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VIRAL HEPATITIS

New Classification of Liver Biopsy Assessment for Fibrosis in Chronic Hepatitis B Patients Before and After Treatment

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Liver fibrosis is the net result of dynamic changes between fibrogenesis and fibrolysis. Evidence has shown that antiviral therapy can reverse liver fibrosis or even early cirrhosis caused by hepatitis B virus. However, current evaluation systems mainly focus on the severity of, but not the dynamic changes in, fibrosis. Here, we propose a new classification to evaluate the dynamic changes in the quality of fibrosis, namely: *predominantly progressive* (thick/broad/loose/pale septa with inflammation); *predominately regressive* (delicate/thin/dense/splitting septa); and *indeterminate*, which displayed an overall balance between progressive and regressive scarring. Then, we used this classification to evaluate 71 paired liver biopsies of chronic hepatitis B patients before and after entecavir-based therapy for 78 weeks. *Progressive, indeterminate*, and *regressive* were observed in 58%, 29%, and 13% of patients before treatment versus in 11%, 11%, and 78% after treatment. Of the 55 patients who showed predominantly regressive changes on posttreatment liver biopsy, 29 cases (53%) had fibrosis improvement of at least one Ishak stage, and, more interestingly, 25 cases (45%) had significant improvement in terms of Laennec substage, collagen percentage area, and liver stiffness despite remaining in the same Ishak stage. *Conclusion*: This new classification highlights the importance of assessing and identifying the dynamic changes in the quality of fibrosis, especially relevant in the era of antiviral therapy.(HEPATOLOGY 2017;65:1438-1450)

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Systems for classification of histological findings in liver biopsy specimens (LBx) from patients with chronic viral hepatitis were first introduced and widely used in 1968 following the discovery of hepatitis B virus (HBV). Several histological classifications of chronic hepatitis have been proposed by different groups of pathologists.[1-7] These systems rapidly became important in assessing and reporting of responses to antiviral therapy in clinical trials and ultimately became a central feature for decision making about who should undergo treatment.[8]

Abbreviations: ALB, albumin; ALT, alanine transferase; AST, aspartate transferase; CHB, chronic hepatitis B; CPA, collagen percentage area; DILI, drug/toxin-induced liver injury; LBx, liver biopsy; HAI, histology activity index; H&E, hematoxylin and eosin; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immuno-deficiency virus; HRC, hepatic repair complex; HVPG, hepatic venous wedge pressure; IQR, interquartile range; kPa, kilopascals; LBx, liver biopsy; LSMs, liver stiffness measurements; P-I-R, predominantly progressive, indeterminate and predominately regressive; PLT, platelet; SHG/TPEF, second harmonic generation/two photon excitation fluorescence; ULN, upper limit of normal.

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However, in later stages of fibrosis, it is recognized that there remain differences that are not captured by traditional staging systems. To address this issue, the Laennec classification was developed to subclassify cirrhosis (designated "4") into 4A, 4B, and 4C based on the semiquantitation of the area of fibrosis in the specimen.[9,10] Similarly, other systems quantified the "proportionate area of collagen" by morphometry.[11] These subclassifications of advanced-stage liver disease showed strong correlations with measures of portal hypertension, in particular, hepatic venous wedge pressure (HPVG), but they have not demonstrated utility in addressing more recent questions being raised in the clinical setting, namely, regarding prognosis following successful viral suppression or eradication. In particular, these refinements have not been demonstrated to be predictive of who will respond completely to successful viral suppression or eradication as opposed to continuing to decompensate despite successful antiviral therapy.

