Improvement of Liver Fibrosis after Long-Term Antiviral Therapy Assessed by Fibroscan in Chronic Hepatitis B Patients With Advanced Fibrosis

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OBJECTIVES: **Performing repeated liver biopsies to assess the improvement of liver fibrosis is impractical. The purpose of this prospective cohort study was to assess the improvement of liver fibrosis during antiviral treatment by serial liver stiffness (LS) measurement using Fibroscan in chronic hepatitis B (CHB) patients with advanced fibrosis.**

METHODS: **Nucleos(t)ide analog-naive CHB patients with advanced fibrosis in histological findings (stage ≥F3), high viral load (hepatitis B virus DNA ≥2,000 IU/ml), and normal liver enzyme levels (<2×upper normal limit) before starting antiviral treatment were included in this study. LS measurement was performed at baseline and annually for 5 years during antiviral treatment. Five-year fibrosis improvement was defined as LS value <7.2 kPa (<F3) at year 5.**

RESULTS: **The mean LS value of 120 patients significantly decreased over time (14.5 kPa at baseline; 11.3 kPa at year 1; 9.6 kPa at year 2; 9.3 kPa at year 3; 8.6 kPa at year 4; and 8.3 kPa at year 5). Multivariate analysis showed that baseline LS value was the only predictor of 5-year fibrosis improvement (odds ratio, 0.907; 95% confidence interval, 0.838–0.980; *P*=0.014). Patients with low baseline LS values (<12.0 kPa) had a greater probability of experiencing significant fibrosis improvement than those with high baseline LS values (≥12.0 kPa) (81.5% vs. 29.0%, *P*<0.001).**

CONCLUSIONS: **In CHB patients with advanced fibrosis receiving antiviral treatment, annual LS measurement revealed that fibrosis improvement slows but continues during treatment. Low LS value (<12.0 kPa) at baseline was a significant predictor for 5-year fibrosis improvement.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Progression of liver fibrosis to cirrhosis increases the risk of various complications of hepatic decompensation, including ascites formation, variceal bleeding, encephalopathy, and hepatocellular carcinoma (HCC) (1–3). Studies have emphasized the suppression of viral replication by using nucleotide analogs (NUCs), which lead to clinical benefits such as reduction of hepatic decompensation and HCC development (4–7). The key mechanism by which viral suppression promotes a favorable clinical outcome is the reversal of liver fibrosis that is induced by active antiviral treatment with NUCs. The regression of fibrosis during antiviral treatment has been shown only recently by several studies in which the fibrotic burden was assessed by liver biopsy (LB) (8–10). LB has been the gold standard for assessing liver fibrosis in the past few decades (11); however, LB can have limited diagnostic accuracy resulting from sampling error and inter-observational variation and is an impractical procedure to repeat (12).

The diagnostic accuracy of transient elastography, a noninvasive tool for assessing liver fibrosis, has been sufficiently validated in various chronic liver diseases (13,14). Because transient elasto-

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graphy is simple, noninvasive, and reproducible, it can be repeated at the beginning and during antiviral treatment to assess the dynamic change of liver fibrosis burden. Several studies have investigated liver fibrosis improvement during antiviral treatment in patients with chronic hepatitis B (CHB) using transient elastography (15–22). However, these studies had only 1–3 years of short-term follow-up data. Additionally, these studies showed only initial and follow-up liver stiffness (LS) values but did not trace annual fibrosis burden. Ogawa *et al.* (23) traced annual LS values, but the study sample was too small for rigorous analysis. Thus, the purpose of this study was to assess the improvement of liver fibrosis during antiviral treatment by annual LS measurement in CHB patients with advanced fibrosis in histology and to investigate the predictors for long-term fibrosis improvement.

METHODS

Patients

This prospective study used a cohort from our group's previous study by Kim *et al.* (24). Briefly, NUC-naive CHB patients with advanced fibrosis in histological findings (stage ≥F3), high viral load (hepatitis B virus (HBV) DNA ≥2,000 IU/ml), and normal liver enzyme levels (<2×the upper normal limit (UNL)) before starting antiviral treatment were considered eligible in the study. Exclusion criteria included: (i) co-infection with hepatitis C, hepatitis D, or human immunodeficiency virus; (ii) heavy alcohol consumption (>21 drinks per week in men and >14 drinks per week in women); (iii) HCC or history of HCC; (iv) hepatic decompensation; and (v) unavailable LS value, LS measurement failure, or unreliable LS values. After baseline enrollment, the following criteria were used to additionally exclude patients during the 5 years of antiviral treatment: (i) lost to follow up, (ii) stopped NUC, (iii) HCC occurrence during 5 years of antiviral treatment, (iv) hepatic decompensation during 5 years of antiviral treatment, or (v) refused LS measurement, LS measurement failure, or unreliable LS values (**Supplementary Figure S1**) online. We excluded patients with HCC occurrence or hepatic decompensation during 5 years of antiviral treatment because LS measurement was no longer feasible in these patients owing to the changes in liver anatomy. The study protocol was consistent with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review board of Severance Hospital.

