Antiviral Research 168 (2019) 61–67

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Longitudinal effects of Nucleos(t)ide analogue therapy in chronic hepatitis B patients and the utility of non-invasive fibrosis markers during treatment: A single-center experience for up to 17 years

Pallavi Surana[a], Devika Kapuria[a], Carly Broadwell[a], Elizabeth C. Wright[b], Varun Takyar[a], David E. Kleiner[c], Marc G. Ghany[a], Gil Ben-Yakov[a], Theo Heller[a], T. Jake Liang[a], Christopher Koh[a,*]

[a] Liver Diseases Branch, National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health, Bethesda, MD, USA
[b] Office of the Director, National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health, Bethesda, MD, USA
[c] Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

ARTICLE INFO

Keywords:
Hepatitis B
Nucleoside analogues
Non-invasive markers of fibrosis
Fibrosis
Inflammation

ABSTRACT

Background: Fibrosis regression has been associated with nucleoside analogue (NA) treatment in chronic hepatitis B (CHB) patients. Although non-invasive fibrosis markers have been evaluated in CHB, their utility for monitoring on-treatment histologic regression has not been evaluated.

Aims: To characterize improvements in disease severity and the utility of non-invasive biomarkers in CHB NA treated patients.

Methods: Histology, labs, AST-to-platelet ratio index, and Fibrosis-4 (Fib-4) from treatment-naïve CHB patients were evaluated at baseline and longitudinally. Relative change from baseline to various time points during treatment were evaluated. Correlative analysis of APRI and Fib-4 with histology was performed longitudinally.

Results: 80 CHB patients (84% male, median age 45 (IQR 32, 54)) with histology up to 17 years (median 6(IQR 3.9, 8.0)) years were studied. Median baseline Ishak fibrosis was 3 (IQR 2, 4), histologic activity index (HAI) inflammation was 9 (IQR 7, 11), and AUROC of fibrosis markers for detecting cirrhosis (Ishak ≥ 5) was > 0.64. HAI improved at a rate of 54% during year 1 and 37% in year 2, both greater than in the remaining follow-up periods. Within the first year, fibrosis improved by 35%, greater than all other time periods. Non-invasive biomarkers began to correlate with histology beyond 4 years (APRI: 4–6 years: r = 0.33, p = 0.03; ≥6 years: r = 0.41, p = 0.009; Fib-4: ≥6 years: r = 0.35, p = 0.03).

Conclusion: Early dynamic changes in histology occur in CHB patients on NA followed by linear improvements. Non-invasive fibrosis biomarkers do not capture these dynamic changes and may demonstrate clinical utility beyond 4 years of treatment.

1. Introduction

Chronic hepatitis B (HBV) infection affects approximately 250 million people worldwide and accounts for up to 30% of cirrhosis cases and 50% of hepatocellular carcinoma (HCC) cases globally (Trépo et al., 2014). Higher levels of HBV DNA and the development of cirrhosis are related to increased complications (i.e. esophageal varices and HCC), thus, lowering serum HBV DNA levels may decrease the risk of complications (Grossi et al., 2017; Younossi et al., 2018; Chen and Yang, 2011). Chronic HBV patients with advanced fibrosis or cirrhosis receive treatment based on guideline-directed therapies and substantial amounts of healthcare resources are spent on screening and surveillance measures (Terrault et al., 2018). With effective HBV nucleoside analogue (NA) therapies, which directly inhibit the reverse transcriptase of HBV polymerase, sustained viral suppression can be achieved, thereby halting disease progression (Chon et al., 2017; Buti et al., 2015; Marcellin et al., 2003; Tsai et al., 2015).

Regression of fibrosis and cirrhosis is of significant clinical importance given that it impacts patient prognosis and management; namely healthcare utilization through HCC and variceal surveillance

* Corresponding author. Liver Diseases Branch, National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health, 10 Center Drive, Bldg. 10, Room 9B-16, MSC 1800, Bethesda, MD, 20892.
E-mail address: Christopher.koh@nih.gov (C. Koh).

https://doi.org/10.1016/j.antiviral.2019.05.007
Received 25 January 2019; Received in revised form 16 May 2019; Accepted 19 May 2019
Available online 21 May 2019
0166-3542/ Published by Elsevier B.V.

Abbreviations

HBV	hepatitis B virus
CHB	chronic hepatitis B
APRI	AST-to-platelet ratio index
Fib-4	Fibrosis-4
TC	time category
HAI	Histologic Activity Index
HCC	hepatocellular carcinoma
DNA	deoxyribonucleic acid
NIH	National Institutes of Health
BTRIS	NIH Biomedical Translational Research Information System
ALT	alanine aminotransferase
AST	aspartate aminotransferase
TARC	time-adjusted relative change
ROC	receiver operating curve
IQR	interquartile range
IU/mL	international units per milliliter
AUROC	area under the curve
SE	standard error
WHO	World Health Organization

(Tsai et al., 2015; Chang et al., 2010; Marcellin et al., 2013; Xu et al., 2015). Liver histology is a useful guide for management, however, in the current era of HBV management, follow-up liver biopsies are not routinely performed. Additionally, while liver biopsy is the gold standard for clinical staging; it can be both superfluous and lead to procedural complications (Dienstag, 2002; Bravo et al., 2001; Poynard et al., 2004). Previous studies have evaluated long-term effects of nucleoside analogue therapy in HBV, however histologic follow-up has only been described out to 5 years (Marcellin et al., 2003; Dienstag et al., 1999; Ozgenc et al., 2004; Lai et al., 2006). Thus, descriptions and long-term characterizations of histologic (fibrosis and inflammation) and biochemical improvement in patients on nucleoside analogue therapy are incomplete.

