Histological responses of peginterferon alpha add-on therapy in patients with chronic hepatitis B with advanced liver fibrosis after long-term nucleos(t)ide analog treatment

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Abstract

Although long-term antiviral treatment with nucleos(t)ide analogs (NAs) can lead to histological improvement in patients with chronic hepatitis B (CHB), a substantial proportion of patients still fail to achieve regression of fibrosis. Here, we investigated whether peginterferon alpha (Peg-IFNα) add-on therapy had benefits on fibrosis regression in patients with sustained severe fibrosis even after long-term NA treatment. We conducted a retrospective analysis of data from 50 patients with CHB receiving 48 weeks of Peg-IFNα add-on therapy. All enrolled patients had advanced fibrosis or cirrhosis (S score ≥ 3) at baseline and underwent NA treatment for at least 1 year before Peg-IFNα addition. Paired liver biopsies before and after Peg-IFNα add-on treatment and laboratory tests at baseline, 24 weeks of treatment, 48 weeks of treatment and long-term follow-up were analysed. Of the 50 patients enrolled in this study, 34 patients (68.0%) had significant regression of fibrosis, and 42 (84.0%) showed significant remission of inflammation after Peg-IFNα add-on treatment. Compared with nonresponders, patients with significant histological improvement showed faster hepatitis B surface antigen (HBsAg) decline and tended to have higher cumulative hepatitis B e antigen (HBeAg) and HBsAg loss rates during long-term follow-up.
1 | INTRODUCTION

Chronic hepatitis B (CHB) is a major global health concern. More than 292 million patients are thought to be chronically infected with hepatitis B virus (HBV) worldwide,1,2 and approximately 90 million individuals are affected in China.3 Liver fibrosis is one of the most common complications of CHB and can eventually progress to cirrhosis owing to sustained viral replication and ongoing liver inflammation.4

Previous studies have shown that long-term antiviral treatment with nucleos(t)ide analogs (NAs) can resolve inflammation and reverse fibrosis in histology; these changes are associated with the suppression of HBV replication.5-11 Although many patients with CHB treated with NAs achieve regression of fibrosis, 12%-26% of patients show no significant histological improvement, even after long-term NA treatment.7,10 Additionally, this histological improvement seemed to be more prominent within the first year of NA therapy, and very little further regression in fibrosis could be achieved thereafter. Indeed, in previous studies, there were no significant differences in fibrosis between biopsies taken after 1 year and after at least 3 years following treatment initiation.8,9

Interferon (IFN) has antiviral, antiproliferative and immunomodulatory effects. Unlike NAs, which only inhibit reverse transcription of HBV, IFN interferes with multiple steps in the HBV lifecycle, including virus entry, uncoating, transcription, translation and nucleocapsid assembly. However, IFN may also enhance both the adaptive humoral response and cell-mediated immunity to contain viral replication in the host, thereby promoting the clearance of HBV-infected hepatocytes. These characteristics may promote the effects of IFN on eradication of intrahepatic covalently closed circular DNA (cccDNA).11-13 In clinical practice, patients treated with Peg-IFNα monotherapy or combination therapy with Peg-IFNα and NAs within a finite period are more likely to achieve sustained virological and serological responses, compared with those treated with NA monotherapy.12,14-17

The addition of Peg-IFNα to ongoing NA treatment (Peg-IFNα add-on therapy) is sometimes used by clinical practitioners when the treatment response to NAs is not satisfactory. Some studies have demonstrated that long-term NA monotherapy can partially restore HBV-specific T-cell responsiveness,18 suggesting that Peg-IFNα addition after restoration of HBV-induced T-cell responsiveness may be beneficial.19,20 However, these previous studies have mainly focused on serological responses,18,21-23 and the histological responses to Peg-IFNα add-on therapy have not yet been explored.

Accordingly, in this study, we investigated whether Peg-IFNα add-on therapy provided benefits on histological analysis in patients with sustained severe fibrosis after long-term NA treatment.