LB and histological assessment

LB was performed at baseline before the patients started antiviral treatment to assess the severity of liver fibrosis and necroinflammation. All patients underwent ultrasonography-guided percutaneous LB. LB specimens were formalin-fixed and paraffin-embedded, and 4 μm sections were stained with hematoxylin and eosin and Masson's trichrome. All liver tissue samples were evaluated by an experienced hepatopathologist who did not have access to the patients' clinical data. Liver fibrosis and necroinflammation were evaluated semi-quantitatively according to the METAVIR scoring system (25). Fibrosis was scored as follows: F0, no definite fibrosis; F1, minimal fibrosis (no septa or rare thin septum; may have portal expansion or mild sinusoidal fibrosis); F2, mild fibrosis (occasional thin septa); F3, moderate fibrosis (moderate thin septa; incomplete cirrhosis); and F4, cirrhosis. Stage F4 had more numerous septa per unit length of biopsy sufficient to display nodules with rounded contours than F3 disease. Activity grade was defined as lobule or periportal necroinflammatory activity, whichever was higher, and the scored as follows: A0, none; A1, mild activity; A2, moderate activity; and A3, severe activity. There was no LB specimen unsuitable for fibrosis assessment (all specimens had LB length >12 mm with more than six portal tracts). The median length of biopsied specimens in the study was 16 mm (range, 12–25 mm).

Laboratory tests

Blood parameters were measured on the same day as LB, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum albumin, total bilirubin, platelet count, and prothrombin time. The UNL of AST and ALT were 36 IU/l and 40 IU/l, respectively. Hepatitis B surface antigen and hepatitis B e-antigen (HBeAg) levels were checked using standard enzyme-linked immunosorbent assays (Abbott Diagnostics, Lake Forest, IL). HBV DNA levels were measured by quantitative polymerase chain reaction assay (Amplicor HBV Monitor Test; Roche Diagnostics, Basel, Switzerland), with a detection limit of 12 IU/ml. Virological response was defined as undetectable HBV DNA levels (<12 IU/ml). Blood parameters analysis and ultrasonography were performed every 3–6 months depending upon the clinicians' medical decision.

LS measurement

Baseline LS measurements were performed on the same day before LB. In several patients who underwent LB first, LS measurements were done at least 3 days after (but within 1 month from) LB, after confirming there were no complications related to LB. Thereafter, LS measurement was repeated annually for at least 5 years during antiviral treatment. The operator positioned the probe on the right lobe of the liver through the intercostal spaces on patients lying in the dorsal decubitus position with the right arm in maximal abduction and then pressed the probe button to obtain the measurement (13). An experienced technician (>20,000 examinations) who was blinded to the patients' clinical data performed all LS measurements. LS values are expressed in units of kPa. The success rate was calculated by dividing the number of valid measurements by the total number of measurements. Interquartile range, corresponding to the interval of LS results containing 50% of the valid measurements between the 25th and 75th percentiles, was used as an index of intrinsic variability of LS measurements. LS measurement failure with no valid shots and unreliable LS values showing an interquartile range to median value ratio >0.3, success rate <60%, or <10 valid measurements were excluded from the analysis (26). LS stages were defined following the cutoff values of Marcellin *et al.* (27): F1 ≥5.5 kPa; F2 ≥7.2 kPa; F3 ≥9.5 kPa; and F4 ≥11.0 kPa.

Fibrosis-4 and AST to platelet ratio index scores

As serum fibrosis markers, Fibrosis-4 (FIB-4) and AST to platelet ratio index (APRI) scores were calculated according to the following

formulas. Both scores were assessed on the same day as LB at baseline and annually for 5 years during antiviral treatment.

$$\text{FIB-4} = (\text{age} \times \text{AST})/(\text{platelet count} \times \sqrt{\text{ALT}})$$

$$\text{APRI} = ([\text{AST/UNL}]/\text{platelet count}) \times 100$$

The UNL of AST was 36 IU/l in this study. An APRI score >2.00 was the cutoff for cirrhosis, and a score <1.00 classified patients as Ishak stage 0–4 (28). FIB-4 score >3.25 was the cutoff for advanced fibrosis, and a score <1.45 classified patients as Ishak stage 0–3 (29).

Definitions

Five-year fibrosis improvement was defined as LS value <7.2 kPa (<F3) at year 5. Hepatic decompensation included ascites, variceal bleeding, hepatic encephalopathy, spontaneous bacterial peritonitis, and hepatorenal syndrome.

Statistical analyses

Data are expressed as the mean±s.d., median (range), or *n* (%), as appropriate. The LS value change of each patient over the 5-year of antiviral therapy was depicted with spaghetti plot. Changes in LS values, FIB-4, and APRI were assessed with repeated measures analysis of variance test. Model-based clustering, a technique for clustering data through the imposition of a mixture modeling framework, was used to analyze the annual LS values (30). LS patterns were classified according to the baseline LS value and the differences in annual LS values, and models for each group were determined. To identify independent predictors of fibrosis improvement, we performed univariate and subsequent multivariate regression analyses. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) are indicated. Time-dependent receiver operating characteristic curves and areas under the receiver operating characteristic curve were used to calculate the optimal LS cutoff value for the prediction of fibrosis improvement, which maximized the sum of sensitivity and specificity. The annual incidence rates of HCC were expressed in person-years. The cumulative incidence rates of HCC were calculated using the Kaplan–Meier method. *P* values less than 0.05 on a two-tailed test were considered statistically significant. Statistical analyses were performed using SPSS software (version 18.0; Chicago, IL; and R-package 3.2.3.) (13). An experienced independent statistician performed all analyses.

