0.748–0.971)	0.016	-	-
Δ(78 wk-baseline)/baseline	0.991 (0.982–0.999)	0.026	-	-
King's				
Δ(78 wk-baseline)	0.979 (0.961–0.997)	0.021	-	-
Δ(78 wk-baseline)/baseline	0.995 (0.990–1.000)	0.071	-	-

OR, odds ratio; CI, confidence interval; APRI, aspartate aminotransferase to platelet ratio index; AAG, a new algorithm attributed to age, alanine aminotransferase, gamma-glutamyl transferase.

12 noninvasive models from baseline to weeks 78 and 260 were shown in Supplementary Figs 4 and 5, respectively. From baseline to week 78, for patients with paired liver biopsies, significant group×time interactions were observed between the regression and non-regression groups for the Mehdi's (p=0.011), AAG (p<0.01), aMAP-LSM cirrhosis (p=0.040) and AP (p=0.026) (Supplementary Fig. 4). From baseline to week 260, for patients with paired liver biopsies, significant group×time interactions were observed between the regression and non-regression groups for the King's (p=0.039) (Supplementary Fig. 5).

A total of five models which were Mehdi's, AAG, aMAP-LSM cirrhosis, AP, and King's underwent further analysis. The change/change rate of Mehdi's (OR=0.701, p=0.021 and OR=0.954, p=0.020), the change/change rate of AAG (OR=0.792, p=0.028 and OR=0.982, p=0.046) were associated with liver fibrosis regression at week 78. The change rate of King's (OR=0.962, p=0.048) was associated with liver fibrosis regression at week 260 (Supplementary Table 6). However, the AUROCs of these noninvasive models for predicting liver fibrosis regression at weeks 78 and 260 were ≤0.700, suggesting suboptimal predictive performance (Supplementary Table 7).

DISCUSSION

Liver fibrosis and even cirrhosis could be reversible following antiviral treatment. Although there are various noninvasive models, the majority originate from chronic hepatitis C cohorts and primarily focus on diagnosing the liver fibrosis stage. In recent years, our team developed the

nReg-model to predict liver fibrosis at week 78 after antiviral therapy (AUROC, 0.75; 95% CI, 0.66 to 0.84), comprised of baseline LSM, FIB-4 and platelet, and change of LSM at month 6 from baseline.[27] However, the AUROC of the nReg-model was not outstanding and lacked external validation. This study demonstrated the dynamic changes of LSM and 12 noninvasive models in regression and non-regression groups from baseline to week 78 and week 260, also evaluated the correlation of the changes of noninvasive models and liver fibrosis regression and inflammation based on Ishak-HAI score.

In recent years, the recommendation of noninvasive tests instead of liver biopsy has gradually been mentioned in guidelines. The most commonly used noninvasive tests include APRI, FIB-4 and LSM, but insufficient clinical evidence has shown their capacity to evaluate liver fibrosis regression accurately. In our multicenter cohort study, participants were stratified into regression and non-regression groups, delineating the dynamic fluctuations of LSM and 12 noninvasive models from baseline to week 78 and week 260 after treatment based on triple liver biopsies. Our analysis showed that the changes of noninvasive models and LSM could not predict liver fibrosis regression at week 78 or week 260 optimally, although in subgroup analysis with exclusion of patients with IFN-α treatment, and adjusted with metabolic factors in BMI and hepatic steatosis.

A study with 17 years of follow-up time showed that the changes of APRI and FIB-4 could not reflect dynamic changes of early histological improvement, but may be related to histological changes 4 years after treatment.[28] Similarly, our research showed APRI had significant group×time interaction between regression and non-regression groups from baseline to week 78 and 260, with lower AUROC of 0.705. Then, FIB-4 had no significant group×time interaction between two groups at week 78 and week 260 ($p>0.05$). Furthermore, research has shown that baseline LSM and the decrease in LSM at week 52 were independent predictors of liver fibrosis regression at weeks 78 and 104.[29,30] However, our findings demonstrated LSM had no significant group variance in regression and non-regression groups from baseline to week 78 or week 260.

Previous study showed BMI and hepatic steatosis ≥5% had no association with liver fibrosis progression in CHB patients with significant baseline fibrosis (Ishak score ≥3).[31] The other study identified persistent steatosis was associated with decreased fibrosis regression in CHB patients (persistent steatosis defined as ≥1 point before and after treatment). Disappeared steatosis failed to show a significant association with fibrosis regression (disappeared steatosis as ≥1 point at baseline and 0 point after treatment).[32] Consequently, metabolic factors were considered to play a pivotal role in the prognosis of patients with CHB. In this study, after adjusting for metabolic factors, no significant change was observed in the results.

The changes in most noninvasive models had a significant correlation with the changes of Ishak-HAI score at weeks 78 and 260. Based on these findings, we hypothesized that the changes in noninvasive models were mainly due to the resolution of inflammation, which limits their ability to predict liver fibrosis regression effectively. Although other studies have shown that changes in noninvasive indicators such as LSM reflect improvement in liver inflammation rather than regression of fibrosis within 1.5 years, no study has evaluated the performance of model changes in predicting regression with week 260.[33]

This multicenter study evaluated the performance of noninvasive models in predicting liver fibrosis regression at week 78 and week 260 after treatment for CHB patients based on triple liver biopsies. The assessment of liver fibrosis regression was more precise by the Ishak fibrosis score and P-I-R classification. We compared group×time interaction effects in LSM and noninvasive models between regression and non-regression groups. Also, we assessed the association between change value and change rate of noninvasive models and liver fibrosis regression at weeks 78 and 260. Furthermore, we evaluated the predictive performance of previously established thresholds for changes in LSM and platelet in assessing liver fibrosis regression; however, none demonstrated superior performance. This study also had several limitations. The included noninvasive models involved overlapping biochemical markers. However, these models were established based on different cohorts, and have not been verified in the same cohort to determine their performance in predictive of liver fibrosis regression. In addition, we could not validate certain noninvasive models, including FibroStage, FibroBox, GIVPR, because our study was unable to measure and calculate unique serological markers required for these models.

In conclusion, although significant time×group interactions were observed across several noninvasive models, such as APRI, AAG, and King's, these changes did not optimally evaluate liver fibrosis regression. Future research should focus on developing a dynamic noninvasive model for predicting liver fibrosis regression, offering a viable alternative to liver biopsy.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Study concept and design: S.C., Y.S., H.Y. Data acquisition: J.Z., B.W., X.W., X.O. Drafting and reviewing of the manuscript: J.Z., S.C., J.Z., B.W., X.W., X.X., X.Z., Y.K., X.O., Y.S., H.Y. Critical revision of the manuscript for important intellectual content: S.C., Y.S., H.Y. Statistical analysis: J.Z., X.X., X.Z., Y.K. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

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