2 | PATIENTS AND METHODS

2.1 | Study design

This was a retrospective single-cohort study generated from real-world practice. The patients in this study were between 18 and 65 years of age and visited the Second People’s Hospital of Yinzhou (China). Eligible patients were diagnosed with CHB and had been on continuous NA treatment for more than 1 year before Peg-IFNα addition. Paired liver biopsies were performed prior to and after Peg-IFNα addition. Among these patients, only those with undetectable plasma HBV DNA, normalized or slightly elevated alanine aminotransferase (ALT) concentrations after long-term NA treatment, and severe fibrosis or cirrhosis at first biopsy were included. The exclusion criteria were as follows: decompensated cirrhosis, pregnancy, immunocompromised or immunosuppressive conditions, autoimmune disorders, other hepatitis virus co-infection and the presence of other aetiologies for liver disease.

The results of laboratory tests were collected either from the databases at the Second People’s Hospital of Yinzhou or from reports provided by other healthcare institutes. Demographic characteristics and medication history were obtained from medical records and were all confirmed by patients.

The study protocol was approved by the Ethical Committee of Huashan Hospital and was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

2.2 | Evaluations

We defined the time of the initiation of Peg-IFNα addition as baseline. Data for clinical and laboratory assessments at baseline, 24 weeks and 48 weeks from baseline were collected. Data during long-term follow-up visits were also collected.

Biopsies were assessed by experienced pathologists from the Second People’s Hospital of Yinzhou; these pathologists were unaware of the patient’s clinical condition and treatment regimen. All biopsies were assessed with a Chinese scoring system for histological inflammation and fibrosis in chronic hepatitis. This evaluation system, which was established in 1998, was based on the Knodell scoring system and Chevalier fibrosis evaluation system and was modified according to the...
pathological characteristics of biopsy samples from Chinese patients with chronic hepatitis. This system is widely used in China to evaluate histological inflammation grades and fibrosis stages in patients with chronic hepatitis. The details of this system are listed in Table S1. The necroinflammatory activity was graded using G scores, and the stage of fibrosis was interpreted based on S score.

2.3 | Efficacy measures

The primary efficacy measure was defined as the histological response at the second biopsy after Peg-IFNα addition, and the second efficacy measures were serological responses at 24 and 48 weeks after Peg-IFNα addition. Significant improvement in fibrosis was defined as a decrease of at least 1 point in the S score.

Because all patients included in our study had already been treated with NAs for at least 1 year and had achieved sustained virologic and biochemical responses (with undetectable DNA and ALT levels less than 2 times the upper limit of normal), DNA suppression and ALT normalization were not used as efficacy measures in this study.

2.4 | Statistical analyses

Data for categorical variables were expressed as counts and percentages, and continuous variables were summarized with medians and ranges. Histological characteristics prior to and after Peg-IFNα addition were compared and analysed with Wilcoxon signed rank tests.

Correlation analyses between continuous variables and categorical variables were performed using Spearman’s and Kendall’s coefficients, respectively. The cohort was then divided into histological responders and nonresponders, and qualitative and quantitative differences between subgroups were compared using chi-square tests with continuity correction or Fisher’s exact tests for categorical parameters and Mann-Whitney tests for continuous parameters. The cumulative hepatitis B e antigen (HBeAg) loss rate and HBeAg seroconversion rate were calculated using Kaplan-Meier’s method and compared using log-rank tests between subgroups. Results with $P$ values of less than 0.05 were considered significant. All analyses were performed using SPSS V.23.0 (SPSS, Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Description of the study population

Of 91 eligible patients, 26 were excluded for missing serological test data, 12 were excluded because the fibrosis was not severe enough (S scores ≤ 2) at baseline, two were excluded for ongoing
NA treatment duration of less than 1 year, and one was excluded because a second biopsy was conducted within 1 year from the previous biopsy (Figure 1).

