



# Platelets' increase is associated with improvement of liver fibrosis in entecavir-treated chronic hepatitis B patients with significant liver fibrosis

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## Abstract

**Background and aim** Reduction of platelet count is often observed in chronic hepatitis B (CHB) patients with significant liver fibrosis. In this cohort study, we investigated whether platelets' increase after entecavir (ETV) therapy was associated with the improvement of liver fibrosis.

**Methods** We collected the data of a cohort 82 CHB patients with paired liver biopsies before and after 78-week ETV therapy, and assessed the platelets' change following the treatment and further investigated the associated clinical factors with platelets' change.

**Results** Platelet count increased after treatment, which occurred mainly in patients with low baseline level of platelet count ( $< 200 \times 10^9/L$ ) or with significant fibrosis (Ishak  $\geq 3$ ). Regression analysis showed that baseline platelet count was the main factor associated with post-treatment increase of platelets ( $\beta = -0.215$ ,  $p = 0.015$ ). In patients with significant fibrosis, correlation and linear regression analysis revealed that post-treatment platelets' increase was correlated with improvement of liver fibrosis assessed by reduction of quantitative collagen percentage area ( $r = 0.392$ ,  $p = 0.006$ ) ( $\beta = 2.449$ ,  $p = 0.035$ ), but no correlation between changes in platelet counts and Ishak fibrosis score. Receiver operating curve analysis showed an increase of  $12.5 \times 10^9/L$  in platelet count could identify improvement of liver fibrosis (AUC = 0.70).

**Conclusion** Platelets' increase after ETV therapy was associated to the improvement of liver fibrosis with reduction of collagen percentage area in CHB patients with significant fibrosis.

**Keywords** Platelets · Liver fibrosis · Reversal · Chronic hepatitis B · Antiviral therapy

## Abbreviations

ALT Alanine aminotransferase  
AST Aspartate aminotransferase  
AUC Area under the curve  
CHB Chronic hepatitis B

CHC Chronic hepatitis C  
CI Confidence interval  
CPA Collagen proportionate area  
ETV Entecavir  
SHG Second-harmonic generation  
TPEF Two-photon excitation fluorescence  
ROC Receiver operating curve

Lin Wang and Bingqiong Wang were co-first authors making an equal contribution.

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## Introduction

Antiviral therapy has been the vital treatment in patients with chronic hepatitis B (CHB) [1–3], and it can significantly improve the outcome of patients. It has been proved that liver fibrosis can be regressed after antiviral therapy, even in cirrhosis patients [4, 5]. Paired biopsies of pre- and post-treatment are still the gold standard of evaluating liver

fibrosis change. The non-invasive assessment is needed, which will be useful to dynamically evaluate the fibrosis change after treatment and improve compliance.

Thrombocytopenia is strongly associated with severe fibrosis in patients with chronic liver diseases; therefore platelet count is often used in diagnostic algorithms to noninvasively identify the presence of significant fibrosis [6, 7]. The relationship between post-treatment change of platelet count and liver fibrosis is still unclear. Previous studies on chronic hepatitis C (CHC) revealed that platelets' increase after sustained virological response was correlated with the improvement of hepatic fibrosis [8–10].

Although it had been reported that the platelets increased after nucleoside treatment in CHB patients [11], there has been no study to determine whether post-treatment platelets' increase had relationship with fibrosis improvement. Besides, previous studies used semi-quantitative Ishak staging system to evaluate the change of liver fibrosis, which might cause bias.

In this study, we evaluated the change of platelet counts after ETV (entecavir) treatment and investigated the factors associated with platelets' change, with focus on whether the post-treatment increase of platelet counts was related with improvement of liver fibrosis evaluated by quantitative collagen percentage area (CPA) assessment before and after treatment.