Noninvasive fibrosis biomarkers have demonstrated clinical utility as an alternative to liver biopsies, however, the applications have mainly been tested in cross-sectional settings and for progression of disease. The most commonly accepted non-invasive serum biomarkers include the Fibrosis-4 Index (Fib-4) and the aspartate aminotransferase (AST) to Platelet Ratio Index (APRI) which have been mainly validated in hepatitis C with some exploration in HBV (Sterling et al., 2006; Wai et al., 2003; Branchi et al., 2014; Castera, 2014). In HBV, there are conflicting reports on the ability of APRI and Fib-4 to adequately predict fibrosis in patients with chronic HBV prior to treatment (Başar et al., 2013; Ray Kim et al., 2016). One meta-analysis found that while Fib-4 can detect significant fibrosis and cirrhosis in HBV, it was suboptimal for excluding fibrosis and cirrhosis (Li et al., 2014). Other studies (Chon et al., 2017; Ray Kim et al., 2016) have described that noninvasive fibrosis markers do not appear to be accurate indicators of fibrosis regression in HBV studies performed for up to five years (Chon et al., 2017; Ray Kim et al., 2016). Additionally, these studies have not evaluated how these non-invasive biomarkers correlate with biopsy proven fibrosis and inflammation over successive, shorter intervals of time.

Despite the controversial evidence and inconclusive study of the utility of serum noninvasive fibrosis biomarkers in HBV, there is a clear clinical need for understanding its applicability as it can potentially simplify long term follow-up and reduce healthcare costs. Based on previously reported data, a majority of patients treated with nucleoside analogues may experience fibrosis regression upon treatment, and no longer require intensive follow-ups (Younossi et al., 2018).. In this longitudinal study, we aimed to characterize the rate of histologic fibrosis regression in a cohort of CHB patients treated with NAs with follow-up for up to 17 years. We also explored the utility of cross-sectional noninvasive fibrosis markers as longitudinal biomarkers to predict fibrosis regression.

2. Methods

2.1. Data collection

This retrospective, longitudinal study was performed at the National Institutes of Health Clinical Center between 1988 and 2017. Patients were enrolled in clinical research studies approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board pertaining to the natural history of liver disease and CHB NA therapeutic studies (NCT: 00001971, 00023309, 00120354, 00001457). Data were collected using the NIH Biomedical Translational Research Information System (BTRIS). The analysis included demographic, laboratory as well pathologic data from adults with CHB mono-infection who underwent a baseline liver biopsy (pretreatment) and were then initiated on a variety of NAs, including lamivudine, adefovir, and tenofovir. Several patients were on more than one NA over their treatment period, due to reasons such as development of viral resistance and per protocol design. All patients had at least one additional biopsy after the baseline biopsy and most patients had multiple biopsies during the follow-up period. Patients with additional etiologies of liver disease were excluded from the study (i.e. HCV, HIV, alcohol abuse, and autoimmune diseases). Additional details about inclusion and exclusion criteria are included in the Supplementary Methods. The unique advantage of this cohort is the availability of data, including liver biopsies as well as longitudinal laboratory results which enabled us to characterize disease phenotype.

2.2. Liver histopathology and non-invasive biomarkers

An independent pathologist (DEK) reviewed all liver biopsies. Fibrosis was staged according to the modified Ishak histological activity index, ranging from 0 to 6, and inflammation was graded according to the Knodell score (Histological activity index, HAI) ranging from 0 to 18 (Ishak et al., 1995; Knodell et al., 1981). Laboratory values within 6 months of the biopsy were collected for all patients and used to calculate APRI and FIB-4 scores. Further explanation pertaining to methodology is in the Supplementary Methods.

2.3. Time category designation

Five time-categories were assigned to follow-up biopsies based on time in years from baseline to the biopsy. Time-categories were established to create standardized time points of measurements. No patients had multiple biopsies within the same time-category. There were comparable numbers of biopsies within each time category. Time-categories are defined as follows:

Time Category 1 (TC1): 0–1 years after baseline biopsy
Time Category 2 (TC2): 1–2 years after baseline biopsy
Time Category 3 (TC3): 2–4 years after baseline biopsy
Time Category 4 (TC4): 4–6 years after baseline biopsy
Time Category 5: (TC5): 6 or more years after baseline biopsy

2.4. Time-adjusted relative rate of change (TARC) calculation

The time-adjusted relative rate of change (TARC) was calculated to measure the relative rate of change per year within each time category. TARC was calculated for histologic measurements, lab values, and

noninvasive biomarkers for each patient using the following formula:

$$TARC = \frac{\frac{(follow\ up\ biopsy\ value - baseline\ value)}{baseline\ value}}{Years\ between\ baseline\ and\ follow\ up\ biopsy}$$

2.5. Statistical methods

Receiver operating curves (ROC) were calculated for Fib-4 and APRI at baseline to assess their ability to identify cirrhosis (Ishak ≥ 5) in treatment naïve CHB patients. Sensitivity, specificity, positive predictive value and negative predicted value were also calculated using validated cut-offs for Fib-4 and APRI. TARC values for each biomarker were first calculated at the patient level and then a mixed model was used to calculate least square means and standard errors for each biomarker within each time category. A repeated measures mixed model accounting for the respective baseline values was used to do pairwise comparisons of the least squares means across time categories. Spearman's correlations were calculated to compare TARC values of histology and non-invasive markers within the same time-category. A p-value < 0.05 indicated significance. All statistical analysis was performed using SAS 9.4 (Cary, NC).

3. Results

3.1. Patient characteristics

Eighty CHB patients with longitudinal histologic follow-up up to 17 years (median 6 years (IQR 3.9, 8.0)) were studied (Table 1). At the time of the initial liver biopsy, 84% were male and the median age was 45 years (IQR 32, 54). 56 (70%) patients were e-antigen negative, median fibrosis by Ishak was 3 (IQR 2, 4) and median inflammation by histologic activity index (HAI) was 9.0 (IQR 7, 11). Median baseline HBV DNA counts were 7.03 log IU/mL (IQR: 5.6, 8.1) and by the end of the study period this was reduced to a median of 2.9 log IU/mL (IQR: 1.4, 3.4). Median ALT and AST values were elevated at baseline (89 IU/mL, 56 IU/mL respectively). At baseline, median platelet counts (182 IU/mL) and median albumin levels (4.0 IU/mL) were within normal limits. Patients were treated for a median of 4 years (IQR 3.7, 7.5). During the study period. At baseline, patients were treated with several different nucleoside analogues: lamivudine (64%), adefovir (20%) and tenofovir (16%). Of the 51 patients started on Lamivudine therapy at the start of the study, 20 (39%) developed lamivudine resistance during the study period.