RESULTS

Baseline characteristics

Among 178 NUC-naive CHB patients with advanced fibrosis in histological findings (stage ≥F3), high viral load (HBV DNA ≥2,000 IU/ml), and normal liver enzyme levels (<2×UNL) who met the eligible criteria, 34 patients were excluded at enrollment and 24 patients were excluded during 5 years of antiviral treatment. Ultimately, a total of 120 patients who underwent annual LS measurement for 5 years during antiviral therapy were selected for analysis (**Supplementary Figure S1**). The baseline characteristics of 120 patients are summarized in **Table 1**. The mean age of the patients (69 males and 51 females) was 50.8 years. Histologically, 103 (85.8%) patients had cirrhosis (F4), and 17 (14.2%) had advanced fibrosis (F3). Baseline HBV DNA and ALT were 8,080,000 IU/ml and 40 IU/l, respectively. All patients started antiviral treatment with either lamivudine (LAM) (*n*=42, 35.0%) or entecavir (ETV) (*n*=78, 65.0%). Mean body mass index was 23.8 kg/m^2, and nine patients had diabetes mellitus. All 120 patients were followed for 5 years, and 24 among them were followed for 6 years.

Change of LS values

The mean baseline LS value was 14.5 kPa for all patients, 9.8 kPa for patients with stage F3 (advanced fibrosis) histology, and 15.3 kPa for patients with stage F4 (cirrhosis) histology. LS value change of each patient over the 5-year was depicted in **Supplementary Figure S2**. Over the 5-year treatment period, fibrosis, as determined by the mean LS value, progressively improved (**Figure 1a**). The proportion of patients with cirrhosis defined by LS value (>11.0 kPa) markedly decreased from 66.7% at baseline to 17.5% at year 5, and the proportion of patients with mild or no fibrosis (LS value <7.2 kPa) increased from 3.3% at baseline to 52.5% at year 5 (**Figure 1b**). Improvement of fibrosis, as determined by a decrease in LS value, occurred in 90.0% (108/120) of patients; decreases in LS value occurred at a similar rate in patients with stage F4 histology compared with patients with stage F3 histology (90.3% (93/103) vs. 82.4% (14/17), *P*=0.394). However, the degree of LS improvement was greater in patients with stage F4 histology than in patients with stage F3 histology (LS changes after 5 years: −6.9 kPa in F4 patients vs. −3.2 kPa in F3 patients; *P*=0.001). In 12 patients who did not show improvement in their LS value, 9 patients showed fluctuating pattern of LS, and 3 patients showed decreasing pattern followed by increasing pattern of LS (**Supplementary Figure S2**).

At baseline, four (3.3%) patients had LS values <7.2 kPa. We excluded these four patients with discordant LS values and histological fibrosis results from further analyses. Five-year fibrosis improvement was documented in 53.4% (62/116) of patients. Patients with F3 histology had a higher rate of 5-year fibrosis improvement (86.7% (13/15)) than patients with F4 histology (48.5% (49/101), *P*=0.048). Twenty-four patients were followed for 6 years. The mean LS values of these patients were 13.9 kPa at baseline, 12.8 kPa at year 1, 10.8 kPa at year 2, 10.4 kPa at year 3, 9.3 kPa at year 4, 8.7 kPa at year 5, and 8.2 kPa at year 6.

Changes in serum fibrosis markers (FIB-4 and APRI)

The mean baseline FIB-4 score was 2.30±1.20, and the mean baseline APRI score was 0.81±0.42 for all patients. Both scores at baseline showed high correlation with each other (ø=0.820, *P*<0.001). The correlations between LS value and both marker values at baseline were also significant (LS value and FIB-4: ø=0.475, *P*<0.001; LS value and APRI: ø=0.436, *P*<0.001).

After 5 years of antiviral treatment, FIB-4 scores significantly improved compared with the baseline values (*P*<0.001); however,

Table 1. Baseline characteristics (*n*=120)

Variables	Values
Demographic variables	
Age (years)	50.8±9.45
Male gender	69 (57.5)
Body mass index (kg/m^2)	23.8±2.6
Normal/overweight/obese[a]	40 (33.3)/43 (35.9)/37 (30.8)
Diabetes mellitus	9 (7.5)
Type of antiviral agent	
Lamivudine/entecavir	42 (35.0)/78 (65.0)
Biochemical parameters	
Aspartate aminotransferase (IU/l)	37±12
Alanine aminotransferase (IU/l)	40±14
Serum albumin (g/dl)	4.3±0.4
Total bilirubin (g/dl)	2.3±0.8
Platelet count (10^9/l)	150±42
Prothrombin time (INR)	1.0±0.1
HBeAg positivity	69 (57.5)
HBV DNA (IU/ml)	8.08E+6±2.26E+5
Liver histology	
Fibrosis stage, 3/4	17 (14.2)/103 (85.8)
Activity grade, 1–2/3–4	99 (82.5)/21 (17.5)
Liver stiffness measurement	
Liver stiffness value (kPa)	14.5±7.2
Interquartile range (kPa)	1.6±2.2
Interquartile range/median value (%)	0.13±0.15

HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; kPa, kilopascal.
Variables are expressed as median (range), mean±(s.d.), or n (%).
[a]BMI is classified according to the BMI criteria for Asians by the World Health Organization.

the annual improvement was not statistically significant (**Figure 2a**). APRI scores also significantly improved compared with baseline values (P<0.001), but annual improvement was not always significant (**Figure 2b**).