## Patients and methods

### Patients

All participating patients were from the “Regression Study” (ClinicalTrials.gov: NCT01938781, NCT01938820). Inclusion criteria included: male or female aged 18–65 years; HBsAg positive for at least 6 months before treatment; HBV-DNA load of more than 20,000 IU/mL in the HBeAg-positive patients before treatment or 2000 IU/mL in the HBeAg-negative patients; with paired liver biopsy before and after 78-week ETV therapy. Exclusion criteria were as follows: co-infection with hepatitis C or human immuno-deficiency virus; the presence of chronic liver disease caused by other etiologies; decompensated cirrhosis; alpha-fetoprotein > 100 ng/mL; Cr > 1.5 times than the upper limit of normal; malignant tumors; severe diseases of heart, lung, kidney, brain, blood system or other organs; pregnant or lactating female.

All patients received ETV therapy after initial evaluation of the liver fibrosis by liver biopsy, and the second liver biopsy was conducted after 78 weeks treatments to evaluate fibrosis change.

### Collection of clinical data

Demographic data were collected at baseline (week 0), and clinical tests were performed at baseline and at every 26 weeks intervals including blood cell counts, HBV-DNA level, serological markers of HBV, liver functional tests, AFP, and liver ultrasonography. HBV serological markers and serum HBV-DNA level were measured centrally using Abbott Architect i2000 (ABBOTT, Wiesbaden, Germany) and Roche COBAS<sup>®</sup>TaqMan<sup>®</sup> HBV Test, respectively.

### Histological evaluation

Liver tissues were formalin fixed, paraffin embedded and sectioned at local centers according to standard techniques. The tissue sections were stained centrally with hematoxylin and eosin, reticulin and Masson trichrome. The stained sections were then independently evaluated by two experienced pathologists. Necroinflammation was assessed by the modified histology activity index (HAI) grading system (a scale of 0–18) and fibrosis was staged by the Ishak fibrosis scoring system (a scale of 0–6) [12].

### Measurement of CPA

Measurement of CPA was performed by second-harmonic generation (SHG)/two-photon excitation fluorescence (TPEF) microscope (Genesis200TM, HistoIndex Pte. Ltd, Singapore) [13, 14]. SHG microscope was used to visualize collagen and TPEF was to identify cell structures. The CPA was measured by the area percentage of TPEF signals normalizing the SHG signals (Supplementary Fig. 1).

### Definition of liver fibrosis improvement

The improvement of liver fibrosis was evaluated by CPA reduction. We defined the fibrosis improvement with a 2.0 CPA reduction after treatment. The receiver operating characteristic curve (ROC) analysis showed that a 2.0 post-treatment reduction of CPA identified the standard with the area under curve (AUC) of 0.68, (Supplementary Fig. 2). The standard was at least 1-point decrease in Ishak score, or was “Predominately Regressive” by “P-I-R classification” when Ishak score was same before and after treatment [15].

### Statistical analysis

Characteristics on pre- and post-treatment were compared using student's paired *t* test for normally distributed continuous variables, the Mann–Whitney test for non-normally distributed variables and the Chi-square test for categorical

variables. Repeated measurement analysis was used to assess the platelets' change following treatment. Linear regression analysis was performed to determine the clinical factors associated with the platelets' change.

Correlations between platelets' increase and reduction of CPA and Ishak score were performed using the Spearman's rank test. For assessing the performance of platelets' increase and increase rate to identify liver fibrosis improvement, the receiver operating characteristic (ROC) curve analysis was carried out to determine the area under the curve (AUC) values. A  $p$  value of less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 20.0 (IBM, Chicago, IL).

