A New Glutamine Synthetase Index to Evaluate Hepatic Lobular Restoration in Advanced Fibrosis During Anti-HBV Therapy

Shuyan Chen

Capital Medical University Affiliated Beijing Friendship Hospital <https://orcid.org/0000-0003-2400-8413>

Bingqiong Wang

Capital Medical University Affiliated Beijing Friendship Hospital

Jialing Zhou

Capital Medical University Affiliated Beijing Friendship Hospital

Xiaoning Wu

Capital Medical University Affiliated Beijing Friendship Hospital

Tongtong Meng

Capital Medical University Affiliated Beijing Friendship Hospital

Hui Liu

Beijing You-an Hospital, Capital Medical University

Tailing Wang

China-Japan Friendship Hospital

Xinyan Zhao

Capital Medical University Affiliated Beijing Friendship Hospital

Shanshan Wu

Capital Medical University Affiliated Beijing Friendship Hospital

Yuanyuan Kong

Capital Medical University Affiliated Beijing Friendship Hospital

Xiaojuan Ou

Capital Medical University Affiliated Beijing Friendship Hospital

Jidong Jia

Capital Medical University Affiliated Beijing Friendship Hospital

Aileen Wee

Capital Medical University Affiliated Beijing Friendship Hospital

Hong You

Capital Medical University Affiliated Beijing Friendship Hospital <https://orcid.org/0000-0001-9409-1158>

Yameng Sun (✉ sunyamenggo@163.com)

Research Article

Keywords: chronic hepatitis B, liver fibrosis, regression, remodeling, portal tract, hepatic vein, Ishak stage, P-I-R score, paired liver biopsy, antiviral treatment

Posted Date: December 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1141631/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

Background

Hepatic lobular architecture distortion is a deleterious turning point and crucial histological feature of advanced liver fibrosis in chronic liver diseases. Regression of fibrosis has been documented in chronic hepatitis B (CHB) patients. However, the restoration of lobular architecture after antiviral therapy is still unclear. Here, we propose a new glutamine synthetase (GS) index (GS-index) to evaluate the extent of architectural disruption and restoration.

Methods

We evaluated 43 pre-and post-treatment liver biopsies of CHB patients with advanced fibrosis (Ishak stage ≥ 4). Glutamine synthetase (GS) is normally expressed by perivenular hepatocytes around hepatic veins (HV). When GS expression is observed in the vicinity of portal tracts (PT), it denotes parenchymal extinction and lobular collapse. We propose the new glutamine synthetase index (GS-index), defined as the percentage of $GS_{HV}/(GS_{HV} + GS_{PT})$, to evaluate the extent of architectural disruption and restoration.

Results

The median GS-index improved from 7% at baseline to 36% at week 78 ($P < 0.001$). When $GS-index_{78w} \geq 50\%$ used to define hepatic lobular restoration, 37% patients (16/43) achieved lobular restoration, with improvement in ALT and AST levels. More importantly, GS-index correlated with fibrosis regression, one stage fibrosis improvement in restored group and no change in non-restored patients ($P = 0.030$).

Conclusion

In the era of antiviral therapy for CHB, restoration of hepatic lobular architecture is achievable. GS-index gives a new evaluation tool to quantitatively assess hepatic lobular status and therapeutic benefits in CHB patients.

Introduction

Hepatic lobular architecture distortion is one of the deleterious pathological changes following severe liver injury with advanced fibrosis. There is loss of normal hexagonal lobular architecture as a result of parenchymal extinction and collapse, bridging portal tract to hepatic vein fibrous deposition, and vascular remodeling.¹ Portal hypertension and liver decompensation may ensue.^{1,2}

The reversibility of hepatitis B virus (HBV)-induced liver fibrosis has been documented in several landmark clinical studies and regressive histological features have been well described by pathologists.^{3,4,5} Bedossa described the crucial events in fibrosis regression which include extracellular matrix degradation, hepatocellular regeneration, and hepatic lobular architecture restoration.¹ Wanless et al. proposed the parameters of hepatic repair complex after observing the serial biopsies of an HBV-induced cirrhotic patient before and after antiviral treatment.⁵ In this study, Wanless et al. also noted that hepatocellular regeneration emerges as minute hepatocyte buds in regions of parenchymal extinction. However, whether with disease improvement and fibrosis regression, hepatic lobular architecture can be restored is uncertain.