It is regarding this area of concern that we highlight a histopathological feature that has not been included in previous classification schema: aspects of regression of advanced-stage (developing or established cirrhosis) liver disease, in particular, as they have been elucidated by Ian Wanless and colleagues in a seminal demonstration of cirrhotic regression after successful anti-HBV therapy.[12] In this article, Wanless et al. described eight parameters indicative of regression of cirrhosis, collectively termed the "hepatic repair complex" (HRC): four describing changes in the qualities of stroma/parenchymal relationships (delicate perforated fibrous septa, isolated thick collagen fibers, delicate periportal fibrous spikes, and hepatocytes within or splitting septa); three describing vascular alterations (portal tract remnants, hepatic vein remnants with prolapsed hepatocytes, and aberrant parenchymal veins); and one describing parenchymal regeneration (minute regenerative nodules of "buds").

We hypothesized that the proportion of progressive scarring compared to the prevalence of HRC parameters in posttreatment LBx might provide prognostically useful information. We suggest that patients who do not improve or continue to decompensate after complete viral suppression or eradication have developed architectural and structural distortions in stroma and

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vascular supply that are self-perpetuating in a positive feedback loop of vascular insufficiency, parenchymal extinction, further scarring, and still more vascular insufficiency. Thus, we believe that the predominance of active scarring over HRC elements in posttreatment LBx would be evidence of self-perpetuating injury and therefore will be prognostic and clinically useful.

Because the HRC vascular parameters are few to absent in most LBx and because the parenchymal buds are best observed in larger tissue samples rather than in needle cores, we focused on the stromal/parenchymal parameters.[13,14] To develop our new "Beijing classification," we examined pretreatment and posttreatment LBx from patients with HBV. We herein suggest that, in addition to traditional assessments of hepatitis activity and stage of scarring, there is utility in assessment of the balance between progressive and regressive liver disease, namely, three classification categories of the qualities of fibrosis: *predominantly progressive, predominately regressive*, and *indeterminate*. Correlation with other clinical features and more specialized techniques of gross (i.e., imaging and liver elastography) and microscopic (i.e., second harmonic generation) liver assessment provides support for our suggested approach.

Patients and Methods

STUDY POPULATION

A total of 112 chronic hepatitis B (CHB) patients with paired liver biopsy before and after treatment were included in this study. Key inclusion criteria were as follows: aged 18-65 years; hepatitis B surface antigen (HBsAg) positivity for at least 6 months before screening; treatment-naïve; HBV-DNA levels higher than 20,000 IU/mL for hepatitis B e antigen (HBeAg)-positive patients or 2,000 IU/mL for HBeAg-negative patients; and paired liver biopsies performed at both baseline and week 78.

Exclusion criteria included coinfection with hepatitis C or human immunodeficiency virus (HIV); the presence of other forms of chronic liver disease; decompensated liver diseases (including ascites, variceal bleeding, or hepatic encephalopathy); alpha-fetoprotein > 100 ng/mL or creatinine > 1.5 × upper limit of normal (ULN); any malignant tumor; any complications of severe heart, lung, kidney, brain, or blood diseases or other important systematic diseases; severe neurological or psychological disease; and pregnant or lactating women.

Patients received entecavir-based treatment for 78 weeks. Liver biopsy was performed at baseline and after 78 weeks of therapy (Fig. 1). Demographic data included sex and year of birth. Clinical laboratory tests were collected at baseline and at every 6-month follow-up.

The study was conducted in accord with the Declaration of Helsinki. The study protocol was approved by the ethics committee of all participating institutions. Written informed consent was provided by all patients. Studies were registered with the ClinicalTrials.gov identifiers NCT01938781 and NCT01938820.

HISTOLOGICAL EVALUATIONS

Liver biopsy specimens were formalin fixed, paraffin embedded, and sectioned by using standard clinical techniques.[15] Five-micrometer sections were stained with hematoxylin and eosin (H&E), reticulin, and Masson's trichrome. All liver biopsy samples were evaluated by an experienced hepatopathologist (N.D. Theise), blinded to treatment assignment, biopsy sequence, biochemical response, and liver stiffness values, in one sitting, independently.