## Results

### Characteristics of the study population

This study enrolled 90 patients receiving ETV treatment. Finally, 82 patients with eligible paired biopsy were included in the final analysis. Most of the patients were male (77%) with a mean age of 41 years. Before treatment, 74% of them were HBeAg-positive with elevated serum ALT and mean HBV-DNA level of 6.30 log IU/mL. After 78 weeks of ETV therapy, HBV-DNA was undetectable ( $< 20$  IU/mL) in 79% patients. Laboratory tests and histology of liver tissue improved, platelets increased from  $155 \pm 53 \times 10^9/L$  to  $182 \pm 52 \times 10^9/L$  and CPA decreased (Table 1). Liver fibrosis improved after treatment. Ishak scores decreased in 46% patients

(Supplementary Fig. 3) and median of CPA measurement also decreased from 4.95 to 2.43 ( $p < 0.01$ ). Among significant fibrosis and cirrhosis patients with unchanged post-treatment Ishak score, 78% of them had fibrosis regression according to "P-I-R criterion" (Supplementary Table).

### Factors associated with post-treatment platelets' increase

Table 2 showed the linear regression analysis about the factors associated with post-treatment platelets' increase. In the univariate analysis, the factors contributing to platelets' increase included worse baseline liver function tests, change of these tests after treatment, pre-treatment inflammatory score and baseline platelet counts. In the multivariate analysis, the baseline level of platelet counts was the only factor related with the post-treatment increase of platelets ( $\beta = -0.215$ ,  $p = 0.015$ ) (Table 2). Platelets' increase was mainly observed in patients with low platelet counts at baseline.

### Impact of baseline platelet counts and fibrosis stage on platelets' increase

Repeated measurement analysis showed that patients with low baseline platelet counts ( $< 200 \times 10^9/L$ ) or with significant fibrosis (Ishak  $\geq 3$ ) had significant platelets' increase after treatment. The mean of platelet counts increased from  $135 \times 10^9/L$  at baseline to  $167 \times 10^9/L$  at week 78 in patients with low baseline platelets, from  $158 \times 10^9/L$  to  $188 \times 10^9/L$  in patients with Ishak 3/4, and

**Table 1** Characteristics of study population before and after treatment

	Baseline	Post-treatment	$p$
$N$	82	82	–
Age (years) <sup>a</sup>	41 $\pm$ 11	–	–
Gender (male) <sup>c</sup>	63 (77%)	–	–
HBeAg (+) <sup>c</sup>	61 (74%)	–	–
HBV-DNA (log <sub>10</sub> IU/mL) <sup>a</sup>	6.30 $\pm$ 1.40	0	$< 0.001$
ALT (U/L) <sup>b</sup>	71 (81)	25 (19)	$< 0.001$
AST (U/L) <sup>b</sup>	53 (49)	22 (10)	$< 0.001$
Albumin (g/L) <sup>a</sup>	42.2 $\pm$ 5.2	45.8 $\pm$ 3.4	$< 0.001$
Bilirubin (mmol/L) <sup>b</sup>	14.9 (7.8)	13.8 (7.2)	0.117
Platelets ( $10^9/L$ ) <sup>a</sup>	155 $\pm$ 53	182 $\pm$ 52	$< 0.001$
Inflammatory score (0–3/4–6/7–9/ $\geq 10$ ) ( $n$ )	7/35/25/15	39/43/0/0	$< 0.001$
Fibrosis score (0–2/3–4/5–6) ( $n$ )	16/46/20	28/40/14	$< 0.001$
CPA <sup>b</sup>	4.95 (6.07)	2.43 (2.31)	$< 0.001$

ALT alanine transaminase, AST aspartate transaminase, CPA collagen percentage area

<sup>a</sup>Variables are expressed as mean  $\pm$  standard deviation

<sup>b</sup>Median (IQR)

<sup>c</sup> $n$  (%)