Hepatic lobular architecture distortion is regarded as one of the morphological features of fibrosis progression in conventional fibrosis staging systems, such as Scheuer and Batts-Ludwig Systems.^{6,7} However, evaluation of architectural distortion is based on the subjective and experiential judgment of the pathologist. In this study, we propose a histological parameter employing glutamine synthetase (GS) immunohistochemical staining to quantify the degree of hepatic lobular architecture restoration.^{8,9} The normal hexagonal liver lobule has peripherally-located portal tracts (PT) with hepatic veins (HV) in the center.¹⁰ Hence, the identification of HVs coupled with the estimation of their distance from PTs serves as a key landmark in establishing normal and abnormal lobular architecture. GS is expressed in perivenular hepatocytes around HVs in normal liver. Currently, zonation profile of its gene namely *GLUL* (central zone) in human hepatocytes is demonstrated by single-cell RNA sequencing (scRNA-seq) methods.^{8,9} Therefore, GS staining can be used to locate HVs in hepatic parenchyma. Based on this pathological characteristic, a histological parameter, named GS-index, is proposed to assess the extent of hepatic lobular architecture restoration.

Methods

Patients

A total of 43 treatment-naïve chronic hepatitis B patients with biopsy-proven advanced fibrosis (Ishak $\geq$ 4) at baseline were enrolled in this study. Patients were enrolled based on the following key criteria: (i) 18 to 65 years of age; (ii) HBV surface antigen (HBsAg) positivity $\geq$ 6 months; (iii) hepatitis B e-antigen (HBeAg)-positive patients with HBV DNA $\geq$ 20,000 IU/mL or HBeAg-negative patients with HBV DNA $\geq$ 2,000 IU/mL; and (iv) paired liver biopsies performed at both baseline and week 78.

The main exclusion criteria were as follows: (i) co-infection with other etiologies; (ii) decompensated cirrhosis characterized by ascites, variceal bleeding, or hepatic encephalopathy; (iii) hepatocellular carcinoma and other malignancies; (iv) concomitant morbidities, such as major systemic diseases.

All patients received continuous entecavir-based treatment. Demographic data, including gender and age, were collected at baseline, while clinical laboratory tests and liver stiffness measurements (LSM) were

collected at baseline and every 6-month follow-up. All patients underwent the second liver biopsy at week 78 after receiving antiviral therapy.

Written informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board of Beijing Friendship Hospital, Capital Medical University, approved the protocol for this study. The ClinicalTrials.gov identifiers of the study were NCT02849132, and NCT03777969.

Histological assessment

The liver specimens were formalin-fixed and routinely processed. Five micrometer-thick paraffin-embedded histological sections were stained with hematoxylin and eosin (H&E), reticulin, Masson trichrome, and GS.¹¹ Immunohistochemistry (IHC) for GS was performed by manual operation according to manufacturer's instructions (GS-6: 1:500, Millipore, Billerica, MA, USA; antigen retrieval solution: citrate buffer for 20 min, Maixin, Fuzhou, China; detection method: DAB kit, Maixin, Fuzhou, China).

Necroinflammatory activity and fibrosis stage of the biopsies were independently assessed by two senior experts, based on Ishak modified histology activity index (HAI) grading system and Ishak fibrosis scoring system.¹² The samples with Ishak stage ≥ 3 were further evaluated according to the P-I-R score of the Beijing classification which was developed during the antiviral therapy era (YS).^{13,14} The P-I-R score has three categories (*predominantly progressive, indeterminate, and predominantly regressive*), providing a new dimension based on dynamic changes of collagen fibers to assess fibrosis regression. Fibrosis reversion was defined as decrease in Ishak fibrosis stage by ≥ 1 after 78 weeks of treatment or R (*predominantly regressive*) by Beijing Classification.

The identification of PTs and the assessment of necroinflammatory score based on modified HAI were dependent on H&E. Fibrosis staging was assessed on reticulin and Masson trichrome. The perivenular expression of GS is utilized to locate HVs (Figure 1A).

GS staining was thus used to determine normal and abnormal lobular architecture. Normal lobular architecture was defined as HVs not approximating PTs (GS_{HV}), while abnormal lobular architecture has HVs approximating/incorporated into PTs (GS_{PT}). Henceforth, the glutamine synthetase index (GS-index) was defined as the percentage of normal HVs among all HVs, i.e., $GS_{HV}/(GS_{HV} + GS_{PT})$ (Figure 1B).

According to the level of the GS-index at week 78, we defined hepatic lobular architecture restoration as $GS\text{-index}_{78w} \geq 50\%$. Patients were thus divided into restored ($GS\text{-index}_{78w} \geq 50\%$) and non-restored groups ($GS\text{-index}_{78w} < 50\%$).