Model-based clustering analysis of serial LS change

Models of serial LS change were determined with model-based clustering analysis in 116 patients with baseline LS value >7.2 kPa. Patients were categorized into two groups according to baseline LS value and annual LS changes (**Figure 3a**). Group 1 showed a higher baseline mean LS value (20.7 kPa) than group 2 (11.4 kPa). Group 1 also had a steeper slope of change in annual LS than group 2. The incidence rate of 5-year fibrosis improvement was higher in group 2 (64.4%) than in group 1 (34.9%; P=0.004; **Figure 3b**).

Predictors associated with 5-year fibrosis improvement

Five-year fibrosis improvement occurred in 53.4% (62/116) of patients. On univariate analysis, factors associated with improvement of fibrosis were high platelet count, low fibrosis stage (F3) at baseline, and low baseline LS value (all P<0.05). By multivariate analysis, after adjusting for baseline platelet count and fibrosis stage, baseline LS value was the only predictor of fibrosis improvement after 5 years of antiviral treatment (OR, 0.907; 95% CI, 0.838–0.980; P=0.014; **Table 2**). The optimal baseline cutoff LS value to predict improvement of fibrosis was calculated as 12.0 kPa with an area under the receiver operating characteristic curve of 0.756 (95% CI, 0.666–0.845; P<0.001; sensitivity, 71.0% (59.7–82.2); specificity, 81.5% (71.8–91.1); positive predictive value, 81.5% (71.1–91.8); and negative predictive value, 71.0% (59.7–82.2)). When using this optimal cutoff value of 12.0 kPa, patients with low baseline LS values (<12.0 kPa) had a greater probability of experiencing a significant improvement of fibrosis than those with high baseline LS values (≥12.0 kPa) (81.5 vs. 29.0%, P<0.001).

Antiviral treatment and clinical outcomes

Forty-two (35%) patients were treated with LAM, and 78 (65%) patients were treated with ETV (**Table 1**). Baseline characteristics, including demographics, biochemical parameters, and histological data, were similar between patients who received LAM and those who received ETV (all P>0.05, data not shown). None of the patients treated with ETV showed antiviral resistance, whereas 35.7% (15/42) of patients treated with LAM developed tyrosine-methionine-aspartic acid-aspartic acid (YMDD)-mutations. During the study period, patients who showed YMDD mutation continued add-on treatment with either adefovir (n=22) or tenofovir (n=5), according to the clinicians' discretion. The virological response rate increased annually (year 1, 46.6%; year 2, 51.7%; year 3, 67.2%; year 4, 81.0%; and year 5, 83.6%). Virological response rates at years 1, 2, 3, or 4 were not a predictor for 5-year fibrosis improvement. The type of antiviral agent and the development of the YMDD mutation also did not influence 5-year fibrosis improvement (all P>0.05; **Table 2**). In 69 HBeAg-positive patients at baseline, HBeAg seroconversion rate annually increased (year 1, 5.8%; year 2, 8.7%; year 3, 15.9%; year 4, 17.4%; and year 5, 21.7%). Each year, HBeAg seroconversion rate in HBeAg-positive patients was not a predictor for 5-year fibrosis improvement (all P>0.05).

Incidence of HCC and hepatic decompensation

Of the 144 patients enrolled at baseline (**Supplementary Figure S1**), we calculated the incidence of HCC and hepatic decompensation. During the median follow-up of 78.5 months (range, 16.9–95.8 months), HCC developed in six (4.2%) patients. Five patients (patient 1, 3, 4, 5, and 6) developed HCC within 5 years (patient 1, at 3.2 years; patient 3, at 4.7 years; patient 4, at 4.8 years; patient 5, at 2.3 years; and patient 6, at 1.4 years after antiviral treatment); however, one patient (patient 2) developed HCC after 6.4 years. The cumulative incidence rates of HCC at 1, 3, and 5 years were 0.7%, 1.5%, and 3.8%, respectively. The annual LS values of the