The characteristics of the study population are described in Table 1. Patients were predominately men (median age: 44 years). Patients had all undergone long-term NA therapy before addition of Peg-IFNα, and the duration of NA therapy ranged from 1 to 6 years (average: 2.4 years). All patients had undetectable HBV DNA levels and normalized or slightly elevated ALT (less than 2 times the upper limit of normal) before Peg-IFNα add-on. In total, 35 (70.0%) patients were HBeAg negative at baseline. Notably, because the broad-range hepatitis B surface antigen (HBsAg) quantification test was not available at our institution before 2014, HBsAg levels were not precisely quantified when higher than 250 IU/mL in some patients at baseline. From our analysis, 37 (74.0%) patients had HBsAg levels higher than 250 IU/mL at baseline. Based on biopsies before Peg-IFNα add-on, patients all showed severe fibrosis and moderate-to-severe inflammation, with S scores greater than or equal to 3 in all patients and G scores greater than or equal to 3 in most patients (68.0%) in the baseline biopsy.

### 3.2 | Histological response

Overall, compared with the baseline biopsies, both S staging and G grading showed significant reductions in secondary biopsies examined after add-on Peg-IFNα therapy ($P < 0.0001$). The proportion of patients with mild-to-moderate fibrosis (S scores of 1-2) increased from 0 at baseline to 40.0% (20/50) at the time of the second biopsy. The proportion of patients with early-stage cirrhosis (S score = 4) was reduced from 72% (36/50) at baseline to 24.0% (12/50) at the second biopsy (Figure 2A). Changes of fibrosis in individuals are shown in Figure 2B. Of 50 patients, only two (4%) showed worsening of fibrosis. Additionally, 34 patients (68.0%) had significant histological improvement (reduction of S staging ≥ 1) and 14 patients (28.0%) remained unchanged, even after Peg-IFNα add-on therapy. Moreover, 42 of 50 patients (84.0%) showed significant resolution of inflammation activity. Fibrosis was strongly correlated with inflammation in histology, with a Kendall $\tau_B$ coefficient of 0.488 ($P < 0.0001$); however, the correlations between regression of fibrosis and relief in inflammation were not significant ($P = 0.543$).

### 3.3 | Serological response

Among 15 patients with positive HBeAg at baseline, four patients (26.7%) achieved HBeAg loss at 24 weeks after Peg-IFNα add-on, and one more achieved HBeAg loss at 48 weeks (n = 5, 33.3%). There was no significant reduction in HBsAg levels either from baseline to 24 weeks ($P = 0.317$) or from 24 to 48 weeks ($P = 0.109$) after Peg-IFNα addition in patients with positive HBeAg at baseline. However, in patients with negative HBeAg at baseline, a significant decrease in HBsAg from baseline was observed ($P = 0.03$). Further analyses showed that the reduction was significant from 24 to 48 weeks ($P = 0.013$), but not from baseline to 24 weeks ($P = 0.721$). These