Liver stiffness measurements

Liver stiffness measurements were obtained through transient elastography by experienced operators, according to the manufacturer's instructions (Fibroscan; Echosens, Paris, France).¹⁵ The LSM (in kPa) was considered reliable when 10 available measurements could be obtained with a success rate greater

than 60% and a ratio of the interquartile range (IQR) of liver stiffness to the median (IQR/med) not more than 0.30.

Laboratory assessments

Laboratory tests, including liver function tests - alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), and total bilirubin (TBIL); blood routine examination - platelet (PLT) counts; serum HBV DNA levels; and alpha-fetoprotein (AFP) which was employed to screen for hepatocellular carcinoma (HCC), were assessed at baseline and at every 6-month follow-up.

Serum HBV DNA levels were assessed using COBAS®TaqMan® HBV Test (Roche Molecular Systems, Branchburg, NJ, USA) with a detection threshold of 20 IU/mL in the central laboratory.

Statistical analysis

Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median (IQR). Categorical variables were measured using counts and percentages. Wilcoxon signed-rank test were used to compare the distribution of GS-index and the changes of clinical characteristics before and after treatment. Mann-Whitney test was used to compare the GS-index in restored and non-restored or reverse and non-reverse patients, and the dynamic changes of clinical and histological characteristics in restored and non-restored patients. In addition, we used the analysis of covariance to adjust the baseline level of the GS-index and assess the dynamic changes of GS-index in restored and non-restored or reverse and non-reverse patients. Chi-square test and likelihood ratios were used to compare the distribution of Ishak fibrosis stage and P-I-R score from baseline to week 78 and the proportions of reverse and non-reverse patients in restored and non-restored groups. The statistical tests conducted were two-sided. Statistical significance was considered when $P < 0.05$. Data were analyzed using SPSS 24.0 (IBM Corporation, Armonk, NY).

Results

Patient enrollment and clinical characteristics

A total of 43 CHB patients with advanced fibrosis (Ishak ≥ 4) at baseline were enrolled in this study. All patients underwent paired liver biopsies at baseline and week 78.

The mean age of the enrolled patients was 39 years and they were predominantly males (84%). After 78 weeks of anti-HBV treatment, HBV DNA significantly decreased from 6.3 log IU/mL to 0 log IU/mL. ALT and AST also decreased from 91.4 U/L to 30.0 U/L and 62.1 U/L to 26.8 U/L, respectively. PLT and LSM improved from 124.0×10^9 /L to 142.0×10^9 /L, and 16.4 kPa to 7.2 kPa, respectively.

The clinical characteristics of the enrolled patients were compared before and after treatment (Table 1). The dynamic changes in the clinical characteristics portrayed in the improved liver function and liver stiffness values demonstrated the effectiveness of antiviral therapy.

Table 1
Clinical and histological characteristics of chronic hepatitis B patients before and after antiviral treatment.

	Baseline (n = 43)	Week 78 (n = 43)	P value
Clinical characteristics			
Age, year	39 ± 10	–	–
Gender (male), n (%)	36 (83.7)	–	–
HBV-DNA, Log IU/mL	6.3 (5.1, 6.9)	0 (0, 0)	<0.001
PLT, 10 ⁹ /L	124.0 (108.0, 170.0)	142.0 (112.0, 204.0)	0.024
ALT, U/L	91.4 (57.0, 140.6)	30.0 (21.0, 37.0)	<0.001
AST, U/L	62.1 (42.0, 95.0)	26.8 (21.0, 34.0)	<0.001
Albumin, g/L	41.2 (35.7, 44.5)	45.6 (43.0, 47.4)	<0.001
TBIL, mmol/L	17.4 (12.7, 23.7)	12.6 (10.0, 16.3)	<0.001
INR	1.1 (1.0, 1.2)	1.0 (1.0, 1.1)	<0.001
AFP, ng/ml	11.0 (5.2, 27.0)	3.0 (2.2, 4.0)	<0.001
Liver stiffness value, kPa	16.4 (12.3, 24.1)	7.2 (5.9, 10.7)	<0.001
Child Pugh	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	0.004
MELD Score	8.0 (7.0, 9.0)	6.5 (6.0, 7.0)	<0.001
Histological characteristics			
Length, cm	1.3 (1.0, 1.5)	1.3 (1.1, 1.5)	0.346
Portal tract, n	9 (5, 13)	8 (6,10)	0.475
Hepatic vein, n	9 (8, 13)	11 (8,15)	0.508
Ishak score, n (%)	n = 43	n = 43	<0.001
0	0 (0)	0 (0)	
1	0 (0)	1 (2.3)	
2	0 (0)	1 (2.3)	
3	0 (0)	13 (30.2)	
4	22 (51.2)	11 (25.6)	
P-I-R score*: Two patients with post-treatment Ishak stage < 3 were not applicable for P-I-R classification.			