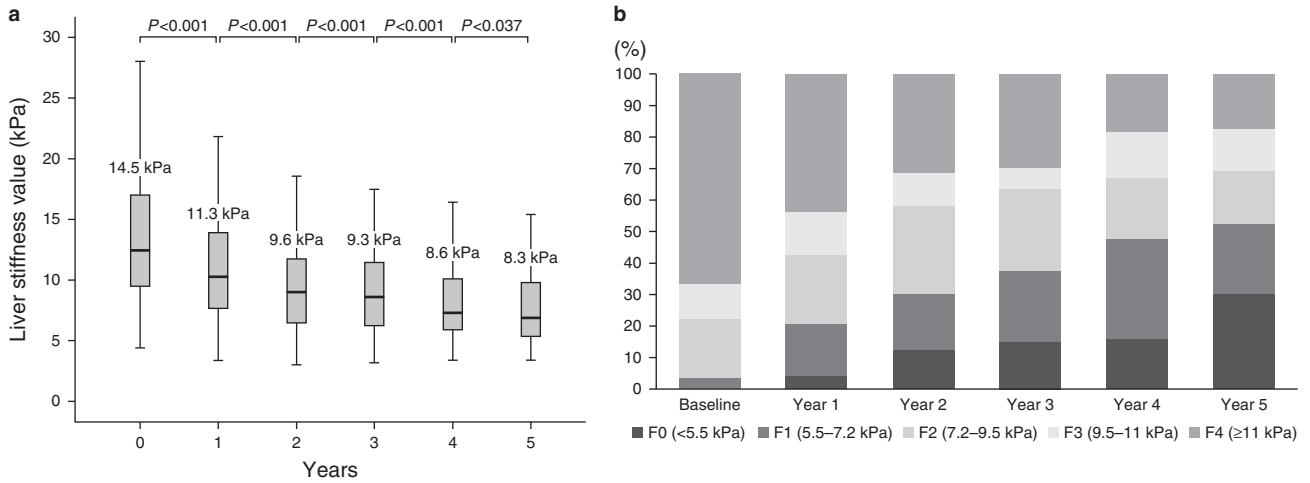

Figure 1. Liver stiffness changes during 5 years of antiviral treatment (*n*=120). (**a**) Over the 5-year treatment period, fibrosis, as determined by the mean LS value, progressively improved. (**b**) The proportion of patients with cirrhosis defined by LS value (>11.0 kPa) markedly decreased from 66.7% at baseline to 17.5% at year 5, and the proportion of patients with mild or no fibrosis (LS value <7.2 kPa) increased from 3.3% at baseline to 52.5% at year 5. LS, liver stiffness.

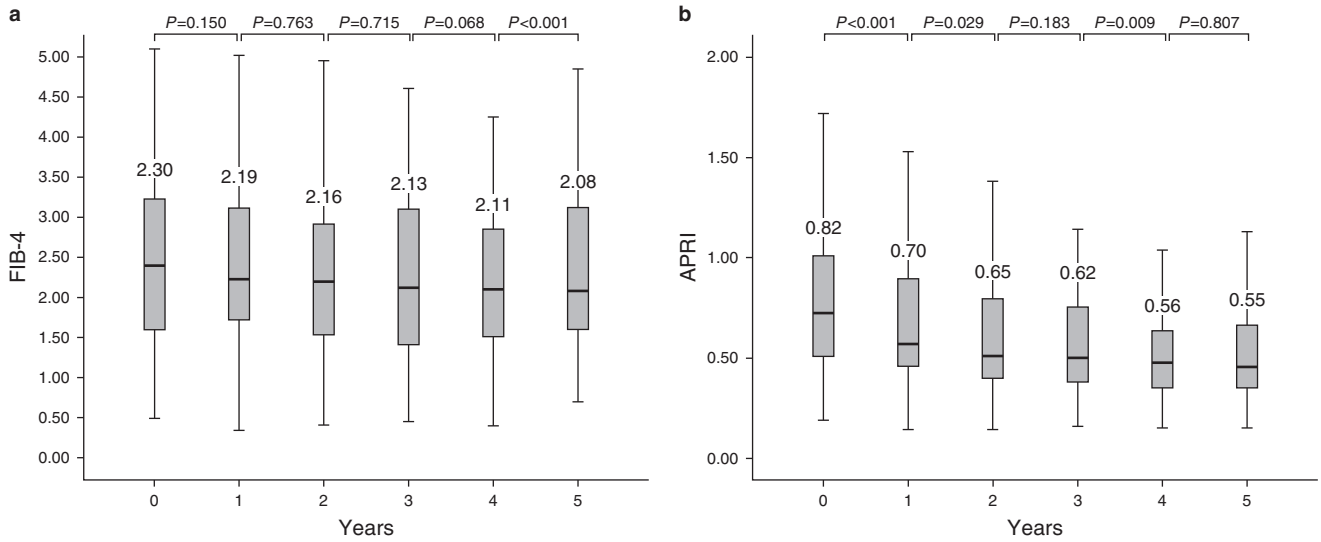

Figure 2. FIB-4 score and APRI score changes during 5 years of antiviral treatment (*n*=120). (**a**) FIB-4 scores significantly improved compared with the baseline values; however, the annual improvement was not statistically significant. (**b**) APRI scores also significantly improved compared with baseline values, but annual improvement was not always significant. APRI, AST to platelet ratio index; FIB-4, fibrosis-4.

six patients with HCC are depicted in **Figure 4**. Among the six patients with HCC, three (patients 1, 2, and 4) showed a pattern of decreasing LS values at the time of HCC development. Hepatic decompensation developed in one (0.7%) patient who showed ascites after 20 months of treatment with ETV.

DISCUSSION

Serial long-term LS data addressing the improvement of fibrosis during antiviral treatment in naive CHB patients with advanced fibrosis have been limited. This is the first prospective study to demonstrate significant fibrosis improvement during 5 years of antiviral treatment, determined by the decline of LS values from 14.5 kPa at baseline to 8.3 kPa at year 5. Over 5 years, the LS value significantly decreased each year, suggesting that NUC treatment resulted in increased fibrosis improvement each year. However, NUC had the greatest effect on fibrosis improvement at year 1 (mean, −3.2 kPa), and the degree of fibrosis improvement decreased over the subsequent years. Although apparent LS change at year 1 may be in part due to reduction of inflammation as well as fibrosis, we think the effect of inflammation was minimal because the patients in our study had normal or slightly elevated liver enzymes. Ultimately, the question of whether NUC will continue to induce fibrosis improvement beyond 5 years and for how long this effect will persist, should be investigated in the future studies.

