An-Luo-Hua-Xian Pill Improves the Regression of Liver Fibrosis in Chronic Hepatitis B Patients Treated with Entecavir

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Abbreviations: ALHX, An-Luo-Hua-Xian; APRI, aspartate aminotransferase to platelet ratio index; CHB, Chronic Hepatitis B; ETV, Entecavir; F, liver histologic fibrosis score; FIB-4, fibrosis-4-score; HAI, liver histologic activity scores of inflammatory; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; OR, odds ratio; TCM, traditional Chinese medicine.

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Abstract

Background and Aims: Chronic hepatitis B (CHB) can cause liver fibrosis and lead to cirrhosis and cancer. As the effectiveness of antiviral therapy to reverse liver fibrosis is limited, we aimed to evaluate the effect of An-Luo-Hua-Xian pill (ALHX) on fibrosis regression in CHB patients treated with entecavir (ETV). Methods: Treatment-naive patients with CHB were randomly treated with ETV alone or combined with ALHX (ETV+ALHX) between October 1, 2013 and December 31, 2020. Demographic, laboratory, and liver histology data before and after 78 weeks of treatment were collected. The Ishak fibrosis score (F) was used and fibrosis regression required a decrease in F of ≥1 after treatment. Results: A total of 780 patients were enrolled, and 394 with a second liver biopsy after treatment were included in the per-protocol population, 132 in ETV group and 262 in ETV+ALHX group. After 78 weeks of treatment, the fibrosis regression rate in the ETV+ALHX group was significantly higher than that of the ETV group at baseline F≥3 patients: 124/211 (58.8%) vs. 45/98 (45.9%), p=0.035. The percentage of patients with a decreased liver stiffness measurement (LSM) was higher in the ETV+ALHX group: 156/211 (73.9%) vs. 62/98 (63.6%), p=0.056. Logistic regression analysis showed that ETV combined with ALHX was associated with fibrosis regression [odds ratio (OR)=1.94, p=0.018], and a family history of hepatocellular carcinoma was on the contrary. (OR=0.41, p=0.031). Conclusions: ETV combined with ALHX increased liver fibrosis regression in CHB patients.

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Introduction

Chronic hepatitis B virus (HBV) infection is a serious public health problem, especially in China.1 Liver fibrosis caused by chronic hepatitis B (CHB) is the key in occurrence of liver cirrhosis and liver cancer. If CHB patients are not diagnosed and treated in time, most of them will die of decompensated liver cirrhosis or HCC. CHB accounts for 30% of all liver cirrhosis deaths and 40% of HCC deaths.2 Many studies have shown that antiviral therapy is effective in improving HBV-related liver fibrosis. The improvement rate of hepatic fibrosis in newly treated CHB patients with entecavir (ETV) for 48 weeks is reported as 32–39%.3 Although liver fibrosis was improved in more than half of patients, there are still no clearly recommended antifibrosis drugs.

In recent decades, through continuous research and experiments, traditional Chinese medicine (TCM) has made remarkable progress in the treatment of chronic liver disease, and many drugs for the treatment of liver fibrosis have been approved by the State Drug Administration of China. Studies have shown that TCM alone or combined with anti-HBV drugs was effective in delaying or reversing liver fibrosis/cirrhosis.4,5 An-Luo-Hua-Xian pill (ALHX, National Medical Products Administration Approval No. Z20010098) is a Chinese patent drug containing more than ten kinds of TCMs.6 The components are Rehmannia glutinosa (Di Huang), Radices pseudo-ginseng (San Qi), Leech (Shui Zhi), Bombix batryticatus (Jiang Can), Pheretima (Di Long), Atractylodes macrocephala ermina (Bai Zhu), Curcumae radix (Yu Jin), Bovis calculus (Niu Huang), Arcaea concha (Wa Neng Zi), Moutan cortex (Mu Dab Pi), Radices rhei (Da Huang), raw Hordei fructus erinates (Sheng Mai Ya), Galli gigerii endothelium corneum (Ji Nei Jin) and powdered buffalo horn extract (Concentrated powder of Shui Niu Jiao). "Luo" in Chinese means collateral branches of the pathway system that runs the "Qi" and blood of the whole body, "Xian" means fibrosis, "An" means to calm or pacify and "Hua" means to soften or alleviate. ALHX regulates immunity, improve liver microcirculation, promote hepatocyte injury and repair collagen synthesis and promote collagen degradation.7 Our previous study found that in CHB patients with significant fibrosis (fibrosis grade ≥3), ETV combined with ALHX significantly improved the regression rate of liver fibrosis/cirrhosis compared with ETV alone.8 Larger studies are needed to confirm the curative effectiveness, develop standard treatment regimens for reversing liver fibrosis, and promote its clinical use. Based on previous investigations, we carried out a prospective randomized controlled trial to further clarify the antifibrosis effectiveness of ALHX in CHB patients.