**Table 2** Linear regression analysis for factors related with post-treatment platelets' increase ( $n = 82$ )

	Univariate analysis			Multivariate analysis		
	$\beta$	SE	$p$	$\beta$	SE	$p$
Baseline						
Age (per year)	- 0.010	0.367	0.979			
Gender (female)	2.406	9.647	0.804			
HBV-DNA (per log IU/L)	0.455	2.850	0.873			
ALT (per U/L)	0.058	0.024	0.021	- 0.342	0.260	0.193
Albumin (per g/L)	- 2.513	0.762	0.001	0.796	1.286	0.538
Bilirubin, per $\mu\text{mol/L}$	1.015	0.228	< 0.001	- 0.084	0.797	0.916
Platelet (per $10 \times 10^9/\text{L}$ )	- 0.246	0.069	0.001	- 0.215	0.086	0.015
Inflammatory score	2.793	1.330	0.039	- 0.385	3.909	0.329
Ishak score	3.141	3.206	0.330			
CPA (%)	1.921	0.835	0.024	- 0.866	2.193	0.694
Changes after treatment						
HBV-DNA clearance	- 0.712	9.868	0.943			
Reduction of ALT	0.059	0.024	0.016	0.325	0.259	0.214
Increase of albumin	2.470	0.778	0.002	1.210	1.314	0.361
Reduction of bilirubin	1.012	0.217	< 0.001	0.854	0.763	0.267
Reduction of inflammatory score	2.666	1.377	0.057	5.039	3.782	0.188
Decrease of CPA	2.857	0.958	0.004	1.982	2.593	0.448

SE standard error,  $\beta$  was regression coefficient

from  $131 \times 10^9/\text{L}$  to  $158 \times 10^9/\text{L}$  in cirrhosis patients (Ishak5/6). There was no difference in platelet counts between pre- and post-treatment among patients with high baseline platelets or mild fibrosis (Table 3).

### Factors associated with platelets' increase in patients with significant fibrosis

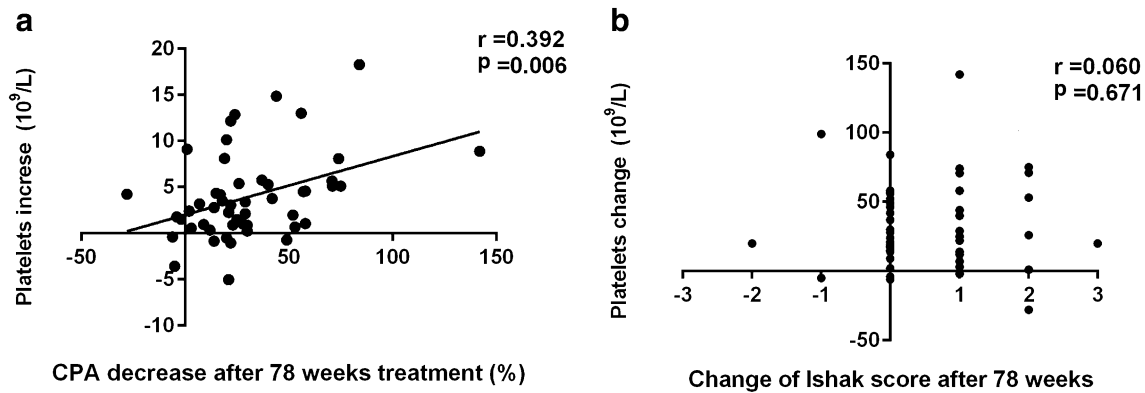
In 53 patients with baseline platelet counts less than  $200 \times 10^9/\text{L}$  and Ishak score  $\geq 3$ , the mean platelet counts increased from baseline  $130 \times 10^9/\text{L}$  to  $163 \times 10^9/\text{L}$  after 78-week treatment. The correlation analysis revealed that there was a positive correlation between post-treatment

platelets' increase and CPA reduction in patients with significant fibrosis ( $r = 0.392$ ,  $p = 0.006$ ), and no correlation between platelets' increase and Ishak score change (Fig. 1). The multivariate linear regression analysis showed that the only factor associated with platelets' increase were post-treatment CPA reduction ( $\beta = 2.449$ ,  $p = 0.035$ ) (Table 4). There was no correlation between post-treatment change of Ishak score and platelets' increase either univariate or multivariate analysis.