### Table 1 Characteristics of the study group

| Baseline characteristics |   |
|--------------------------|--|
| Demographic characteristics |   |
| Age (y, range) | 44 (24-62) |
| Male (n, %) | 40 (80.0%) |
| Serological characteristics |   |
| PLT (×10^9/L, range) | 130.5 (53-231) |
| ALT (U/L, range) | 35.5 (11-66) |
| Undetectable HBV DNA (n, %) | 50 (100%) |
| HBeAg negative (n, %) | 35 (70.0%) |
| HBsAg > 250 IU/mL (n, %) | 37 (74.0%) |
| Histological characteristics |   |
| Inflammation (G scores) |   |
| G ≥ 3 (n, %) | 34 (68.0%) |
| Fibrosis (S scores) |   |
| S = 3 (n, %) | 14 (28.0%) |
| S = 4 (n, %) | 36 (72.0%) |
| On-treatment characteristics at 24 wk after PEG-IFN addition |   |
| Serological characteristics |   |
| PLT (×10^9/L, range) | 83.5 (29-243) |
| ALT (U/L, range) | 45 (12-148) |
| Undetectable HBV DNA (n, %) | 50 (100%) |
| HBeAg negative (n, %) | 39 (78.0%) |
| HBsAg > 250 IU/mL (n, %) | 32 (64.0%) |
| Treatment Response |   |
| HBsAg loss (n, %) | 1 (2.0%) |
| Among patients with positive HBeAg at baseline (n = 15) |   |
| HBeAg loss (n, %) | 4 (26.7%) |
| Post-treatment characteristics at 48w after PEG-IFN addition |   |
| Serological characteristics |   |
| PLT (×10^9/L, range) | 114 (47-228) |
| ALT (U/L, range) | 24 (9-893) |
| Undetectable HBV DNA (n, %) | 50 (100%) |
| HBeAg negative (n, %) | 40 (80.0%) |
| HBsAg > 250 IU/mL (n, %) | 28 (56.0%) |
| Histological characteristics |   |
| Inflammation (G scores) |   |
| G ≥ 3 (n, %) | 6 (12.0%) |
| Fibrosis (S scores) |   |
| S = 3 (n, %) | 18 (36.0%) |
| S = 4 (n, %) | 12 (24.0%) |
| Treatment Response |   |
| HBsAg loss (n, %) | 4 (8.0%) |
| Among patients with positive HBeAg at baseline (n = 15) |   |
| HBeAg loss (n, %) | 5 (33.3%) |
| Histological response |   |
| S decrease ≥ 1 (n, %) | 34 (68.0%) |
findings suggested the reduction in HBsAg induced by the addition of Peg-IFNα was not a rapid response and mainly occurred during the late stage of Peg-IFNα add-on therapy. Among all patients, four achieved HBsAg loss (8.0%) at 48 weeks after Peg-IFNα addition. However, only one achieved HBsAg loss at 24 weeks after Peg-IFNα addition. The patterns of changes in serological tests are shown in Figure 3.

### 3.4 Factors correlated with fibrosis regression

Next, we attempted to identify the factors correlated with fibrosis regression. We divided patients into two subgroups according to their histological response in fibrosis. Baseline characteristics, characteristics during treatment and endpoint characteristics are shown in Table 2.

Alanine aminotransferase levels at 24 weeks after Peg-IFNα addition were higher in histological responders \( (P = 0.043) \), which may be related to the more intense immunological response induced by Peg-IFNα. Additionally, the decreases in HBsAg from baseline to 48 weeks after Peg-IFNα addition were sharper in patients with fibrosis regression \( (P = 0.001) \). This finding suggested that failure to achieve histological improvement may be associated with persistent high-level HBsAg.

No other significant differences were identified for any parameters, including demographic and serological characteristics, histological inflammation activity at baseline and changes during add-on Peg-IFNα therapy. Moreover, although patients with worse fibrosis at baseline (S score = 4) seemed to have lower histological response rate than patients with less severe fibrosis, this difference was not significant \( (P = 0.474; \text{Figure 2C}) \). There were also no differences between groups with and without fibrosis regression in terms of inflammation resolution \( (P = 0.456; \text{Figure 2B}) \).

Finally, we calculated the cumulative HBeAg and HBsAg loss rates in long-term follow-up (up to 4 years after add-on of Peg-IFNα). The curve showed a trend for more rapid HBeAg loss in patients without histological improvement, but higher cumulative HBeAg loss rates in patients with histological improvement during long-term follow-up (Figure 4A). Additionally, the curve also showed a tendency for faster and higher cumulative HBsAg loss rates during long-term follow-up in histological responders (Figure 4B). However, there were no significant differences for either parameter \( (P = 0.851 \text{ for HBeAg loss and } P = 0.284 \text{ for HBeAg seroconversion})\).