	Baseline (n = 43)	Week 78 (n = 43)	P value
5	15 (34.9)	16 (37.2)	
6	6 (14.0)	1 (2.3)	
P-I-R score*	n = 43	n = 41	<0.001
P	39 (90.7)	6 (14.6)	
I	3 (7.0)	14 (34.1)	
R	1 (2.3)	21 (51.2)	
P-I-R score*: Two patients with post-treatment Ishak stage < 3 were not applicable for P-I-R classification.			

Histological assessment based on fibrosis staging (Ishak and P-I-R score)

The distribution of fibrosis stage is shown in Table 1. The proportion of cases with advanced liver fibrosis (Ishak $\geq$ 4) decreased from 100% at baseline to 65% at week 78.

Based on Ishak fibrosis score, 23 of 43 patients (53%) had fibrosis regression (decrease $\geq$ 1 stage), 17 of 43 (40%) revealed no change, and 3 of 43 (7%) showed fibrosis progression (increase $\geq$ 1 stage).

Based on the P-I-R score at week 78 (2 cases with post-treatment Ishak stage<3 were not applicable for P-I-R classification), the proportion of predominantly regressive cases (R) was 21 of 41 (51%), indeterminate (I) was 14 of 41 (34%), and predominantly progressive (P) was 6 of 41 (15%). Thus, based on the P-I-R score, the proportion of reverse (R) and non-reverse (P/I) patients were 21 of 41 (51%) and 20 of 41 (49%), respectively.

Hepatic lobular architecture restoration based on GS expression

The paired liver biopsies were evaluated based on the GS-index. Median value of GS-index was significantly increased from 7% at baseline to 36% at week 78 ($P<0.001$) (Figure 2A).

The changes of GS-index after week 78 (Δ GS-index_{78w-0w}) are of interest. A total of 51% (22/43) of patients achieved significant GS-index improvement from 0% at baseline to 55% at week 78, while 49% (21/43) of them had almost no change, from 17% at baseline to 20% at week 78 ($P<0.001$) (Figure 2B). These findings imply that even after effective treatment, differing rates of tissue repair and restoration may occur in different individuals.

To further compare the restored versus non-restored groups after treatment, we defined the cutoff for hepatic lobular architecture restoration as at least 50% of lobules restored, i.e., half of the HVs do not approximate PT at week 78 ($\text{GS-index}_{78\text{w}} \geq 50\%$). Patients were divided into restored ($\text{GS-index}_{78\text{w}} \geq 50\%$, $n=16$) and non-restored ($\text{GS-index}_{78\text{w}} < 50\%$, $n=27$) groups. The dynamic changes of GS-index increased from 7–67% in restored patients and 10–20% in non-restored patients, at baseline to week 78 ($P < 0.001$) (Figure 3A). The median changes of GS-index from baseline to week 78 were 60% in restored and 10% in non-restored patients ($P < 0.001$) (Figure 3B).

Dynamic changes of clinical data before and after treatment were compared between restored and non-restored patients (Table 2). The decrease in ALT levels was greater in the restored patients (restored vs. non-restored: 91.0 vs. 34.7 U/L, $P=0.060$); similarly, for the decrease in AST levels (restored vs. non-restored: 49.5 U/L vs. 14.0 U/L, $P=0.006$). The median level of Ishak fibrosis score after 78 weeks of treatment decreased by 1 stage in restored patients vs. 0 stage in non-restored patients ($P=0.030$).

Table 2

Differences in the dynamic changes of clinical characteristics between restored (GS-index_{78w} ≥ 50%) and non-restored (GS-index_{78w} < 50%) chronic hepatitis B patients from baseline to week 78 after antiviral therapy.