Methods

Study design and patients

This multicenter, open-label prospective randomized controlled trial was conducted in 33 hospitals in mainland China between October 1, 2013 and December 31, 2020. Eligible patients consented to participate in the study. The inclusion criteria were (1) 18–70 years of age, (2) hepatitis B surface antigen (HBsAg)+positive for ≥6 months or with pathologically confirmed chronic HBV infection, (3) HBV DNA positive, and (4) having regular follow-up. Exclusion criteria were (1) other types of viral hepatitis, i.e. hepatitis C virus, hepatitis D virus, or human immunodeficiency virus co-infection; and other chronic liver diseases (e.g., autoimmune hepatitis, drug-induced liver damage, genetic, nonalcoholic fatty liver); (2) decompensated manifestations of liver cirrhosis, including ascites, hepatic encephalopathy, gastrointestinal bleeding, hepatorenal syndrome, spontaneous bacterial peritonitis and other complications of liver cirrhosis or primary liver cancer; (3) unstable diabetes, hypertension, thyroid diseases and autoimmune diseases; (4) patients with serious diseases of heart, lung, kidney, brain, blood and other important organs with dysfunction; (5) patients with severe neurological and mental diseases (e.g., epilepsy, depression, mania, schizophrenia, etc.); (6) pregnancy or lactation.

Patients were randomly assigned 2:1 to receive either ETV or ALHX or ETV group by simple randomization with no stratification. Randomized treatment was open-label. The study coordinator assigned patients serial numbers that were linked to a computer-generated randomization list assigning the treatment regimens. ALHX 6 g/bid was administered orally. All patients signed informed consent forms before enrollment. Study procedures followed the ethical principles of the Helsinki Declaration and were approved by The Ethical Committees of Peking University First Hospital. The complete protocol for the clinical trial was registered at ClinicalTrials.gov (NCT01962155 and NCT03568578).

Liver biopsy and scoring system

Liver histological specimens (paraffin embedded) were collected by percutaneous ultrasound-guided liver biopsy and transported to a central laboratory for interpretation. Specimens were considered adequate for scoring if they were more than 2.0-cm long and contained at least 11 portal tracts. They were assessed by two professional liver pa-
thologists from Capital Medical University affiliated Beijing You-An Hospital under double-blind conditions. If the results of the two pathologists were different, or the interpretation results differed with the local pathology, the results were determined by joint discussion with a third pathologist.

The evaluation of liver fibrosis (F) was divided into six stages (0–6), and necroinflammatory scores were assigned using a modified histological activity index (HAI) as described by Ishak et al. F ≥ 3 was considered significant fibrosis. Fibrosis regression after treatment required a decrease in F ≥ 1, and progression required an increase in F of at least one stage. Histological improvement required a decrease of the HAI of at least two grades and no fibrosis progression.

Endpoints

The primary endpoints were liver fibrosis regression and reduction of liver stiffness measurement (LSM). LSM was divided into four levels, 7.4 ≤ LSM < 9.4, 9.4 ≤ LSM < 12.4, and ≥ 12.4. LSM reduction required a decrease of at least one level after treatment. Secondary endpoints included histological improvement and noninvasive fibrosis index reduction, biochemical, virological, and serological responses. Noninvasive fibrosis indexes were fibrosis-4-score (FIB-4) and aspartate aminotransferase to platelet ratio index (APRI) were calculated from biochemical data. FIB-4 was divided into three levels, FIB-4 < 1.45, FIB-4 ≥ 1.45 and an intermediate level, FIB-4 reduction required a decrease on one level, the same as LSM. APRI was divided into three levels, < 1.00, ≥ 1.00 and an intermediate level, APRI reduction also required a decrease of at least one level. Qualitative assay of HBsAg was performed with available enzyme-linked immunosorbent assays (Roche Diagnostics Co., Penzberg, Germany), HBV DNA was assayed with Roche COBAS AmpliPrep/COBAS TaqMan assay (Roche Co., Penzberg, Germany) with a lower limit of detection of 20 IU/mL, and assay of serum hepatitis B core antibody (anti-HBc) was with a chemiluminescent particle immunoassay (Wantai Co., Xiamen, China). All assays were performed by a central laboratory at Peking University First Hospital. Biochemical data and transient elastography results (i.e. LSM) were collected at local study centers.