### 4 DISCUSSION

Several recent clinical studies have shown that long-term NA monotherapy can lead to regression of fibrosis and cirrhosis, which are associated with sustained viral suppression during treatment. However, some subgroups of patients show no histological changes (12%–26%, depending on the number of patients included, baseline HBeAg status and time between the two biopsies), and some patients show histological progression (approximately 5%, similar in different studies).\(^5\)\(^–\)\(^10\)

Further regression in fibrosis is limited during prolonged long-term NA therapy; indeed, previous studies showed that there were no significant differences in fibrosis between biopsies collected at 1 year and
at long-term follow-up (at least 3 years) after treatment initiation. These results were consistent with our observations from clinical practice, that is most patients could show histological improvement in long-term NA treatment, but histological nonresponders could also be detected. In histological nonresponders, little histological improvement was observed, even after years of treatment. This phenomenon may be associated with the mechanism of fibrosis.

Fibrosis is a result of accumulation of extracellular matrix (ECM) components caused by imbalances in their production, deposition and degradation. Advanced liver fibrosis was previously thought to be irreversible; however, recent studies have shown that cirrhosis can be reversed if the underlying cause of liver injury is controlled. Although IFN can relieve inflammation and suppress virus replication, the degradation and resorption of already deposited ECM require matrix metalloproteinases produced by host cells, and the regression of liver fibrosis is a time-consuming process. When cirrhosis progresses to a certain level, it can still be irreversible. This could explain our observation that there was always a subgroup of patients who could not achieve histological responses, even after Peg-IFNα add-on therapy, and that no further histological improvements occurred, even after long-term antiviral treatment.

The role of Peg-IFNα in antiviral therapy in patients with CHB has been an interest of investigators for many years. The benefits of Peg-IFNα monotherapy in combination with NAs, as well as addition to ongoing NAs, have been reported in different studies; however, these studies have primarily evaluated serological changes.
### TABLE 2  Characteristics of histological responders and nonresponders

|                             | Improvement in fibrosis (n = 34) | No Improvement in fibrosis (n = 16) | P value |
|-----------------------------|----------------------------------|------------------------------------|---------|
| **Baseline characteristics**|                                  |                                    |         |
| Demographic characteristics |                                  |                                    |         |
| Age (years, range)          | 44 (26–62)                       | 44 (24–54)                         | 0.574   |
| Male (n, %)                 | 28 (82.4%)                       | 12 (75.0%)                         | 0.820   |
| **Serological characteristics**|                                  |                                    |         |
| PLT (*10^9/L, range)        | 136 (53–178)                     | 110 (56–220)                       | 0.234   |
| ALT (U/L, range)            | 33 (11–66)                       | 39 (18–62)                         | 0.179   |
| HBeAg negative (n, %)       | 25 (73.5%)                       | 10 (62.5%)                         | 0.643   |
| HBsAg > 250 IU/mL (n, %)    | 23 (67.6%)                       | 14 (87.5%)                         | 0.251   |
| **Histological characteristics**|                                  |                                    |         |
| Inflammation (G scores)     |                                  |                                    |         |
| G ≥ 3 (n, %)                | 21 (61.8%)                       | 13 (81.3%)                         | 0.088   |
| Fibrosis (S scores)         |                                  |                                    |         |
| S = 3 (n, %)                | 8 (23.5%)                        | 6 (37.5%)                          | 0.491   |
| S = 4 (n, %)                | 26 (76.5%)                       | 10 (62.5%)                         | 0.491   |
| **On-treatment characteristics at 24w after PEG-IFN addition**|                        |                                    |         |
| Serological characteristics |                                  |                                    |         |
| PLT (*10^9/L, range)*       | 82 (29–243)                      | 84 (63–159)                        | 0.397   |
| ALT (U/L, range)*           | 48.5 (13–148)                    | 34 (12–66)                         | 0.043   |
| HBeAg negative (n, %)       | 28 (82.4%)                       | 11 (68.8%)                         | 0.105   |
| HBsAg > 250 IU/mL (n, %)    | 19 (55.9%)                       | 13 (81.3%)                         | 0.153   |
| Treatment response          |                                  |                                    |         |
| HBsAg loss (n, %)           | 1 (2.9%)                         | 0 (0%)                             | >0.999  |
| HBsAg reduction (folds, range) | 1.225 (0.58–37.5)               | 1 (0.86–9.08)                      | 0.242   |
| Among patients with positive HBeAg at baseline (n, %) | (n = 9)                              | (n = 6)                           |         |
| HBeAg loss (n, %)           | 3 (33.3%)                        | 1 (16.7%)                          | 0.905   |
| **Post-treatment characteristics at 48w after PEG-IFN addition**|                        |                                    |         |
| Serological characteristics |                                  |                                    |         |
| PLT (*10^9/L, range)        | 99.5 (47–228)                    | 120 (75–208)                       | 0.133   |
| ALT (U/L, range)            | 25.5 (9–893)                     | 22 (10–95)                         | 0.749   |
| HBeAg negative (n, %)       | 28 (82.4%)                       | 12 (75.0%)                         | 0.820   |
| HBsAg > 250 IU/mL (n, %)    | 16 (47.1%)                       | 12 (75.0%)                         | 0.121   |
| Treatment Response          |                                  |                                    |         |
| HBsAg loss (n, %)           | 4 (11.8%)                        | 0 (0%)                             | 0.383   |
| HBsAg reduction (folds, range)** | 1.95 (0.3–7627.67)               | 0.83 (0.05–6.25)                   | 0.001   |
| Among patients with positive HBeAg at baseline (n = 9) | (n = 6)                              | (n = 6)                           |         |
| HBeAg loss (n, %)           | 3 (33.3%)                        | 2 (33.3%)                          | >0.999  |
| Histological response       |                                  |                                    |         |
| G score decrease ≥ 1 (n, %) | 27 (79.4%)                       | 15 (93.7%)                         | 0.456   |