	Restored (n = 16)		Non-restored (n = 27)		P value
	Baseline	Week 78	Baseline	Week 78	Δ(78w-0w)
Age, year	36 ± 11	–	40 ± 10	–	0.145
Gender (male), n (%)	13 (81.3)	–	23 (85.2)	–	1.000
HBeAg loss*	–	6/15 (40.0%)	–	6/21 (28.6%)	0.473
HBV-DNA, Log IU/mL	5.9 (4.0, 7.7)	0 (0, 0)	6.4 (5.9, 6.9)	0 (0, 0)	0.660
PLT, 10 ⁹ /L	156.3 (118.0, 204.8)	169.5 (130.8, 210.0)	115.6 (106.0, 150.0)	132.0 (104.0, 167.0)	0.920
ALT, U/L	120.5 (85.2, 169.3)	25.5 (17.3, 30.8)	74.3 (53.0, 115.4)	35.4 (22.0, 41.4)	0.060
AST, U/L	67.0 (53.8, 123.5)	22.7 (19.5, 29.4)	46.0 (36.4, 79.1)	28.8 (21.2, 36.6)	0.006
Albumin, g/L	41.1 (37.7, 42.9)	45.3 (43.2, 48.0)	41.4 (35.2, 45.3)	45.8 (42.3, 47.1)	0.744
TBIL, mmol/L	17.9 (12.9, 26.3)	11.7 (7.9, 13.6)	17.0 (12.7, 23.7)	14.5 (10.2, 16.7)	0.183
INR	1.1 (1.0, 1.1)	1.0 (0.9, 1.0)	1.1 (1.0, 1.2)	1.0 (1.0, 1.1)	0.492
Liver stiffness value, kPa	12.7 (11.1, 14.3)	5.9 (4.4, 6.8)	18.6 (13.6, 26.7)	8.0 (6.8, 11.9)	0.576
Child Pugh	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 6.0)	5.0 (5.0, 5.0)	0.597
MELD Score	7.0 (7.0, 9.0)	6.0 (6.0, 7.0)	8.0 (7.0, 9.0)	7.0 (6.0, 7.0)	0.966
Necroinflammation score, n (%)					0.685
0-3	0 (0)	5 (31.3)	1 (3.7)	7 (25.9)	
4-6	3 (18.8)	11 (68.8)	7 (25.9)	19 (70.4)	
7-9	7 (43.8)	0 (0)	10 (37.0)	1 (3.7)	
HBeAg loss*: The serum level of HBeAg was tested at baseline and week 104.					

	Restored (n = 16)		Non-restored (n = 27)		P value
	Baseline	Week 78	Baseline	Week 78	$\Delta(78w-0w)$
≥ 10	6 (37.5)	0 (0)	9 (33.3)	0 (0)	
Ishak score, n (%)					0.030
1	0 (0)	1 (6.3)	0 (0)	0 (0)	
2	0 (0)	1 (6.3)	0 (0)	0 (0)	
3	0 (0)	9 (56.3)	0 (0)	4 (14.8)	
4	11 (68.8)	1 (6.3)	11 (40.7)	10 (37.0)	
5	3 (18.8)	3 (18.8)	12 (44.4)	13 (48.1)	
6	2 (12.5)	1 (6.3)	4 (14.8)	0 (0)	
HBeAg loss*: The serum level of HBeAg was tested at baseline and week 104.					

Hepatic lobular architecture restoration versus liver fibrosis regression

The proportions of reverse and non-reverse patients in restored and non-restored groups are shown in Figure 4. Based on the cutoff value of $GS-index_{78w}=50\%$ (Figure 4A and B), the proportions of reverse patients were 75% (12/16) in restored and 41% (11/27) in non-restored groups, according to Ishak score. The proportions of non-reverse patients were 25% (4/16) in restored and 59% (16/27) in non-restored patients ($P=0.029$). In addition, the proportions of R and I/P patients were 79% (11/14) and 21% (3/14) in restored patients, and 37% (10/27) and 63% (17/27) in non-restored patients, according to P-I-R score ($P=0.012$).

Based on Ishak score, the median value of the GS-index increased from 0% (baseline) to 53% (week 78) in reverse patients, and 14% (baseline) to 31% (week 78) in non-reverse patients ($P=0.009$). Changes of GS-index from baseline to week 78 ($\Delta GS-index_{78w-0w}$) were 41% and 10% in reverse and non-reverse patients ($P=0.002$). Based on P-I-R score at week 78, the median of GS-index increased from 0 and 16% (baseline) to 50% and 25% (week 78) in reverse and non-reverse patients, respectively ($P=0.004$). The values of $\Delta GS-index_{78w-0w}$ were 43% in reverse patients and 10% in non-reverse patients ($P=0.001$) (Supplementary Figure 1). Therefore, the improvement of GS-index is more significant in reverse patients than in non-reverse patients after antiviral therapy, regardless of Ishak or P-I-R score assessment.