Statistical analysis

Quantitative variables were reported as medians and lower and upper quartiles or means±standard deviations (SDs). Categorical variables were reported as numbers and percentages. The t-test or Kruskal-Wallis test were used to compare continuous variables. Chi-square or Fisher’s exact test were used to compare categorical variables. Logistic regression was used to analyze factors associated with fibrosis regression. P-values < 0.05 was considered statistically significant. The statistical analysis was performed with SPSS 24.0 (IBM Corp., Armonk, NY, USA).

Results

Study population

A total of 1,328 HBsAg (+) patients were screened between October 1, 2013 and December 31, 2020 and 780 CHB patients with liver stiffness were randomized in the intention-to-treat (ITT) population, 258 patients in the ETV group and 522 patients in the ETV+ALHX group. A total of 394 patients with a second liver biopsy after treatment were finally enrolled in the per-protocol (PP) population, 132 in the ETV group and 262 in the ETV+ALHX group. The enrollment protocol is shown in Figure 1. In the PP population, both groups included mainly male patients with an average age of about 40 years, and a body mass index (BMI) of 23–24 kg/m².

There were no significant differences between two groups in the baseline demographic and clinical characteristics except for HAI grade. The baseline characteristics of the PP population are shown in Table 1, the characteristics of the PP population with significant fibrosis are shown in Supplementary Table 1 and those of ITT population are shown in Supplementary Table 2.

Factors related to fibrosis regression

We divided significant fibrosis patients (baseline F ≥ 3) into the ETV+ALHX group had a significantly higher rate of fibrosis regression (124/211, 58.8%) than those in the ETV group (45/98, 45.9%) after 78 weeks of treatment ( p = 0.035, Table 2). Patients in ETV+ALHX group also has a higher fibrosis regression rate than those in the ETV group [130/262 (49.6%) and 53/132 (40.2%); Table 2] but the difference was not significant ( p = 0.075). The distribution of F stage at baseline and after 78 weeks treatment for both groups are shown in Figure 2. Change in fibrosis after treatment compared with baseline is shown in Supplementary Table 3. We also measured fibrosis regression in LSM, APRI and FIB-4. Patients with a baseline F ≥ 3, LSM reduction rate in ETV+ALHX group (73.9%) was also higher than in the ETV group (63.3%), p = 0.056. FIB-4 and APRI in the two groups before and after treatment are also shown in Table 2. The distribution of LSM, APRI and FIB-4 at baseline and after treatment and changes in the two groups before and after treatment are shown in Figure 3.

Secondary endpoints: The rates (i.e. percentages) of patients with normalized ALT and those with a HBV DNA decrease ≥ 2-times the log value, HBV DNA < 20 IU/mL, HBsAg clearance, and HBeAg seroconversion in the ETV+ALHX group and ETV group were not significantly different (Table 2). The percentage of patients with baseline F ≥ 3 and an HAI decrease ≥ 2 grades was significantly higher in the ETV+ALHX group than in the ETV group (67.3% vs. 53.1%, p = 0.016). Histological improvement rate in ETV+ALHX group also has a higher trend in both all patients (55.3% vs. 46.2%) and F3 patients (58.3% vs. 49.0%, Table 2).

Safety

Two patients in the ETV+ALHX group had mild diarrhea, but after evaluation by the attending physician and symptomat-
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Discussion

In this study, we found that ALHX was effective for the regression of liver fibrosis in CHB patients using ETV, especially in those with baseline F≥3 (p=0.035). Using transient elastography to estimate liver fibrosis, the LSM degradation rate was higher in the ETV+ALHX group than the ETV group in baseline F≥3 patients (73.9% vs. 63.3%, p=0.056), non-invasive fibrosis indexes such as FIB-4 and APRI, the degradation rates in ETV+ALHX group were also higher, but more patients may be needed to reach statistical significance. We calculated patients with no or mild liver fibrosis (F<3) and the effect of adding ALHX may be more inclined to maintain liver in mild fibrosis: Fibrosis was stable in 9 (9/34, 26.5%) patients in the ETV group and in 29 (29/51, 56.9%) in the ETV+ALHX group, p=0.006. Fibrosis progressed in 17 (17/34, 50.0%) ETV patients and 16 (16/51, 31.4%) in ETV+ALHX patients, p=0.084. In significant fibrosis pa-