The number of biopsies each patient had ranged from 2 to 5. 16 patients (20%) had just 2 biopsies, 33 patients (41%) had three biopsies, 28 patients (35%) had 4 biopsies and, 3 patients (4%) had 5 biopsies. Mean time between the baseline and second biopsy was 2.06 years (2.30). 41 patients (51%) had a biopsy 6 or more years after the start of treatment. During follow-up, four patients developed HCC and two patients died due to causes unrelated to liver diseases (Supplemental Table 1).

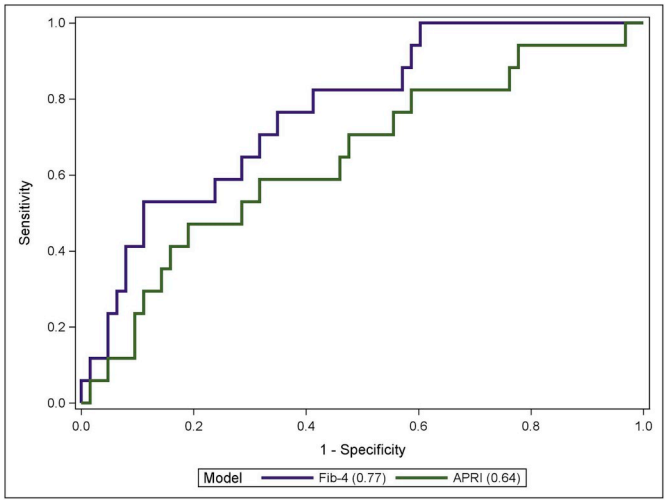


Fig. 1. Receiver Operating Characteristic Curves for Baseline Performance of APRI and Fib-4. Both Fib-4 and APRI perform adequately at baseline for detecting cirrhosis (Ishak ≥ 5). Areas under the curve are indicated in parenthesis in the figure legend.

APRI and Fib-4 performance was tested at baseline (treatment naïve samples) and was found to perform adequately in detecting cirrhosis (Ishak ≥ 5). At baseline, Fib-4 demonstrated better performance characteristics than APRI (AUROC of 0.77 vs 0.64) (Fig. 1). Although APRI demonstrated slightly a higher specificity (87.2 vs 82.9), Fib-4 had demonstrated a largely superior sensitivity (70.0 vs 36.4). Taken together, Fib-4 had a more balanced performance.

3.2. Histologic changes during therapy

Mean Ishak fibrosis and HAI inflammation scores at baseline and within subsequent time categories show an overall improvement in histology after the start of treatment with nucleoside analogues (Fig. 2).

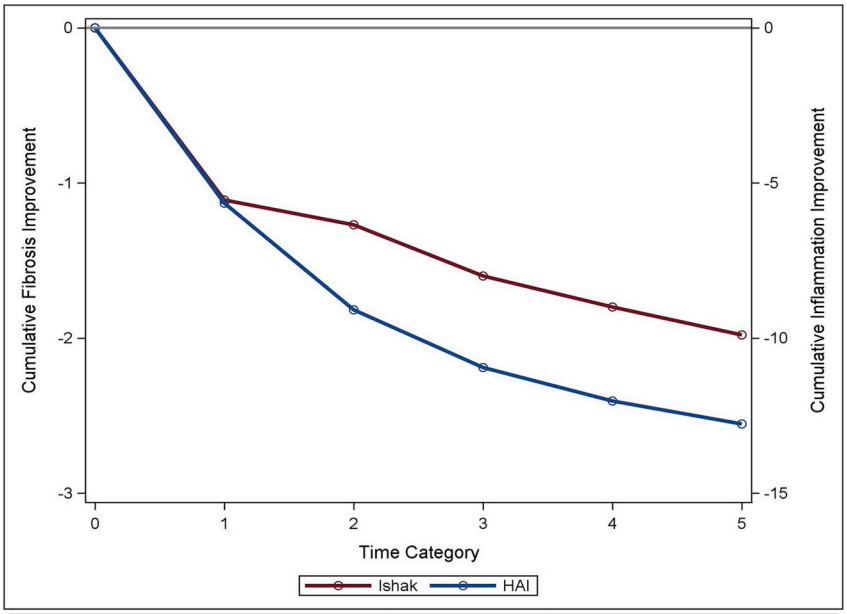
3.2.1. Fibrosis

In the first year after of treatment, Ishak fibrosis score decreased from baseline by an average of 1.1 (SE: 0.15) Ishak unit. The greatest decline in Ishak fibrosis score occurred in time category 1, however, Ishak fibrosis continued to decrease during the follow up period, albeit at different rates (Fig. 2). The improvement in Ishak fibrosis in the first year of treatment was significantly greater ($p < 0.0001$) than improvement in all other time categories (Supplemental Table 2). Though improvement in later time categories was not significantly different from each other, the average decline in Ishak units does appear to

Table 1
Baseline and follow-up characteristics of the chronic hepatitis B patients treated with nucleoside analogues.