**Table 3** Platelet counts of pre- and post-treatment in groups divided by different baseline platelets and fibrosis ( $n = 82$ )

Group	$N$	Baseline ( $10^9/\text{L}$ )	26 week ( $10^9/\text{L}$ )	52 week ( $10^9/\text{L}$ )	78 week ( $10^9/\text{L}$ )	$p$
Total	82	155 $\pm$ 53	169 $\pm$ 57	176 $\pm$ 56	182 $\pm$ 52	< 0.001
Platelets at baseline						
$\geq 200 \times 10^9/\text{L}$	17	231 $\pm$ 23	226 $\pm$ 48	223 $\pm$ 49	235 $\pm$ 39	0.652
< $200 \times 10^9/\text{L}$	65	135 $\pm$ 38	156 $\pm$ 51	163 $\pm$ 51	167 $\pm$ 45	< 0.001
Fibrosis at baseline (Ishak)						
Mild (F0-2)	16	175 $\pm$ 40	189 $\pm$ 66	204 $\pm$ 74	196 $\pm$ 52	0.223
Significant (F3/4)	46	158 $\pm$ 56	174 $\pm$ 55	177 $\pm$ 49	188 $\pm$ 48	< 0.001
Cirrhosis (F5/6)	20	131 $\pm$ 47	143 $\pm$ 49	151 $\pm$ 48	158 $\pm$ 55	< 0.001

Repeated measurement analysis was used to evaluate whether platelet counts had difference between before and after treatment



**Fig. 1** Correlation between platelets' increase and fibrosis improvement evaluated by CPA and Ishak. There was positive correlation between CPA reduction and platelets' increase ( $r = 0.392$ ,  $p < 0.001$ ) (a), and no relation between change of Ishak score and platelets' change (b)

**Table 4** Factors associated with post-treatment platelets' increase in patients with baseline platelets  $< 200 \times 10^9/L$  and Ishak score  $\geq 3$  by linear regression analysis ( $n = 53$ )

	Univariate analysis			Multivariate analysis		
	$\beta$	SE	$p$	$\beta$	SE	$p$
Baseline						
Age (per year)	0.265	0.393	0.504			
Gender (female)	- 1.136	11.149	0.919			
ALT (per U/L)	0.072	0.024	0.004	- 0.426	0.211	0.050
Albumin (per g/L)	- 3.263	0.706	$< 0.001$	- 0.477	1.204	0.694
Bilirubin, per $\mu\text{mol/L}$	0.966	0.188	$< 0.001$	0.794	0.786	0.319
Platelets (per $10 \times 10^9/L$ )	- 0.203	0.102	0.053	- 0.136	0.087	0.125
Inflammatory score	2.892	1.430	0.048	- 7.021	4.799	0.152
Ishak score	0.986	0.208	0.836			
CPA (%)	1.332	0.821	0.112			
Changes after treatment						
Reduction of ALT	0.074	0.023	0.002	0.418	0.209	0.053
Increase of albumin	3.320	0.688	$< 0.001$	1.120	1.181	0.349
Reduction of bilirubin	0.912	0.180	$< 0.001$	- 0.227	0.765	0.769
Reduction of inflammatory score	2.007	1.469	0.178			
Reduction of Ishak score	0.986	0.029	0.836			
Decrease of CPA	2.449	0.847	0.006	2.048	0.937	0.035

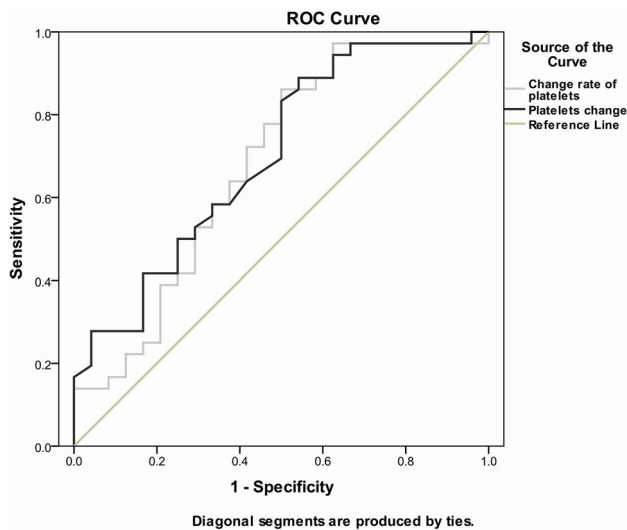
### Performance of platelets' increase to identify fibrosis improvement