Necroinflammation activity and fibrosis stage were assessed by the Ishak modified histology activity index

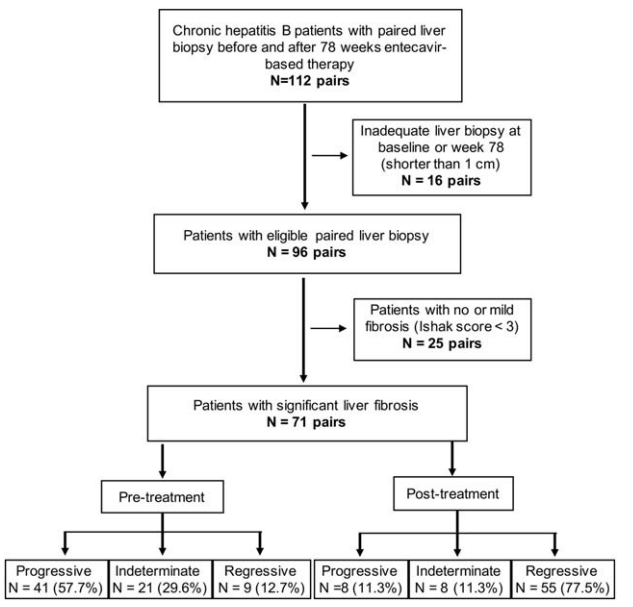

FIG. 1. Flow chart for patient enrollment and evaluation. A total of 71 patients with satisfactory paired biopsies were enrolled in the study. Inflammation HAI score, Ishak fibrosis score, and new classification of fibrosis quality were evaluated for each biopsy.

(HAI) grading and staging system.[4] Cirrhosis (Ishak stage 5-6) was further subclassified into 4A, 4B, and 4C according to the Laennec system.[9,10]

In addition, a new classification was proposed to evaluate the qualities of fibrosis activity based on the balance between progressive and regressive liver disease. This approach was applied for LBx with at least focal fibrous septa formation or higher stages of scarring. The classification consisted of three categories of fibrosis quality: *predominantly progressive, indeterminate* and *predominately regressive* (P-I-R score). These were based on low power ($2\times$ or $4\times$) examination of the H&E, trichrome, and reticulin stains LBx:

- **Predominantly progressive**: defined as *most* (more than 50%) fibroseptal stroma in the LBx showing wide/broad, loosely aggregated collagen fibers, often a mix of light and dark staining fibers on trichrome, which are moderately to markedly cellular containing, variably, inflammatory cells, macrophages, and ductular reactions (Figs. 2A,B and 3A).
- **Indeterminate**: defined as an uncertain mix/balance between progressive and regressive scarring. If the examining pathologist cannot come to a conclusion as to whether progressive or regressive scarring predominates, or is tempted to go to higher magnification in order to assess the balance, the specimen was categorized as indeterminate (Fig. 3B).
- **Predominately regressive**: defined as *most* (more than 50%) fibroseptal stroma in the LBx showing features of HRC, namely, thin, densely compacted stroma, largely darkly staining on trichrome, which are largely acellular (Figs. 2C,D and 3C).

Fibrosis regression was defined as Ishak decreasing $\geq$1 score after treatment. "Absolutely reversing or advancing" was defined as same directionality implied by both change of Ishak score and posttreatment P-I-R score; and "probably reversing or advancing" was defined as only one parameter showed directionality.

INTEROBSERVER VARIATION ASSESSMENT

For interobserver variation assessment, seven pathologists (six self-identified as specialized hepatopathologists, one self-identified as a general surgical pathologist without focused liver pathology training or practice) and five hepatologists and one nonphysican scientist (engineer; listed in Acknowledgements). Participants were trained on-site in English and in Chinese (N.D.T., H.Y.) with review of figures from this article, question and answers from participants, and review of nine teaching cases with open discussion and consultation. Then, 30 coded cases of LBx of this cohort using P-I-R score were presented. All members were blinded to LBx sequence and clinical data except for the presence of CHB. Each pathologist, hepatologist, and the one engineer reviewed the 30 cases of slides independently, without consultation or discussion.