Median (IQR)	Baseline (n = 81)	TC1 (n = 21)	TC2 (n = 38)	TC3 (n = 38)	TC4 (n = 41)	TC5 (n = 41)
Male/Female (%)	83/17	85/15	85/15	78/22	85/15	88/12
Age (years)	45.4 (32.4, 53.7)	43.2 (33.7, 50.1)	47.4 (33.9, 58.5)	46.8 (36.2, 57.6)	52.1 (36.4, 60.8)	53.6 (41.5, 62.0)
ALT (IU/L)	88.0 (55, 147)	29.0 (24.0, 40.0)	35.0 (26.0, 51.0)	29.5 (22.0, 54.0)	36.0 (24.0, 55.0)	34.0 (25.0, 45.0)
AST (IU/L)	56.0 (39.0, 90.0)	27.0 (25.0, 34.0)	29.5 (23.0, 41.0)	26.0 (22.0, 39.0)	30.0 (23.0, 39.0)	28.0 (22.0, 36.0)
Albumin (g/dL)	4.0 (3.70, 4.10)	4.0 (3.75, 4.30)	4.0 (3.80, 4.20)	4.10 (3.90, 4.20)	4.0 (3.70, 4.30)	3.90 (3.70, 4.20)
Alk Phos (IU/L)	74.5 (63.5, 97.0)	66.0 (61.0, 77.0)	69.0 (53.0, 84.0)	70.5 (56.0, 77.0)	75.0 (60.0, 90.0)	79.5 (66.5, 99.0)
Tbili (mg/dL)	0.80 (0.60, 1.0)	0.7 (0.50, 1.40)	0.80 (0.60, 1.10)	0.80 (0.60, 1.00)	0.70 (0.60, 0.90)	0.70 (0.50, 1.10)
Platelets (K/uL)	182.0 (156, 205)	170.0 (156, 191)	185.5 (147, 211)	182.0 (156, 211)	168.0 (143, 215)	172.0 (138, 196)
HBV DNA (log IU/mL)	7.0 (5.6, 8.1)	5.7 (4.3, 6.5)	3.5 (1.7, 5.0)	2.3 (1.4, 7.5)	4.3 (1.4, 7.5)	2.9 (1.4, 3.4)
Ishak	3.0 (2.0, 4.0)	3.0 (1.0, 4.0)	3.0 (1.0, 4.0)	1.0 (0.0, 3.0)	2.0 (0.0, 4.0)	1.0 (0.0, 3.0)
HAI	9.0 (7.0, 11.0)	3.0 (3.0, 5.0)	5.0 (3.0, 7.0)	2.0 (1.0, 3.0)	3.0 (1.0, 6.0)	3.0 (1.0, 5.0)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Alk Phos, alkaline phosphatase; Tbili, total bilirubin; HAI, Histologic Activity Index.



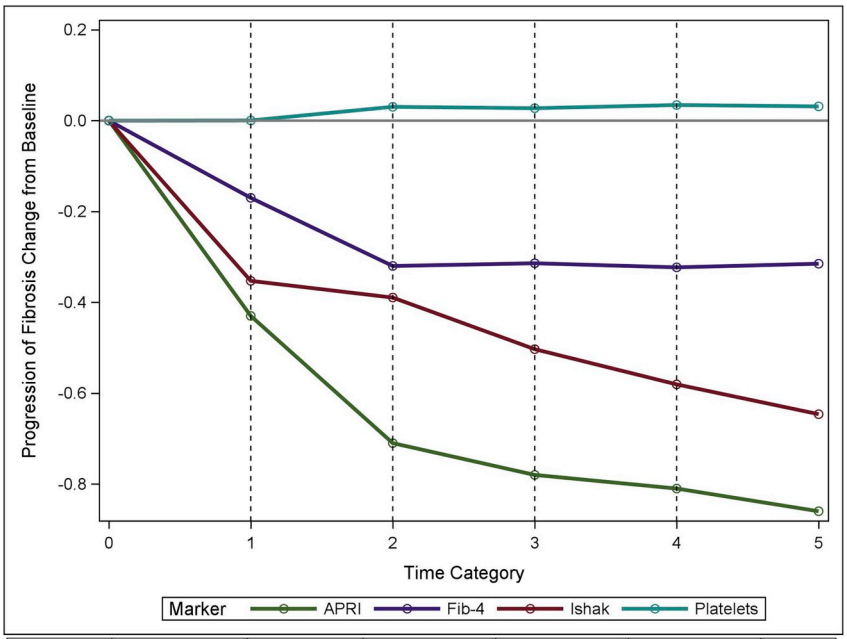
	TC1	TC2	TC3	TC4	TC5
Ishak	-1.11(0.15)	-0.16 (0.11)	-0.33 (0.11)	-0.20 (0.11)	-0.18 (0.11)
HAI	-5.65 (0.36)	-3.44 (0.26)	-1.86 (0.26)	-1.08 (0.25)	-0.74 (0.25)

*Mean (SE)

Fig. 2. Cumulative Rate of Histologic Change from Baseline. Data points demonstrate the cumulative changes from time-category 1 to time-category 5. Slopes (shown in table) between data points indicate adjusted means of absolute changes and are in Ishak units of change or HAI units of change where appropriate. Fibrosis (red line) dramatically improves during the first year of treatment and becomes more linear from year 2 onwards. Improvements in inflammation (blue line) are more dynamic during the first two years of treatment followed by a more linear pattern in the remaining years.

decrease (TC2 = −0.16, TC3 = −0.33, TC4 = −0.20, TC5 = −0.18). By time category 5, the average rate of Ishak fibrosis decline was 0.18 Ishak units per year from baseline. Additionally, an analysis comparing those who regressed histologically in the first two time-categories did not reveal any significant factors associated with regression.