Fig. 1. Flowchart of enrollment.
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Table 1. Baseline demographic and clinical characteristics

|                          | ETV   | ETV+ALHX | p-value |
|--------------------------|-------|----------|---------|
| N                        | 132   | 262      | 0.831   |
| Age, year                | 40.68±11.58 | 40.92±10.07 | 0.831   |
| <40, n (%)               | 63 (47.7) | 125 (47.7) |         |
| ≥40, n (%)               | 69 (52.3) | 137 (52.3) |         |
| Male sex, n (%)          | 104 (78.8) | 190 (72.5) | 0.177   |
| BMI (kg/m²)              | 23.52±3.03 | 24.00±3.27 | 0.163   |
| Family history of HBV infection, n (%) | 57 (43.2) | 131 (50.4) | 0.177   |
| Male sex, n (%)          | 104 (78.8) | 190 (72.5) | 0.177   |
| BMI (kg/m²)              | 23.52±3.03 | 24.00±3.27 | 0.163   |
| Family history of HBV infection, n (%) | 57 (43.2) | 131 (50.4) | 0.177   |

All values shown are based on available data, and are means±SDs or medians (range). AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; Cr, creatinine; DBil, direct bilirubin; F, liver fibrosis score; GGT, glutamyl transferase; HAI, liver histologic activity scores of inflammatory; HBV, hepatitis B virus; HCC, hepatocellular carcinoma HGB, hemoglobin; INR, international normalized ratio; LSM, liver stiffness measurement; LY%, percentage of lymphocyte; PLT, platelet; qAnti-HBc, quantitative anti-hepatitis B core antigen; TBil, total bilirubin; TCHO, total cholesterol; TG, triglyceride; WBC, white blood cell.
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Table 2. Treatment efficacy of the ETV group and ETV+ALHX group

|                                | ETV   | ETV+ALHX | p-value |
|--------------------------------|-------|----------|---------|
| **All patients, n**            | 132   | 262      |         |
| ALT normalization, n (%)       | 83/90 (92.2) | 159/178 (88.8) | 0.449   |
| HBV DNA decreased ≥2×log, n (%)| 118 (89.4) | 238 (90.8) | 0.646   |
| HBV DNA≤20 IU/mL, n (%)        | 100 (75.8) | 203 (77.5) | 0.702   |
| HBeAg clearance, n (%)         | 26 (19.7) | 44 (16.9) | 0.498   |
| HBeAg seroconversion, n (%)    | 9 (6.8) | 11 (13.3) | 0.271   |
| Histological improvement1, n (%)| 61 (46.2) | 145 (55.3) | 0.087   |
| HAI decreased ≥2, n (%)        | 74 (56.1) | 173 (66.0) | 0.053   |
| **Fibrosis changes**2         |       |          |         |
| Regression, n (%)              | 53 (40.2) | 130 (49.6) | 0.075   |
| Progression, n (%)             | 30 (25.1) | 45 (17.2) | 0.185   |
| LSM degradation3, n (%)        | 80 (60.6) | 179 (68.7) | 0.109   |
| FIB-4 degradation4, n (%)      | 47 (35.6) | 96 (36.6) | 0.840   |
| APRI degradation5, n (%)       | 47 (35.6) | 107 (40.8) | 0.315   |
| **F2 patients, n**             | 98    | 211      |         |
| ALT normalization, n (%)       | 65/70 (92.9) | 126/144 (87.5) | 0.235   |
| HBV DNA decreased ≥2log10, n (%)| 90 (91.8) | 191 (90.5) | 0.708   |
| HBV DNA ≤20 IU/mL, n (%)       | 75 (76.5) | 166 (78.7) | 0.672   |
| HBeAg clearance, n (%)         | 14 (14.3) | 33 (15.8) | 0.733   |
| HBeAg seroconversion, n (%)    | 1 (1.0) | 8 (3.8) | 0.174   |
| Histological improvement1, n (%)| 48 (49.0) | 123 (58.3) | 0.125   |
| HAI decreased ≥2, n (%)        | 52 (53.1) | 142 (67.3) | 0.016   |
| **Fibrosis changes**2         |       |          |         |
| Regression, n (%)              | 45 (45.9) | 124 (58.8) | 0.035   |
| Progression, n (%)             | 13 (13.3) | 29 (13.7) | 0.909   |
| LSM degradation3, n (%)        | 62 (63.3) | 156 (73.9) | 0.056   |
| FIB-4 degradation4, n (%)      | 33 (33.7) | 77 (36.5) | 0.630   |
| APRI degradation5, n (%)       | 37 (37.8) | 88 (41.7) | 0.510   |