*P < 0.05.

**P < 0.01.
In this study, we explored whether Peg-IFNα therapy could benefit patients with no significant improvement in fibrosis after long-term NA treatment. Significant regression of fibrosis and inflammation was observed after Peg-IFNα add-on therapy, and further serological responses were also observed. However, a group of patients (n = 16, 32.0%) had no significant histological improvement. We used different methods to compare several parameters between the two subgroups and investigated time-dependent changes in the patterns of these parameters. A sharper HBsAg decline was identified in patients with significant histological improvement. Because previous studies have already demonstrated the associations between HBsAg and cccDNA,13 we expected that the constant cccDNA activity may have led to the persistent fibrosis observed in nonresponders. Indeed, higher cumulative HBeAg and HBsAg loss rates were observed in long-term follow-up, although the results were not statistically significant.

Our study had some limitations. First, this was a single-cohort study; accordingly, there was no control group to demonstrate that the histological improvement we observed in the Peg-IFNα therapy group was the effect of Peg-IFNα addition. Therefore, to avoid this bias, we limited our study patients to patients who showed persistence of severe fibrosis, even after long-term NA treatment. Second, this was a respective study, and the data were from real-world practice. Thus, the heterogeneity was significant in our patients. We also attempted to set strict inclusion and exclusion criteria in order to minimize heterogeneity and reduce bias. However, these criteria yielded a limited sample size. Third, the sample size of this study was small, and missing data were also noted. This limitation may have resulted in some abnormal findings, potentially concealing correlations or risk factors.

In summary, significant histological improvement was observed after Peg-IFNα add-on therapy in patients with CHB with long-term NA treatment. The histological response may be associated with HBsAg reduction. Additionally, higher cumulative HBeAg and HBsAg loss rates were observed in patients with significant histological improvement compared with nonresponders. Overall, our results suggested that Peg-IFNα add-on therapy may benefit patients who did not achieve histological improvement in long-term NA therapy. Notably, the histological response to Peg-IFNα therapy may be correlated with serological responses. Further independent studies with larger numbers of cases are needed.

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CONFLICTS OF INTEREST
All authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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