Because absolute declines cannot be directly compared, we calculated and compared relative changes. When looking at relative declines, or TARC, Ishak fibrosis improved by 35% from baseline in the first year (Fig. 3). Relative Ishak fibrosis decline from baseline in the first year was significantly greater when compared to TARCs in subsequent years of follow up (TC2 = 4%, TC3 = 11%, TC4 = 8%, TC5 = 7%, $p < 0.001$) (Fig. 3, Supplemental Table 3). These results



Marker	TC1	TC2	TC3	TC4	TC5	P-value
Ishak	-0.35 (0.07)	-0.04 (0.05)	-0.11 (0.05)	-0.08 (0.05)	-0.07 (0.05)	SIG
Platelets	0.0004 (0.02)	0.03 (0.01)	-0.003 (0.01)	0.007 (0.01)	-0.003 (0.01)	NS
Fib-4	-0.17 (0.04)	-0.15 (0.03)	0.006 (0.03)	-0.009 (0.03)	0.008 (0.03)	SIG
APRI	-0.43 (0.08)	-0.28 (0.06)	-0.07 (0.06)	-0.03 (0.06)	-0.05 (0.06)	SIG

*Mean(SE)

Fig. 3. Cumulative Time-adjusted Rate of Fibrosis Changes by Ishak and Non-invasive Markers. Data points demonstrate the cumulative time-adjusted changes from time-category 1 to time-category 5. Slopes (shown in table) between data points indicate time-adjusted means of relative changes (TARC values). “SIG” and “NS” indicate the p-value of a repeated measures mixed model. “SIG” indicates that a difference did exist among the time categories and “NS” indicates that there was no difference. Supplemental Table 3 indicates which pairwise comparisons of time categories were significantly different. While Ishak (red line) shows dynamic time-adjusted improvement in the first year of treatment, similar to APRI and Fib-4, the rates of change are not correlated. APRI and Fib-4 show greater change during both time-categories 1 (0–1 years of treatment) and 2 (1–2 years of treatment).

indicate that the greatest improvement in fibrosis occurs in the first year after the start of treatment with nucleoside analogues. On average the improvement in fibrosis continues, but at a slower rate. These findings mirror those of the absolute changes in Ishak fibrosis.

3.2.2. Inflammation

Similar to what was demonstrated with fibrosis, total HAI inflammation decreased the most during time category 1. The average HAI score decline was 5.7 units (SE: 0.36) (Periportal: 2.5(0.18), Lobular: 1.8(0.15), Portal 1.3(0.09)) in the first year of treatment (Fig. 2). Like the pattern seen in Ishak fibrosis decline, the decline in HAI inflammation was significantly greater in the first year after the start of treatment when compared to all other time categories ($p < 0.0001$) (Supplemental Table 4). Unlike in fibrosis, significant decline in HAI inflammation continued in the second year after the start of treatment (TC2 = −3.4 units) and this decline was significantly greater than the decline seen in subsequent time categories ($p < 0.0001$) (Supplemental Table 4). While the average trends in each time category show a decline in HAI inflammation, the rates of decline slow overtime. By time category 5 (6 or more years from baseline biopsy) the mean decline from baseline was 0.74 (SE: 0.25) HAI inflammation units per year.

HAI inflammation improved with a TARC of 54% per year in the first year (TC1) and 37% in the second year (TC2) (Fig. 4). Each of these rates was significantly greater than rates in the remaining time categories (TC3 = 19%, TC4 = 12%, TC5 = 8%, $p < 0.0001$) (Supplemental Table 4). Additionally, TC1 and TC2 differed significantly from each other ($p = 0.0006$). These relative rates of change show a similar pattern as the absolute rates of change. Periportal inflammation improved by 57% in time category 1 and 37% in time category 2, with improvement slowing in later time categories (TC3 = 18%, TC4 = 15%, TC5 = 8%, $p < 0.0001$) (Supplemental Table 4). Relative change in periportal inflammation was significantly greater in the first two years compared to all later time categories. Lobular and portal inflammation TARC followed a similar pattern with significantly greater relative declines in the first two years of treatment compared to later years ($p < 0.0001$ for both) (Supplemental Table 4). Compared to periportal and portal inflammation (TC1 = 54%), lobular inflammation had the slowest rate of change in the first year (TC1 = 47%). In subsequent years the rates of change were comparable (Lobular: TC2 = 32%, TC3 = 17%, TC4 = 8%, TC5 = 6% Portal: TC2 = 31%, TC3 = 19%, TC4 = 11%, TC5 = 6%).

3.3. Laboratory values and non-invasive markers

Changes in laboratory values such as platelet counts, ALT, and AST were evaluated. ALT and AST values show a general trend of decline after the start of treatment. In TC1 the relative decline of both ALT and AST is 43%. TARC values tend to decrease in later time categories for both liver enzymes (ALT TC2: 27%, AST TC2: 29%); however, these values are not significantly different than TC1 TARC values ($p > 0.10$) (Fig. 4). Interestingly, patients who normalized ALT (≤41 IU/mL) in the first two time categories also had significantly increased HAI regression in the first two time categories compared to patients who did not normalize ALT. A mixed model to compare the TARC values across time categories showed that TC1 and TC2 are significantly greater than in subsequent time categories (Supplementary Table 4).

The time-adjusted relative rate of changes of platelet counts remained relatively stable throughout all time categories. In time category 1, platelet counts improved at a rate of 0.04%. However, in time category 2, platelet counts improved at a rate of 3%, with leveling off in later time categories. In time category 3 and onwards, platelets change by negligible amounts (TC3 = − 0.3%, TC4 = 0.7%, TC5 = − 0.3%). The calculated average TARCs for platelets at each time category were