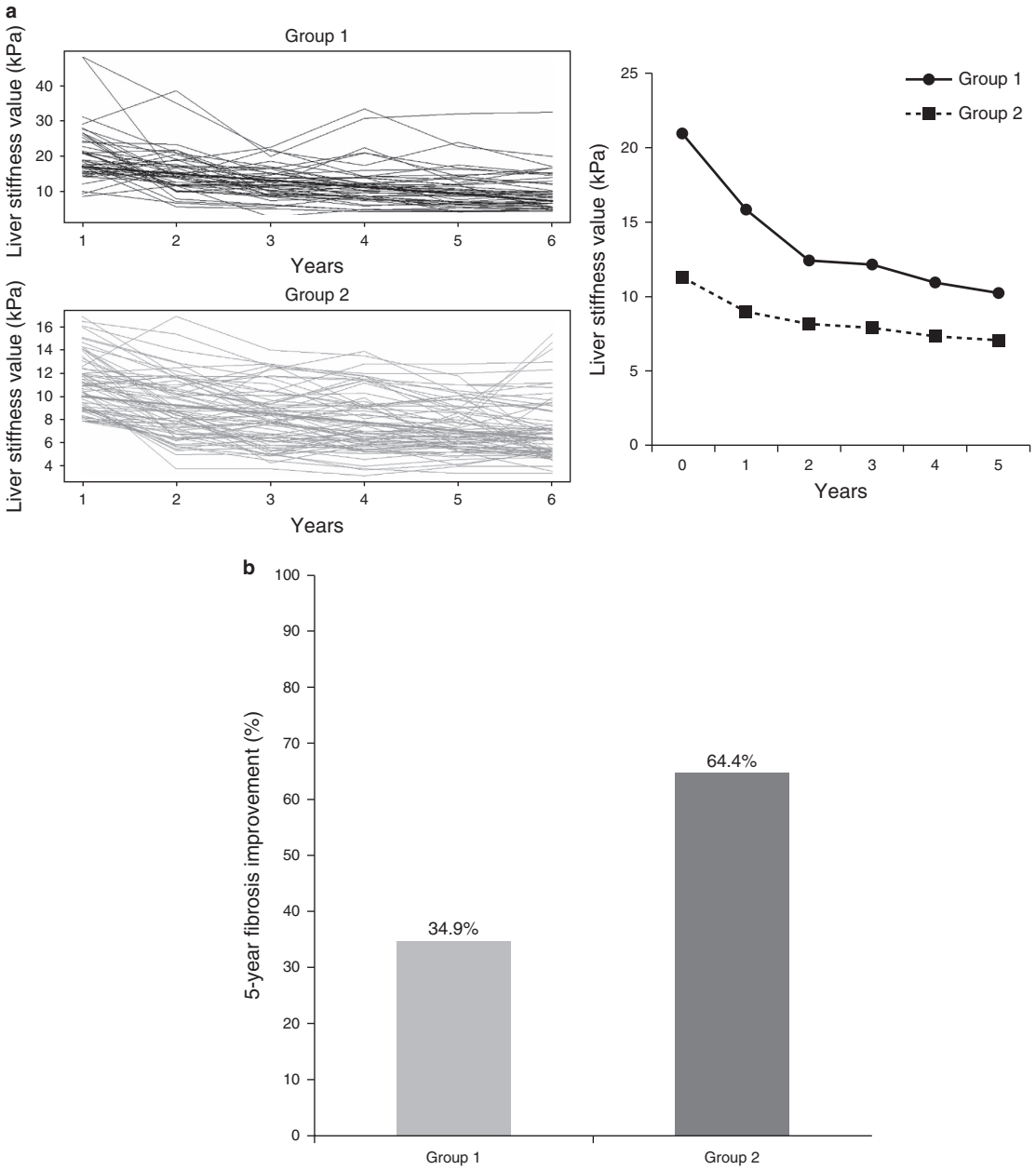

Figure 3. Model-based clustering analysis of serial LS change (n=116). (**a**) Patients were categorized into two groups according to baseline LS value and the annual LS changes. (**b**) Incidence rate of 5-year fibrosis improvement was higher in group 2 (64.4%) than in group 1 (34.9%) (P=0.004). LS, liver stiffness.

In our study, 90.0% of patients showed decreased LS values at year 5 compared with baseline, and 53.4% achieved 5-year fibrosis improvement. Although direct comparison is impossible, our findings are similar to those of Marcellin *et al.* (31) who reported the histological regression of fibrosis as 51% and histological improvement as 87% in patients who underwent 5 years of tenofovir treatment. Moreover, Marcellin *et al.* (31) showed that the histological improvement rate was greater in patients treated with tenofovir who had the highest liver injury scores, and we likewise found that the degree of improvement of fibrosis was greater in patients with histological cirrhosis (F4) than those with F3 fibrosis. However, the rate of 5-year fibrosis improvement, the final end point of our study, was significantly higher in patients with histological F3 disease than those with cirrhosis.