1HAI decreased ≥2 grades and fibrosis stage was not increased after treatment. 2Regression required a decrease of ≥1 in fibrosis stage after treatment; Progression required an increase of ≥1 in fibrosis stage after treatment. 3LSM was divided into four grades: <7.4, 7.4≤LSM <9.4, 9.4≤ LSM <12.4 and ≥12.4, grade decreased ≥1 after treatment. 4FIB-4 was divided into three grades: <1.45, 1.45≤FIB-4<3.25, and ≥3.25, grade decreased ≥1 after treatment. 5APRI was divided into three grades: <1.00, 1.00≤ APRI<2.00, and ≥2.00, grade decreased ≥1 after treatment. ALT, alanine aminotransferase; HAI, liver histologic activity scores of inflammatory; HBV, hepatitis B virus; ALHX, An-Luo-Hua-Xian; APRI, aspartate aminotransferase to platelet ratio index; ETV, Entecavir; F, liver histologic fibrosis score; FIB-4, fibrosis-4-score.

ALT, alanine aminotransferase; HAI, liver histologic activity scores of inflammatory; HBV, hepatitis B virus; ALHX, An-Luo-Hua-Xian; APRI, aspartate aminotransferase to platelet ratio index; ETV, Entecavir; F, liver histologic fibrosis score; FIB-4, fibrosis-4-score.

1HAI decreased ≥2 grades and fibrosis stage was not increased after treatment. 2Regression required a decrease of ≥1 in fibrosis stage after treatment; Progression required an increase of ≥1 in fibrosis stage after treatment. 3LSM was divided into four grades: <7.4, 7.4≤LSM <9.4, 9.4≤ LSM <12.4 and ≥12.4, grade decreased ≥1 after treatment. 4FIB-4 was divided into three grades: <1.45, 1.45≤FIB-4<3.25, and ≥3.25, grade decreased ≥1 after treatment. 5APRI was divided into three grades: <1.00, 1.00≤ APRI<2.00, and ≥2.00, grade decreased ≥1 after treatment. ALT, alanine aminotransferase; HAI, liver histologic activity scores of inflammatory; HBV, hepatitis B virus; ALHX, An-Luo-Hua-Xian; APRI, aspartate aminotransferase to platelet ratio index; ETV, Entecavir; F, liver histologic fibrosis score; FIB-4, fibrosis-4-score.

Other Chinese patent drugs such as Biejia-Ruangan (BR) and Fu-Cheng-Hua-Yu (FZHY) have an antifibrosis effect in clinical therapy. Rong et al., in a study of 1,000 CHB patients (705 with a second biopsy), reported that the rate of fibrosis regression after 72 weeks of treat-
ment was significantly higher in the ETV+BR group than in the ETV group (40% vs. 31.8%, \( p = 0.0069 \)). Huang et al.\(^2\) found that BR inhibited hepatic collagen deposition and improved liver injury in rats with \( \text{CCl}_4 \)-induced hepatic fibrosis, which was associated with downregulation of the transforming growth factor beta-\( \beta \)-Smad pathway. As early as 2005, a multicenter clinical trial confirmed the antifibrosis effectiveness and safety of FZHY in CHB patients.\(^2\) A single-center clinical study by Gui et al.,\(^2\) which included 46 CHB patients with a second liver biopsy, reported that ETV+ FZHY had a significantly higher rate of fibrosis regression (82% vs. 54%, \( p < 0.05 \)). In mice with fibrosis induced by \( \text{CCl}_4 \) and dimethylnitrosamine, it was observed that FZHY not only was effective against fibrosis, it also improved \( \text{CCl}_4 \) and dimethylnitrosamine-induced sinus capillary formation and expression of angiogenesis and angiogenesis-related factors.\(^2\) Hepatic sinusoidal capillarization and angiogenesis in the fibrous septum connecting the portal vein and the central hepatic vein are two key events leading to liver cirrhosis.\(^2\) Further controlled studies are needed to compare the efficacy of these traditional Chinese medicines.