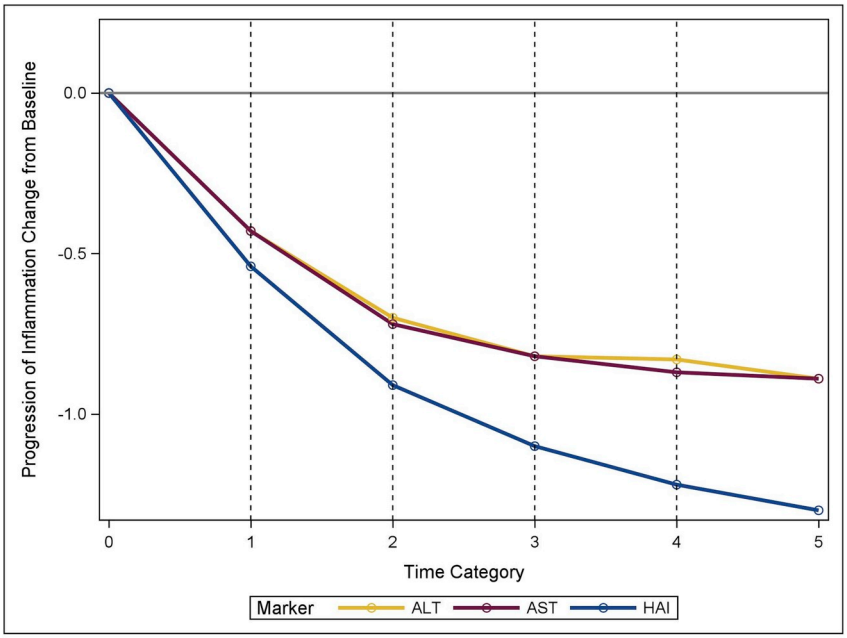


Fig. 4. Cumulative Time-adjusted rate of Inflammation Changes by Histologic Activity Index, ALT, and AST. Data points demonstrate the cumulative time-adjusted changes from time-category 1 to time-category 5. Slopes (shown in table) between data points indicate time-adjusted means of relative changes (TARC values). "SIG" and "NS" indicate the p-value of a repeated measures mixed model. "SIG" indicates that a difference did exist among the time categories and "NS" indicates that there was no difference. Supplemental Table 4 indicates which pairwise comparisons of time categories were significantly different. ALT and AST appear to mirror improvements in HAI during the first two years of treatment. While ALT and AST continue to decrease in later years, they do not decrease as dramatically as HAI scores.

Marker	TC1	TC2	TC3	TC4	TC5	P-value
Total HAI	-0.54(0.04)	-0.37 (0.03)	-0.19 (0.03)	-0.12 (0.03)	-0.08 (0.03)	SIG
Periportal	-0.57 (0.06)	-0.37 (0.04)	-0.18 (0.04)	-0.15 (0.04)	-0.08 (0.04)	SIG
Lobular	-0.47 (0.05)	-0.32 (0.03)	-0.17 (0.03)	-0.08 (0.03)	-0.06 (0.03)	SIG
Portal	-0.54 (0.05)	-0.31 (0.04)	-0.19 (0.04)	-0.11 (0.04)	-0.07 (0.04)	SIG
ALT	-0.43 (0.11)	-0.27 (0.08)	-0.12 (0.08)	-0.01 (0.08)	-0.06 (0.08)	SIG
AST	-0.43 (0.08)	-0.29 (0.06)	-0.10 (0.06)	-0.05 (0.05)	-0.05 (0.05)	SIG

*Mean(SE)

Table 2
Spearman correlations between Ishak fibrosis and non-invasive markers of fibrosis.

Marker	TC1	TC2	TC3	TC4	TC5
Platelets	0.23	0.02	−0.04	−0.10	−0.30*
Fib-4	−0.01	0.17	0.05	0.16	0.35**
APRI	0.20	0.19	−0.04	0.33**	0.41**
ALT	0.34	0.23	0.05	0.40**	0.37**
AST	0.22	0.24	0.10	0.32**	0.39**

*p < 0.10 **p < 0.05.

not significantly different from one another (Fig. 3).

Fib-4 and APRI demonstrated the greatest relative declines, 17% and 43% respectively, in time category 1 (Fig. 3). In time category 2, Fib-4 has a TARC of −15% and APRI has a TARC of −0.28%. TARC values in time categories 3, 4, and 5 indicate similarly slow relative changes from baseline. Changes in time category 1 were significantly greater than changes in time category 3 onwards for both APRI and Fib-4 (Supplementary Table 3).

3.4. Comparing changes in non-invasive markers and histology

Spearman's correlations between the TARCs of APRI, Fib-4, and platelets with Ishak fibrosis demonstrated no correlation in time categories 1, 2, and 3 (Table 2). However, at time category 4 the TARCs of Ishak and APRI began to show some significant correlation ($r = 0.33$, $p = 0.04$). At time category 5 the TARC values of both APRI and Fib-4 correlated with Ishak fibrosis TARC (APRI: $r = 0.41$, $p = 0.009$; Fib-4 $r = 0.35$, $p = 0.03$). TARC in platelets and Ishak fibrosis trended towards correlation at 6 years of follow-up (TC5 $r = -0.30$, $p = 0.06$).

In general, ALT and AST improvement correlated strongly with HAI throughout treatment and was strongest in the first year (ALT $r = 0.61$, $p = 0.001$; AST $r = 0.68$, $p < 0.0001$) (Table 3). Table 3 shows that changes in ALT and AST had weaker R values in time category 2 and time category 3; but, the R values began the strengthen again in time category 4 onwards. By time category five ALT correlates with HAI with an R of 0.58 and AST correlates with an R of 0.60. Ishak fibrosis TARC also correlated with ALT and AST TARCs; however, the TARC values did not begin to correlate until several years after the start of treatment (Table 2). Ishak fibrosis and AST TARC began to correlate in time category 4 ($r = 0.32$, $p = 0.04$) and continued to correlate in time category 5 ($r = 0.39$, $p = 0.02$). Ishak fibrosis and ALT TARC followed a similar pattern of correlation (TC4: $r = 0.40$, $p = 0.01$; TC5: $r = 0.37$, $p = 0.02$).

4. Discussion

In this longitudinal study of 81 chronic hepatitis B patients treated with long-term nucleoside analogue therapy for up to 17 years, we characterized the evolution of changes in histology and biochemistry after the start of therapy along with understanding the clinical utility of non-invasive fibrosis markers when used longitudinally to monitor on-therapy HBV patients. As expected, the most dramatic improvements in histologic inflammation occurs within the first year which is mirrored by significant improvements in ALT and AST during the same timeframe and histologic inflammation normalizes by about four years of treatment. Interestingly, fibrosis regression does not appear to be linear, where the most dramatic improvements occur during the first year, followed by subtle linear improvement from two years onwards. Additionally, platelet counts do not appear to mirror the improvements seen in histologic fibrosis. While non-invasive fibrosis biomarkers correlate with pre-treatment histology, once therapy is initiated, its clinical utility becomes limited due to a lack of correlation with histology until after 4 years of therapy.