ROC analysis showed that platelets' increase and increase rate could identify fibrosis improvement which defined as at least 2.0 reduce in CPA. A post-treatment  $12.5 \times 10^9/L$  increase in platelet counts distinguished fibrosis improvement with 89% sensitivity, 46% specificity, and an AUC of 0.70 (95% CI 0.54–0.82). It was similar to 9% increase rate of platelet count to identify fibrosis improvement (sensitivity: 86%, specificity: 50%, and AUC: 0.68) (Fig. 2).

### Discussion

In this cohort study, we found that the platelets increased after ETV therapy in CHB patients, especially in those patients with low pre-treatment platelet counts and significant fibrosis. Among patients with significant fibrosis, platelets' increase after treatment was correlated with improvement of liver fibrosis measured by CPA reduction.

Our results were in line with previous studies of CHC patients receiving antiviral therapy. In a study including CHC patients mostly with mild fibrosis, the mean level of platelet counts was similar between pre- and post-treatment ( $232 \times 10^9/L$  vs.  $235 \times 10^9/L$ ) [16]. In contrast, the platelet counts after treatment were significantly increased



**Fig. 2** ROC curves of post-treatment platelets' increase and increase rate for diagnosis the liver fibrosis improvement. They were indicated by a gray line and a black line, respectively. The definition of fibrosis improvement was a 2.0 post-treatment reduction of CPA

compared with pre-treatment level in studies which included patients mostly with significant fibrosis [17, 18].

We found that post-treatment platelets' increase had a positive correlation with fibrosis improvement in patients with significant fibrosis, and the fibrosis improvement should be evaluated by CPA. There was no correlation when fibrosis change was measured by Ishak score. Similarly, Kim et al. also found that improvement in non-invasive measurements of liver fibrosis was not associated with liver fibrosis change assessed by Ishak staging in CHB patients [11]. The reason might be that the semi-quantitative Ishak scoring system was not sensitive enough to evaluate the post-treatment change of liver fibrosis. The quantitative CPA was suitable in assessing the subtle change of liver fibrosis. Some studies had confirmed that CPA can be used to effectively monitor the liver fibrosis change in CHC patients [19, 20]. The results of our study suggested that post-treatment change of platelets counts might be useful tool to evaluate the subtle fibrosis change measured by CPA.

One limitation of this cohort study was that most of patients were followed only for 78 weeks. Fortunately, patients in the cohort will be followed successively for another 5 years, and then we can verify the results in longer follow-up interval. The cohort of present study was not large-scale. Hopefully, the strict inclusion and exclusion criteria could minimize the confounding factors.

In conclusion, ETV therapy can increase platelet counts of CHB patients with significant liver fibrosis, and this platelets' increase had positive correlation with fibrosis improvement that need be evaluated by CPA.

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## Compliance with ethical standards

**Conflict of interest** Lin Wang, Bingqiong Wang, Hong You, Xiaoning Wu, Jialing Zhou, Xiaojuan Ou, and Jidong Jia do not have anything to disclose regarding potential conflicts of interest.

**Ethical approval** The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki (2008 revision), and the protocol of study was approved by the Ethics Committee of Beijing Friendship Hospital. Our study had been registered in the ClinicalTrials.gov (NCT01938781 and NCT01938820).

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**Informed consent** The written informed consent was obtained from every patient prior to initiating any study procedures.

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