Loomba et al.\(^2\) found that a family history of HCC multiplied the risk of HCC associated with HBV infection. The cumulative risk was 15.8% with vs. 7.5% without a family history (\( p < 0.001 \)). Our study found that a family history of HCC affected the regression of liver fibrosis, so it may lead to the progression of fibrosis and eventually develop into liver cirrhosis and HCC. Therefore, in guidelines for the diagnosis and treatment of CHB, it is suggested that patients with a family history of HCC should start antiviral treatment.\(^2\) Univariate and multivariate analysis in our study also showed that in patients with significant fibrosis, there was a negative correlation between histological evidence of fibrosis regression and a family history of HCC. As we know, HBV has at least nine different genotypes. Genotypes B and C are the most prevalent in China, and genotype C is associated with earlier progression to HCC.\(^2\) Our study provides additional support for the guideline recommendation for starting combined antifibrosis and antiviral treatment of these CHB patients as soon as possible. However, the relationship between a family history of HCC

![Fig. 2. Distribution of fibrosis scores in both study groups before and after 78 weeks of treatment.](image-url)
and lack of fibrosis regression and the incidence of HCC needs further study to clarify the cause.

This study has several limitations. Firstly, nearly half the patients with a first liver biopsy had a second one after treatment. We tried our best to communicate with patients, but partly because of COVID-19, some patients could not return to hospital for follow-up. Secondly, ALHX is a patent Chinese drug containing more than ten kinds of traditional Chinese medicines. We have not studied the antifibrosis effectiveness of single components. Our study showed that ALHX combined with antiviral therapy reversed liver fibrosis, but its specific antifibrosis components need to be studied. Thirdly, our study did not use a placebo in the ETV group and only observed the results of 78 weeks of treatment. We need longer patient follow-up and are working on it.

Conclusion

In CHB patients using ETV, combined treatment with ALHX increased the rates of liver fibrosis regression after 78 weeks treatment in baseline F≥3 patients as shown by invasive liver biopsy or noninvasive methods such as LSM, etc. Logistic regression analysis found that ALHX was associated with the regression outcome and that a family history of HCC had the opposite association. In patients with significant fibrosis, we recommend combining antiviral therapy and ALHX as soon as possible.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (Liu YQ, Zhao H, Wang GQ), methodology (Liu YQ, Zhang C, Li JW), formal analysis and investigation, (Liu YQ, Zhang C, Li JW), writing - original draft preparation (Liu YQ), writing - review and editing (Zhuang H, Hong Zhao, Zhao H, Wang GQ), funding acquisition (Wang GQ), material preparation and data collection (Cao LH, Zhang ZQ, Zhao WF, Shang QH, Zhang DZ, Ma AL, Xie Q, Gui HL, Zhang G, Liu YX, Shang J, Xie SB, Li J, Zhang QX, Zou Q, Chen YP, Zhang Z, Zhang MX, Cheng J, Zhang FC, Huang LH, Li JB, Meng QH, Yu HB, Mi YQ, Peng YZ, Wang ZJ, Chen LM, Meng FP, Ren WH, Bai L, Zeng YL, Fan R, Lou XZ, Liang WF, Liu H; Supervision: Zhao H, Wang GQ).
Table 3. Analysis of factors associated with fibrosis regression in F≥3 patients