Table 3
Spearman Correlations between HAI inflammation and Non-invasive Markers of Inflammation.

Marker	TC1	TC2	TC3	TC4	TC5
ALT	0.61**	0.47**	0.30*	0.61**	0.58**
AST	0.68**	0.42**	0.37**	0.64**	0.60**

*p < 0.10 **p < 0.05.

The early improvements in histologic fibrosis seen in our study have been described in the early seminal studies evaluating the utility of nucleoside analogue therapy for chronic HBV (Grossi et al., 2017; Marcellin et al., 2013;). However, these studies reported single follow-up biopsies on patients on therapy only out to two years (Shin et al., 2015). In this study of patients with sequential biopsies of patients out to 17 years, we identified that the most dramatic improvement in fibrosis regression likely occurs during the first year of treatment. Thereafter, while there appears to be a continued improvement in fibrosis, this improvement appears to be less dramatic. To date, several other studies describe “long-term” histologic changes with nucleoside analogue treatment, however the maximum follow-up time of these studies is five years (Dienstag et al., 2003; Marcellin et al., 2013). These studies also demonstrate early improvement in histologic inflammation and fibrosis. A plausible explanation for this finding is that early dynamic improvement in hepatic fibrosis is reflective of the dynamic and complex process of fibrogenesis. Wanless et al. observed that cirrhosis regression favors the most recently formed sub septae and older septae may resist regression (Wanless et al., 2000). Matrix cross-linking may further contribute to the inability to achieve complete fibrosis regression and the limited improvement observed in alter time categories may indicative of fibrotic features that resist fibrosis degradation (Iredale et al., 2013).

In addition to the cross-sectional evaluation of noninvasive fibrosis markers, we also evaluated the ability of these markers to predict histology longitudinally for a period of 17 years. While APRI and FIB-4 performance at baseline (prior to treatment) is similar to what has been described by others, longitudinal correlatives with histologic fibrosis did not recur until time category 4 (4–6 years after start of treatment), at which point only APRI weakly correlated with fibrosis (Kim et al., 2010; Yuen et al., 2015). FIB-4 begins to correlate with changes in histology at a later timepoint, 6 years after the start of treatment. It is worth noting that in Fig. 2, APRI appears to closely follow Ishak fibrosis at time category 1 and Fib-4 appears to closely follow Ishak fibrosis at time category 2; however, this visual trend is not seen in the Spearman R values (Table 2). The Spearman rank correlation coefficients are relatively weak; however, this could be a result of the smaller sample size with biopsies extending beyond 4 years. The significant correlation during later years is likely because a large component of these markers relies on AST which is influenced by hepatocellular inflammation. Normalization of AST and ALT during later time-categories may account for the increased correlation between non-invasive markers of fibrosis and histologic change. APRI's stronger correlation with changes in histology provides additional evidence to previous studies that APRI may be an effective non-invasive method monitoring fibrosis in patients treated with nucleoside analogues; however, these studies have shorter periods of follow-up and lack sequential biopsies (Peng et al., 2016; Stasi et al., 2017).

A more systematic prospective evaluation of this may be able recapitulate and further elucidate this finding beyond a period of 4 years, which would provide substantial clinical utility given the prevalence of long-term nucleoside analogue therapy in CHB patients and the absence of good clinical biomarkers describing fibrosis regression. Additionally, such a took would enable re-allocation of precious resources as well as save patients from unnecessary testing and healthcare related expenditure in resource limited settings.

This study has several limitations. First, the findings in this study

need to be seen within the limitation of a retrospective study. Second, while all subjects had at least one pre- and post-treatment biopsy, not all subjects underwent liver biopsies in each of the defined time categories. Thirdly, not all patients were maintained on the same nucleoside analogue therapy during the duration of the study, due to the development of on-treatment viral resistance or protocol (Supplemental Table 1). Finally, while significant correlations were identified with histology and non-invasive fibrosis markers starting at 4 years after starting therapy, this needs to be confirmed in another cohort of nucleoside analogue treated chronic HBV patients.

In conclusion, long-term nucleoside analogue therapy in patients infected with chronic hepatitis B leads to dynamic histologic improvement within the first four years of therapy. Histologic improvement continues in later years; however, the rate of improvement appears to be linear. Commonly utilized non-invasive fibrosis biomarkers demonstrate clinical utility in the pre-treatment period, however, their performance after the initiation of nucleoside analogue therapy for up to 4 years does not appear to accurately reflect histologic changes. Beyond 4 years of therapy, non-invasive fibrosis markers appear to become more accurate in identifying fibrosis regression, however further prospective evaluation is warranted.

Conflicts of interest

None of the authors has financial interests or conflicts of interest related to this research.

Financial Support

This research was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.05.007.

Table 1 displays the median (interquartile range) of the indicated parameters at baseline and at the later time categories.

Table 2 indicates the Spearman R between TARC values for Ishak fibrosis and the indicated marker of fibrosis. The asterixis indicates whether the spearman correlation was significant of trending towards significance.

Table 3 indicates the Spearman R between TARC values for HAI and the indicated marker of inflammation. The asterixis indicates whether the spearman correlation was significant of trending towards significance.