Two representative cases of restored and non-restored patients with paired liver biopsies are shown (Figure 5). For the restored patient, GS-index increased dramatically from 0% (baseline) to 75% (week 78) ($GS-index_{78w}=75\%$); Ishak fibrosis score decreased from 5 to 3; and the level of LSM decreased from 12.5

kPa to 6.8 kPa. For the non-restored patient, the dynamic changes observed for GS-index were 0% (baseline) to 13% (week 78) ($\text{GS-index}_{78\text{w}}=13\%$). Ishak fibrosis score remained at stage 5 after 78 weeks of treatment although LSM slightly decreased from 30.4 kPa (baseline) to 25.7 kPa (week 78).

Discussion

In this study, we proposed a histological parameter (GS-index) to quantify hepatic lobular architecture restoration in CHB patients in the era of antiviral therapy. Patients in the restored group were characterized by better clinical and morphological improvements than in the non-restored group. In addition, we observed through the GS-index that reverse patients attain more significant hepatic architecture restoration than non-reverse patients.

The GS-index was measured by the proportion of normal HVs in all HVs. Recently, Wanless updated the observation of the parenchymal extinction sequence (PE sequence), which consists of 9 levels in the natural history of parenchymal extinction lesions (PELs)¹⁶; this can be easily quantified by GS staining. Based on PELs, he proposed the VPA index which was defined as the proportion of PTs attaching to adjacent HVs. Thus, both the GS-index and the VPA index indicate that the approximation of HVs and PT is a key histological feature of advanced liver fibrosis.

Loss of normal lobular architecture is one of crucial functional consequences of liver fibrosis progression leading to portal hypertension and liver decompensation.^{1,2} In a study of HCV cirrhotic patients with paired liver biopsies, D'Ambrosio et al. observed that the proportion of patients with normal lobular metabolic zonation was increased by 50% (19/38) after 61 months of sustained virological response (SVR), when compared to pretreatment biopsies.¹⁷ For CHB patients with advanced liver fibrosis, our investigations revealed that hepatic lobular architecture restoration and clinical characteristics, such as liver function and LSM, can both be improved by efficacious antiviral treatment.

It is well documented that liver fibrosis can be reversed after successful suppression of HBV replication by antiviral treatment^{18,19}. Our study illustrated the relationship between hepatic lobular architecture restoration and fibrosis regression. There is corresponding improvement in the health of the patients with degradation of collagen fibers and elongation of the distance between HVs and PT to achieve hepatic lobular zonation restoration. We also observed that the extent of restoration is different between reverse and non-reverse patients during longitudinal follow-up period.

In this study, the definition of normal lobular architecture is that HVs do not approximate PTs. The results of our analysis indicated that the elongation of the distance between HVs and PTs is a key histological feature of hepatic lobular architecture restoration and liver fibrosis regression. Hadi et al. proposed that PT-HVs approximation remains after fibrosis regression, manifesting as fibrous septa resorption.²⁰ The enrollment criteria for this study included regression cases with the morphological feature of veno-portal approximation, which may lead to selection bias. In comparison with this cross-sectional study, our

research is based on a national and multicenter cohort of patients in which sequential liver biopsies were performed during longitudinal follow-up period, making our results more robust.

The $GS\text{-index} \geq 50\%$ threshold of lobular restoration was proposed after long deliberation based on the following considerations: i) based on this cutoff value, restored patients can attain additional improvement in both clinical and histological characteristics compared to non-restored patients; ii) since there is no gold standard of hepatic lobular restoration assessment, in analogy with the previous semi-quantification assessment of liver fibrosis (Ishak system), which defined the “quantitative” indicators (“few”, “some” and “most”) based on the threshold of half of the portal tract or central area in routine practice,²³ we attempted to defined lobular restoration as the half of the HVs in the whole liver biopsy not approximating to PT at week 78. The definition of hepatic lobular restoration needs to be confirmed in further investigation with the data of longer prospective follow up and clinical endpoints.

The novelty of this study is the type of insight being proposed. The GS-index has two important clinical implications. Firstly, the utility of GS-index facilitates the quantification of hepatic lobular zonation restoration. Secondly, the dynamic changes of GS-index add a new dimension, which is independent of fibrosis severity and inflammatory activity, for disease evolution assessment.

Our study has certain limitations. First, loss of GS expression following loss of endothelium-hepatocyte proximity or contact was observed in severe cirrhosis, especially Laennec 4B or 4C, leading to underestimation of HVs.^{8,21-24} Second, thick collagen fibers and contiguous liver parenchymal collapse can interfere with the perception of HVs and PTs. Third, the opening up of new HVs in cirrhosis regression is uncertain; therefore, we may have to approach this dilemma by counting the increase in the number of new veins rather than separating them from PTs. Last, overstaining in some parts of the specimens might lead to confusion in terms of identifying the HVs.