COLLAGEN PERCENTAGE MEASUREMENT

Total collagen percentage area (CPA) was measured using second harmonic generation/two photon excitation fluorescence (SHG/TPEF) technology-based microscope (Genesis200TM; HistoIndex Pte. Ltd, Singapore).[16] Image acquisition was performed by a $20\times$ objective on unstained liver biopsy samples with 512×512 pixel resolution of each $200\times200\ \mu m^2$ tile. In order to cover all the sample areas and to avoid missing information, each biopsy tissue was fully scanned with multiple adjacent images, which were stitched to form a whole slide scan. SHG microscopy was used to visualize collagen, whereas TPEF microscopy was used to identify other cell structures.[17] Collagen was edited to exclude collagenous structures, including image artifacts and structural collagen in large portal tracts and blood vessel walls. The area percentage of TPEF signals in the image was used to normalize SHG signals, so the normalized total collagen percentage can be compared in further analysis.

LIVER STIFFNESS MEASUREMENTS AND LABORATORY ASSESSMENTS

Liver stiffness measurements (LSMs) taken by transient elastography (Fibroscan; Echosens, Paris, France) were performed by experienced operators according to described methods.[18] Only LSM values with at least 10 valid measurements, a success rate $\geq$60%, and the interquartile range (IQR)-to-liver stiffness ratio $\leq$ 30% were considered reliable. Liver stiffness is expressed in kilopascals (kPa).

Laboratory tests, including liver biochemistries and platelet count, were performed at local laboratories according to standard procedures. Serum HBV-DNA

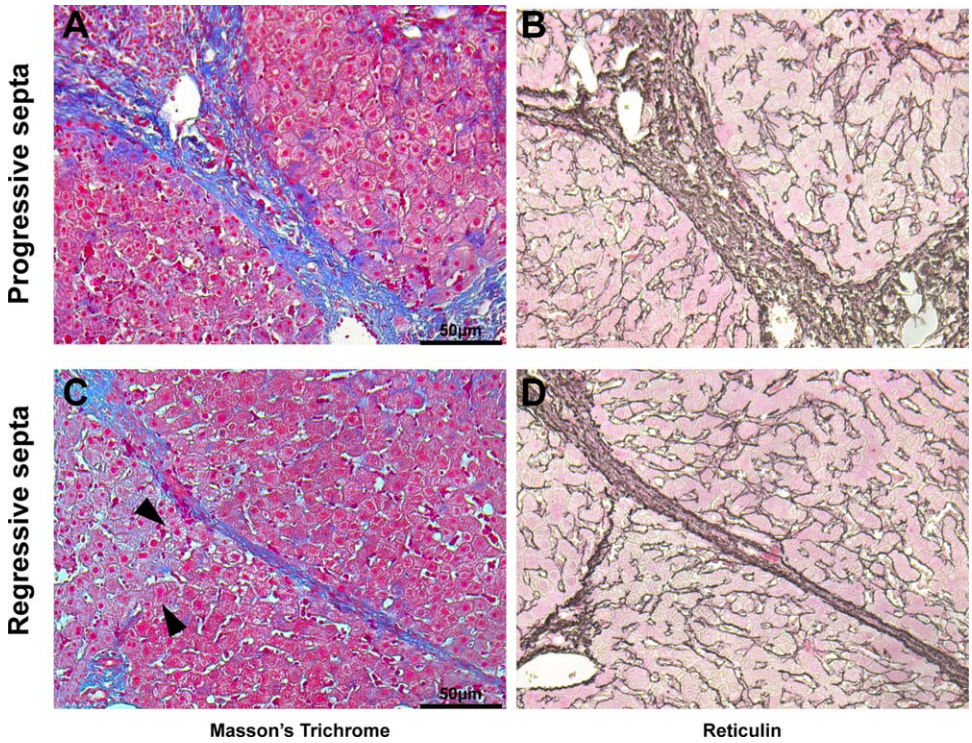

FIG. 2. Liver biopsy samples of patients with progressive and regressive septa. (A,B) Progressive fibrosis septa are wide/broad, loosely aggregated collagen fibers, often a mix of light and dark staining fibers on trichrome. (C,D) Regressive features are scars with thin, densely compacted stroma, largely darkly staining on trichrome. Septum can be fragmented and interrupted by hepatocytes (arrowhead).