We monitored the linear improvement of fibrosis using LS values measured by Fibroscan and found that improvement of fibrosis occurred each year during 5 years of ETV treatment. LS measurement was a useful method for monitoring long-term improvement of fibrosis in CHB patients in our study, as this parameter is accurate, noninvasive, and reproducible. Improvement of fibrosis was also assessed by two established serum fibrosis scores (FIB-4 and APRI score) recommended by the World Health Organization CHB guidelines (32,33). These two markers showed high correlation with LS value at baseline, and these markers decreased in

Table 2. Predictors for 5-year fibrosis improvement (LS value <7.2 kPa at year 5) (*n*=116)

	Univariate		Multivariate	
Variables	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**
Age (years)	NS			
Male gender	NS			
Body mass index (kg/m^2)	NS			
Normal/overweight/obese[a]	NS			
Diabetes mellitus	NS			
Lamivudine/entecavir	NS			
Resistance	NS			
Biochemical parameters				
Alanine aminotransferase (IU/l)	NS			
Serum albumin (g/dl)	NS			
Total bilirubin (g/dl)	NS			
Platelet count (10^9/l)	0.022	1.011 (1.002–1.021)	0.302	1.006 (0.995–1.016)
Prothrombin time (INR)	NS			
HBeAg positivity	NS			
HBV DNA (IU/ml)	NS			
Liver histology at baseline				
Fibrosis stage, 4 vs. 3	0.014	0.145 (0.031–0.676)	0.076	0.239 (0.049–1.016)
Activity grade, 3–4 vs. 1–2	NS			
Liver stiffness measurement (kPa)				
Liver stiffness value at baseline	0.001	0.880 (0.816–0.949)	0.014	0.907 (0.838–0.980)
Liver stiffness value at year 1	<0.001	0.776 (0.690–0.872)		
Liver stiffness value at year 2	<0.001	0.683 (0.582–0.800)		
Liver stiffness value at year 3	<0.001	0.581 (0.474–0.712)		
Liver stiffness value at year 4	<0.001	0.511 (0.400–0.653)		
Virological response at year 5	NS			

HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; CI, confidence interval; kPa, kilopascal; NS, not significant; OR, odds ratio.
[a]BMI is classified according to the BMI criteria for Asian by World Health Organization.

a linear fashion, but not significantly every year, during antiviral treatment. Although a recent study (34) reported that APRI and FIB-4 scores do not accurately assess fibrosis during antiviral therapy, that study used LB as a standard, which is limited by sampling error, inter-observer variability, and intra-observer variability (12). To determine the usefulness of LS measurement, FIB-4, and APRI scores, future research is necessary to investigate whether changes in theses markers can predict the development of HCC or cirrhosis-related complications.

In our study, we introduced a new analytic approach of model-based cluster analysis, a method which aims to identify unobserved heterogeneity in a population based on the observed data. Model-based cluster analysis assumes that the distribution is made up of a number of multivariate component distributions, and it utilizes the expectation-maximization algorithm to maximize the likelihood estimation (35). As a result, a model-based cluster analysis finds out a number of explicit groups of objects that are similar to one another within a group but sufficiently different from members of other groups. In our study, we found out, two distinctive subgroups according to LS dynamics in terms of baseline LS value and change of LS over the time. Compared with group 2 patients, group 1 patients had high baseline LS values and responded more quickly to NUC, resulting in steeper LS slopes in all periods; however, the final LS value at year 5 was lower in group 2 patients, who had low baseline LS values, than in group 1 patients. At year 5, more patients in group 2 experienced improvement of fibrosis than in group 1, which is in concordant result with the following multivariate regression analysis showing low baseline LS value was the predictor of 5-year fibrosis improvement.

Viral factors such as HBeAg positivity; HBV DNA level at baseline; and virological response or HBeAg seroconversion rate at year

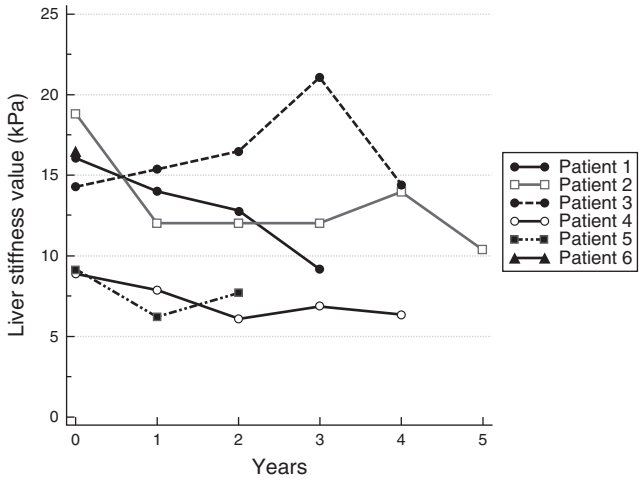

Figure 4. The annual LS values of the six patients with HCC. Among six patients with HCC, three patients (patient 1, 2, and 4) showed decreasing pattern of LS values at the time of HCC development (patient 6 underwent only baseline LS measurement, because the patient developed HCC at 1.4 years after starting antiviral treatment). LS, liver stiffness.

1, 2, 3, or 4 were not predictive factors for 5-year fibrosis improvement. Antiviral factors, such as type of NUC, development of resistance to LAM, or high body mass index, also were not predictive of 5-year fibrosis improvement. Baseline LS value was the only predictor for 5-year fibrosis improvement, and an LS value <12 kPa was the optimal cutoff. In general, guidelines for treatment of CHB recommend starting NUC therapy in patients with liver enzymes two times higher than the UNL and with HBV DNA ≥20,000 IU/ml for HBeAg-positive patients and HBV DNA ≥2,000 IU/ml for HBeAg-negative patients (36–39). However, according to our findings, CHB patients with advanced fibrosis or cirrhosis with normal or slightly elevated liver enzymes (AST or ALT <2×UNL) treated with NUC experienced significant annual improvement of fibrosis. Additionally, patients with low LS values (<12 kPa) treated with NUC are 2.8 times more likely to experience 5-year fibrosis improvement than patients with high LS values (≥12 kPa). Therefore, we should cautiously consider early intervention with NUC therapy in patients with sufficiently high HBV DNA and normal or slightly elevated liver enzymes (AST or ALT <2×UNL) in early stages of advanced fibrosis (LS value <12 kPa).