In conclusion, our study draws attention to the hepatic lobular architecture restoration assessment for CHB patients and the relationship to liver fibrosis regression. Restored patients can achieve greater improvements in histological and clinical features than non-restored patients. Hepatic lobular architecture can be restored through liver fibrosis regression.

Abbreviations

HBV, hepatitis B virus; CHB, chronic hepatitis B; GS, glutamine synthetase; GS-index, glutamine synthetase index; PT, portal tract; HV, hepatic vein; scRNA-seq, single-cell RNA sequencing; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e-antigen; LSM, liver stiffness measurement; H&E, hematoxylin and eosin; IHC, immunohistochemistry; HAI, histology activity index; P-I-R, predominantly progressive, indeterminate, and predominately regressive; GS_{HV} , HVs not approximating to PTs; GS_{PT} , HVs approximating/incorporated into PTs; IQR, interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; PLT, platelet; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; SD,

standard deviation; PE sequence, parenchymal extinction sequence; PELs, parenchymal extinction lesions; SVR, sustained virological response.

Declarations

Funding: This study was funded by National Science and Technology Major Project (2018ZX10302204, 2018ZX10302204-004) and National Natural Science Foundation of China (81800535 and 81970524).

Conflicts of interest: All authors have nothing to disclose.

Ethics approval: The study was conducted in accordance with the principles enshrined in the Declaration of Helsinki and the Good Clinical Practices. The Ethics Committee of all participating centers approved the study protocol (2016-P2-021-02).

Consent to participate: All patients gave written informed consent prior to their enrollment.

Consent for publication: All authors had access to the study data and reviewed and approved the final manuscript.

Availability of data and material: Not applicable.

Code availability: Not applicable.

Author Contribution:

Study design: HY and JDJ

Liver biopsy assessment: TLW, HL, YMS, AW, XYZ, SYC

Data collection: JLZ, YMS, SYC, XNW, TTM, BQW, XJO

Statistical analysis: SYC, YMS, SSW, YYK

Manuscript writing: SYC

Critical revision of the manuscript: YMS, AW, HY

References

1. Bedossa P. Reversibility of hepatitis B virus cirrhosis after therapy: who and why? *Liver Int* 2015;35:78-81.
2. Garcia-Pagan JC, Gracia-Sancho J, Bosch J. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. *J Hepatol* 2012;57:458-461.

3. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013;381:468-475.
4. Kong Y, Sun Y, Zhou J, Wu X, Chen Y, Piao H, et al. Early Steep Decline of Liver Stiffness Predicts Histological Reversal of Fibrosis in Chronic Hepatitis B Patients Treated With Entecavir. *J Viral Hepat* 2019;26:576-585.
5. Wanless IR, Nakashima E, Sherman M. Regression of Human Cirrhosis. Morphologic Features and the Genesis of Incomplete Septal Cirrhosis. *Arch Pathol Lab Med* 2000;124:1599-1607.
6. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991;13:372-374.
7. Batts KP, Ludwig J. Chronic Hepatitis. An Update on Terminology and Reporting. *Am J Surg Pathol* 1995;19:1409-1417.
8. Fleming KE, Wanless IR. Glutamine Synthetase Expression in Activated Hepatocyte Progenitor Cells and Loss of Hepatocellular Expression in Congestion and Cirrhosis. *Liver Int* 2013;33:525-534.
9. Aizarani N, Saviano A, Sagar, Mailly L, Durand S, Herman JS, et al. A Human Liver Cell Atlas Reveals Heterogeneity and Epithelial Progenitors. *Nature* 2019;572:199-204.
10. Kiernan F. The anatomy and physiology of the liver. *Pilos Trans R Soc Lond* 1833;123:711-770.
11. Lefkowitz JH. Scheuer's Liver Biopsy Interpretation. 9th ed. Edinburgh: Elsevier, 2015:11-16.
12. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-699.
13. Sun Y, Zhou J, Wang L, Wu X, Chen Y, Piao H, et al. New classification of liver biopsy assessment for fibrosis in chronic hepatitis B patients before and after treatment. *Hepatology* 2017;65:1438-1450.
14. Theise ND, Jia J, Sun Y, Wee A, You H. Progression and regression of fibrosis in viral hepatitis in the treatment era: the Beijing classification. *Mod Pathol* 2018;31:1191-1200.
15. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008;48:835-847.
16. Wanless IR. The Role of Vascular Injury and Congestion in the Pathogenesis of Cirrhosis: the Congestive Escalator and the Parenchymal Extinction Sequence. *Curr Hepatol Rep* 2020;19:40-53.
17. D'Ambrosio R, Aghemo A, Rumi MG, Ronchi G, Donato MF, Paradis V, et al. A Morphometric and Immunohistochemical Study to Assess the Benefit of a Sustained Virological Response in Hepatitis C Virus Patients With Cirrhosis. *Hepatology* 2012;56:532-543.
18. Sun Y, Wu X, Zhou J, Meng T, Wang B, Chen S, et al. Persistent Low Level of Hepatitis B Virus Promotes Fibrosis Progression During Therapy. *Clin Gastroenterol Hepatol* 2020;18:2582-2591.e6.
19. Wang J, Yu Y, Li G, Shen C, Meng Z, Zheng J, et al. Relationship between serum HBV-RNA levels and intrahepatic viral as well as histologic activity markers in entecavir-treated patients. *J Hepatol* 2018;68:16-24.