|                | Regression     | No regression | Uni  | p-value\(^1\) | Multi | p-value\(^2\) |
|----------------|----------------|---------------|------|---------------|-------|---------------|
| N              | 169            | 140           |      |               |       |               |
| ETV+ALHX group | 124 (73.4)     | 87 (62.1)     | 4.46 | 0.035         | 1.94  | (1.12, 3.37)  | 0.018 |
| Age (years)    | 41.12±10.51    | 42.34±10.72   | -1.00| 0.316         |       |               |
| <40, n (%)     | 79 (46.7)      | 58 (41.4)     | 0.88 | 0.349         |       |               |
| Male sex, n (%)| 121 (71.6)     | 102 (72.9)    | 0.06 | 0.81          |       |               |
| Family history of HBV infection, n (%) | 81/167 (48.5) | 72 (51.4)     | 0.26 | 0.610         |       |               |
| Family history of HCC, n (%)   | 13/168 (7.7)   | 24 (17.1)     | 6.39 | 0.011         | 0.41  | (0.18, 0.92)  | 0.031 |
| History of drinking, n (%)     | 5 (3.0)        | 4 (2.9)       | 0.00 | 0.958         |       |               |
| HBsAg positive duration, year | 11 (4.20)      | 10 (2.20)     | -0.26| 0.794         |       |               |
| BMI (kg/m²)    | 23.98±3.43,23.62| 24.22±3.04,24.22| -0.64| 0.524         |       |               |
| WBC (×10⁹/L)   | 5.47±1.58,5.30 | 5.32±1.56,5.18| 0.77 | 0.443         |       |               |
| LY%            | 34.93±7.84,34.6| 34.83±9.61,35.9| 0.11 | 0.918         |       |               |
| HGB (g/L)      | 143.91±17.38,147| 146.22±16.76,148| -1.19| 0.237         |       |               |
| PLT (×10⁹/L)   | 157.57±52.26,155.00| 144.86±46.07,143.00| 2.25 | 0.026         | 1.00  | (1.00, 1.01)  | 0.012 |
| ALT (U/L)      | 56.00 (38.00, 101.28)| 56.00 (35.00, 101.28)| 1.16 | 0.265         |       |               |
| AST (U/L)      | 41.00 (28.00, 70.00)| 41.00 (30.09, 69.26)| 1.11 | 0.239         |       |               |
| ALP (U/L)      | 87.15 (71.05, 109.22)| 89.32 (71.28, 114.91)| -0.11| 0.914         |       |               |
| GGT (U/L)      | 46.00 (28.50, 80.00)| 42.78 (24.91, 83.81)| -0.16| 0.870         |       |               |
| ALB (g/L)      | 42.46±4.91,43.00| 41.68±4.81,42.00| 1.41 | 0.161         | 0.943 |               |
| TBil (μmol/L)  | 14.70 (11.40, 20.80)| 15.90 (11.45, 20.30)| 1.27 | 0.177         | 0.217 |               |
| DBil (μmol/L)  | 4.70 (3.21, 7.50)| 4.70 (3.40, 7.65)| 1.71 | 0.065         | 0.077 |               |
| Cr (μmol/L)    | 66.97±14.13,66.00| 65.81±12.93,65.40| 0.75 | 0.457         |       |               |
| TCHO (mmol/L)  | 4.42±0.93,4.31| 4.23±0.74,4.20| 1.85 | 0.060         | 0.688 |               |
| TG (mmol/L)    | 1.19±0.64,1.01| 1.08±0.50,1.02| 1.59 | 0.113         | 0.798 |               |
| AFP (ng/mL)    | 4.53 (2.94, 12.98)| 5.00 (3.10, 16.45)| -0.08| 0.940         |       |               |
| INR            | 1.10±0.11,1.08| 1.11±0.15,1.10| -1.43| 0.154         | 0.10  | (0.01, 0.87)  | 0.037 |
| HBV DNA (log₁₀ IU/mL) | 5.62±1.90,5.68| 5.62±1.75,5.83| -0.26| 0.797         |       |               |
| HBsAg (log₁₀ IU/mL) | 3.32±0.69,3.38| 3.22±0.66,3.31| 1.32 | 0.189         | 0.092 |               |
| HBeAg (+), n (%)| 93/167 (55.7)  | 80/136 (58.8)  | 0.30 | 0.583         |       |               |
| qAnti-HBc (log₁₀ IU/mL) | 3.88±0.69,3.90| 3.81±0.83,3.82| 0.75 | 0.448         |       |               |
| LSM (kPa)      | 11.90 (8.50, 18.55)| 13.45 (8.90, 19.90)| -0.64| 0.520         |       |               |
| HAI            | 2.42 ± 0.490   |               |      |               |       |               |

**Note:** ETV, Entecavir; ALHX, An-Luo-Hua-Xian; F, liver histologic fibrosis score; HAI, liver histologic activity scores of inflammatory; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; Cr, creatinine.
All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. Informed consent was obtained from all patients for being included in the study.

Data sharing statement

Data supporting the findings of this study are available within the article and its supplementary materials.