References

Başar, Ö., Yimaz, B., Ekiz, F., et al., 2013. Non-invasive tests in prediction of liver fibrosis in chronic hepatitis B and comparison with post-antiviral treatment results. Clin. Res. Hepatol. Gastroenterol. 37, 152–158.
Branchi, F., Conti, C.B., Baccarin, A., et al., 2014. Non-invasive assessment of liver fibrosis in chronic hepatitis B. World J. Gastroenterol. 20, 14568–14580.
Bravo, A.A., Sheth, S.G., Chopra, S., 2001. Liver biopsy. N. Engl. J. Med. 344, 495–500.
Buti, M., Fung, S., Gane, E., et al., 2015. Long-term clinical outcomes in cirrhotic chronic hepatitis B patients treated with tenofovir disoproxil fumarate for up to 5 years. Hepatol. Int.l 9, 243–250.
Castera, L., 2014. Hepatitis B: are non-invasive markers of liver fibrosis reliable? Liver Int. 34 (Suppl. 1), 91–96.
Chang, C., Martin, P., Fotiadu, A., et al., 2010. A patient with chronic hepatitis B and regression of fibrosis during treatment. Semin. Liver Dis. 30, 296–301.
Chen, C.J., Yang, H.I., 2011. Natural history of chronic hepatitis B REVEALed. J. Gastroenterol. Hepatol. 26, 628–638.
Chon, Y.E., Park, J.Y., Myoung, S.-M., et al., 2017. Improvement of liver fibrosis after long-term antiviral therapy assessed by fibroscan in chronic hepatitis B patients with advanced fibrosis. Am. J. Gastroenterol. 1–10.
Dienstag, J.L., 2002. The role of liver biopsy in chronic hepatitis C. Hepatology 36, S152–S160.
Dienstag, J.L., Schiff, E.R., Wright, T.L., et al., 1999. Lamivudine as initial treatment for chronic hepatitis B in the United States. N. Engl. J. Med. 341, 1256–1263.
Dienstag, J.L., Goldin, R.D., Heathcote, E.J., et al., 2003. Histological outcome during long-term lamivudine therapy. Gastroenterology 124, 105–117.
Grossi, G., Viganò, M., Loglio, A., et al., 2017. Hepatitis B virus long-term impact of antiviral therapy nucleot(s)ide analogues (NUCs). Liver Int. 37 (Suppl. 1), 45–51.
Iredale, J.P., Thompson, A., Henderson, N.C., 2013. Extracellular matrix degradation in liver fibrosis: biochemistry and regulation. Biochim. Biophys. Acta 1832, 876–883.
Ishak, K., Baptista, A., Bianchi, L., et al., 1995. Histological grading and staging of chronic hepatitis. J. Hepatol. 22, 696–699.
Kim, B.K., Kim, D.Y., Park, J.Y., et al., 2010. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. Liver Int. 30, 546–553.
Knodell, R.G., Ishak, K.G., Black, W.C., et al., 1981. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1, 431–435.
Lai, C.L., Shouval, D., Lok, A.S., et al., 2006. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N. Engl. J. Med. 354, 1011–1020.
Li, Y., Chen, Y., Zhao, Y., 2014. The diagnostic value of the FIB-4 index for staging hepatitis B-related fibrosis: a meta-analysis. PLoS One 9, e105728.
Marcellin, P., Chang, T.T., Lim, S.G., et al., 2003. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N. Engl. J. Med. 348, 808–816.
Marcellin, P., Gane, E., Buti, M., et al., 2013. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 381, 468–475.
Ozgenc, F., Arikan, C., Sertoz, R.Y., et al., 2004. Effect of long-term lamivudine in chronic hepatitis B virus-infected children. Antivir. Ther. 9, 729–732.
Peng, J., Cai, S., Yu, T., et al., 2016. Aspartate aminotransferase to platelet ratio index - a reliable predictor of therapeutic efficacy and improvement of Ishak score in chronic hepatitis B patients treated with nucleoside analogues. Scand. J. Clin. Lab. Invest. 76, 133–142.
Poynard, T., Imbert-Bismut, F., Munteanu, M., et al., 2004. Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. Comp. Hepatol. 3, 8.
Ray Kim, W., Berg, T., Asselah, T., et al., 2016. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. J. Hepatol. 64, 773–780.
Shin, S.K., Kim, J.H., Park, H., et al., 2015. Improvement of liver function and non-invasive fibrosis markers in hepatitis B virus-associated cirrhosis: 2years of entecavir treatment. J. Gastroenterol. Hepatol. 30, 1775–1781.
Stasi, C., Salomoni, E., Arena, U., et al., 2017. Non-invasive assessment of liver fibrosis in patients with HBV-related chronic liver disease undergoing antiviral treatment: a preliminary study. Eur. J. Pharmacol. 806, 105–109.
Sterling, R.K., Lissen, E., Clumeck, N., et al., 2006. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 43, 1317–1325.
Terrault, N.A., Lok, A.S.F., McMahon, B.J., et al., 2018. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 67, 1560–1599.
Trépo, C., Chan, H.L.Y., Lok, A., 2014. Hepatitis B virus infection. The Lancet 384, 2053–2063.
Tsai, N.C., Marcellin, P., Buti, M., et al., 2015. Viral suppression and cirrhosis regression with tenofovir disoproxil fumarate in Asians with chronic hepatitis B. Dig. Dis. Sci. 60, 260–268.
Wai, C.T., Greenson, J.K., Fontana, R.J., et al., 2003. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 38, 518–526.
Wanless, I.R., Nakashima, E., Sherman, M., 2000. Regression of human cirrhosis. Morphologic features and the genesis of incomplete septal cirrhosis. Arch. Pathol. Lab Med. 124, 1599–1607.
Xu, B., Lin, L., Xu, G., et al., 2015. Long-term lamivudine treatment achieves regression of advanced liver fibrosis/cirrhosis in patients with chronic hepatitis B. J. Gastroenterol. Hepatol. 30, 372–378.
Younossi, Z.M., Ttepanova, M., Younossi, I., et al., 2018 Sep 27. Long-term effects of treatment for chronic HBV infection on patient-reported outcomes. Clin. Gastroenterol. Hepatol. https://doi.org/10.1016/j.cgh.2018.09.041. pii: S1542-3565(18)31073-5. [Epub ahead of print].
Yuen, M.F., Ahn, S.H., Lee, K.S., et al., 2015. Two-year treatment outcome of chronic hepatitis B infection treated with besifovir vs. entecavir: results from a multicentre study. J. Hepatol. 62, 526–532.