Theoretically, reversal of fibrosis reduces the chance for developing hepatic decompensation and HCC. However, the cumulative incidence rates of HCC in our study were similar to those in another study with a similar patient cohort without NUC therapy (40), and we found no distinctive LS value pattern for patients with HCC compared to patients without HCC (**Figure 4**). These findings are probably due to the short follow-up period for HCC occurrence and the small number of patients with HCC occurrence. A large, well-designed prospective study by Jung *et al.* (41) reported that LS values can predict future HCC development in CHB patients. Another study by Kim *et al.* (42) showed that CHB patients without clinically evident CHB-related cirrhosis and with a subcirrhotic LS value ≤13 kPa had a lower risk of HCC than those with higher LS values (>13 kPa). LS measurement appeared to accurately estimate the fibrotic burden and predict HCC development. In a study by Lampertico *et al.* (43), after 10 years of NUC therapy, HBeAg-negative patients with compensated cirrhosis experienced significant regression of pre-existing esophageal varices, and had a negligible risk of developing *de novo* esophageal varices, or progression of pre-existing esophageal varices. This finding suggests the necessity of long-term HBV DNA suppression with NUC therapy because improvement of fibrosis following antiviral treatment reduces the chance of developing cirrhosis-related complications. Therefore, LS should be measured annually beyond 5 years to assess the improvement of fibrosis. More importantly, our future research will investigate whether patients with low baseline LS values who achieved low LS values at year 5 have a lower chance of developing HCC and cirrhosis-related complications than patients with high baseline LS values. If future studies confirm this hypothesis, NUC treatment may benefit CHB patients with normal liver enzyme levels who are in early advanced fibrosis stages before progression to cirrhosis.

Our study had several limitations. First, because we did not have paired LB data at year 5, the criterion for improvement of fibrosis was defined using only the LS value. Also of note, several studies have suggested that LS values during antiviral treatment can underdiagnose liver cirrhosis because the macronodular pattern of cirrhosis with thin fibrous septa can estimate low LS value (44). Although we do not have follow-up LB results for patients with 5-year fibrosis improvement (n=62), we investigated the evidence of clinical liver cirrhosis ((i) a platelet count <100,000/ml and ultrasonographic findings suggestive of cirrhosis (a blunted, nodular liver edge accompanied by splenomegaly (>12 cm)) or (ii) esophageal or gastric varices or (iii) evidence of hepatic decompensations) (41) in these patients at year 5. Only one among 62 patients (1.6%) showed ultrasonographic evidence of cirrhosis. Therefore, further research is needed to substantiate our result that the serial LS value can be used to precisely monitor the improvement of fibrosis.

However, to our knowledge, this is the first prospective study to demonstrate significant fibrosis improvement by using Fibroscan to measure annual LS values over 5 years. The LS values in our patient cohort are highly reliable because this study enrolled patients with normal or slightly elevated liver enzymes, which avoids overestimation of liver fibrosis caused by necroinflammation, a strong confounder for assessing fibrosis with Fibroscan. Another strength of our study is that a single highly experienced operator performed LS measurement over 5 years, which prevents the variability of LS values, a major limitation of Fibroscan, resulting from operator dependence (45).

In conclusion, this prospective study showed that annual fibrosis improvement as represented by LS value slows but continues during long-term antiviral treatment in CHB patients with advanced fibrosis. Low LS values (<12.0 kPa) at baseline were a significant predictor for 5-year fibrosis improvement. Future research should focus on investigating the development of cirrhosis-related complications and HCC in these patients who show improvement of fibrosis monitored by Fibroscan. Meanwhile, screening for HCC is imperative in CHB patients regardless of the amount of fibrosis.

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CONFLICT OF INTEREST

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WRITING ASSISTANCE

The English in this document has been checked by at least two professional editors, both native speakers of English, from Bioscience Writers.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Because progression of liver fibrosis to cirrhosis increases the risks of various complications of hepatic decompensation and hepatocellular carcinoma (HCC), suppression of viral replication by using nucleotide analogues (NUC) to reduce the risks has been emphasized.
- ✓ However, the improvement of fibrosis during antiviral treatment has been shown only recently by several studies in which the fibrotic burden was assessed by liver biopsy (LB).
- ✓ Serial long-term data of liver stiffness (LS) regarding improvement of fibrosis during antiviral treatment in chronic hepatitis B (CHB) patients have been limited.

WHAT IS NEW HERE

- ✓ In CHB patients with advanced fibrosis or cirrhosis with normal or slightly elevated liver enzymes (AST or ALT <2×UNL) treated with NUC, the mean LS value significantly decreased during 5 years of antiviral treatment.
- ✓ NUC had the greatest effect on fibrosis improvement at year 1 (mean, −3.2 kPa), and the degree of fibrosis improvement decreased over the subsequent years.
- ✓ Low LS values (<12.0 kPa) at baseline were a significant predictor for 5-year fibrosis improvement.

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