20. Hadi R, Shin K, Reder N, Alpert L, Koch L, Choi WT, et al. Utility of glutamine synthetase immunohistochemistry in identifying features of regressed cirrhosis. *Mod Pathol* 2020,33:448-455.
21. Kuo FC, Darnell JE Jr. Evidence that interaction of hepatocytes with the collecting (hepatic) veins triggers position- specific transcription of the glutamine synthetase and ornithine aminotransferase genes in the mouse liver. *Mol Cell Biol* 1991,11:6050-6058.
22. Kim MY, Cho MY, Baik SK, Park HJ, Jeon HK, Im CK, et al. Histological subclassification of cirrhosis using the Laennec fibrosis scoring system correlates with clinical stage and grade of portal hypertension. *J Hepatol* 2011,55:1004-1009.
23. Saxena R. *Practical Hepatic Pathology: A Diagnostic Approach*, Philadelphia, Saunders, 2011, 201-213.
24. Kim SU, Oh HJ, Wanless IR, Lee S, Han KH, Park YN. The Laennec staging system for histological sub-classification of cirrhosis is useful for stratification of prognosis in patients with liver cirrhosis. *J Hepatol* 2012,57:556-563.

Figures

[illegible]

Definition of “GS-index”. A. GS expression in normal human liver. B. The definition of GS-index. GSHV (blue boxes) is defined as GS-positive perivenular hepatocytes around hepatic vein (HV) in the center of lobules, GSPT (red boxes) is defined as GS-positivity approximating portal tract (PT), green dotted lines indicate portal tract. “GS-index” is defined as percentage of GSHV/(GSHV+GSPT).

Figure 2

Distribution and dynamic changes of GS-index in paired liver biopsies from CHB patients at baseline and week 78. A. Distribution of GS-index in all patients. B. Dynamic changes of GS-index showing significant changes of GS-index (solid lines) and non-significant changes (dotted lines).

Based on $GS-index_{78w} = 50\%$

Figure 3

Dynamic changes of GS-index in lobular architecture restored and non-restored CHB patients at baseline and week 78 based on $GS-index_{78w}$. A. Dynamic changes of GS-index in restored and non-restored patients. B. The $\Delta GS-index_{78w-0w}$ in restored and non-restored patients. Restored patients (blue) are defined as $GS-index_{78w} \geq 50\%$, non-restored patients (red) are defined as $GS-index_{78w} < 50\%$.

Based on GS-index_{78w} = 50%

Figure 4

Proportions of reverse and non-reverse patients, according to Ishak and P-I-R score (Beijing classification) at week 78, in restored and non-restored groups. A. The proportions of reverse and non-reverse patients based on Ishak scoring of the fibrosis stage. B. The proportions of reverse and non-reverse patients based on P-I-R scoring of the degree of fibrosis. Fibrosis reverse (green) is defined as decrease in Ishak fibrosis score or R (predominantly regressive), fibrosis non-reverse (orange) is defined as Ishak fibrosis score increase by ≥ 0 or P/I (predominantly progressive/indeterminate).

Figure 5

Paired liver biopsies from restored and non-restored CHB patients illustrating changes in hepatic lobular architecture and GS-index between baseline and week 78. A. Restored patient shows vastly improved GS-index with corresponding decrease in Ishak fibrosis scores and LSM values. B. Non-restored patient shows no improvement in GS-index, stable Ishak fibrosis score but decrease in LSM values.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure1.tiff](#)