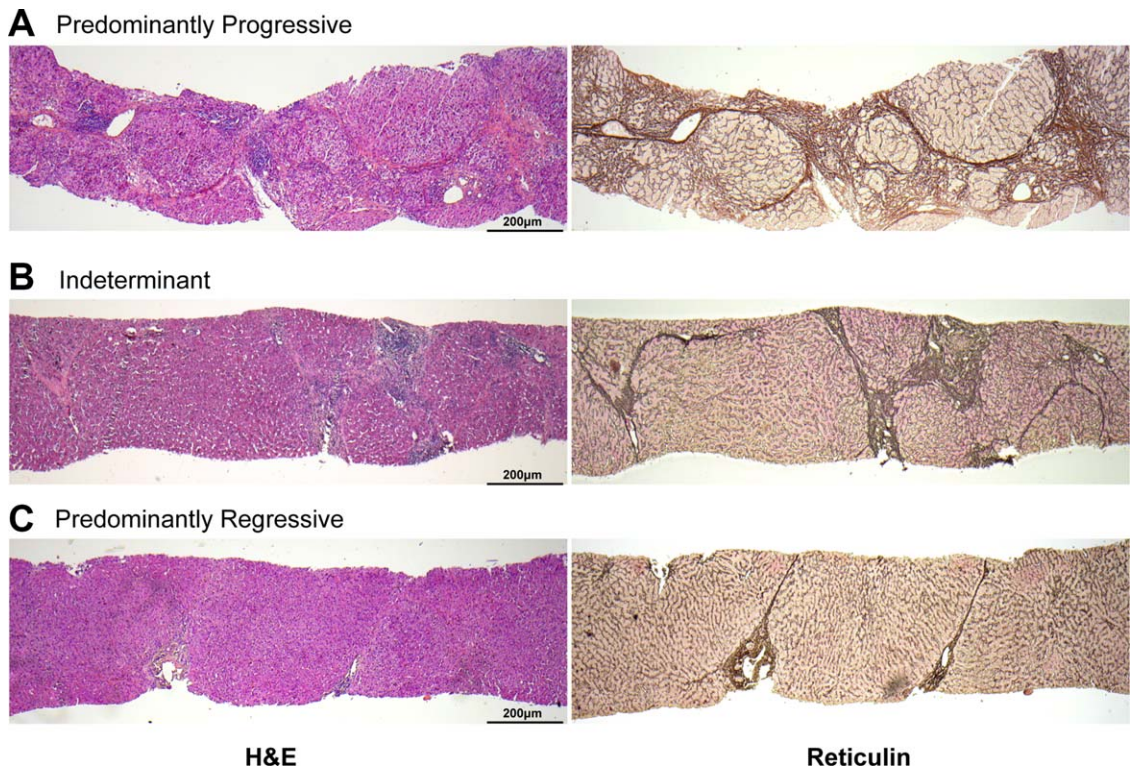

FIG. 3. New fibrosis quality classification. According to the morphological balance between progressive and regressive of fibrosis/scarring. There were three classification categories: predominantly progressive (A), indeterminate (B), and predominately regressive (C).

level was measured centrally with the Roche COBAS TaqMan HBV Test, a real-time TaqMan PCR assay (lower limit of quantification = 20 IU/mL). HBV serological markers (HBsAg, hepatitis B surface antibody, HBeAg, hepatitis B e antibody, and hepatitis B core antibody) were measured by Abbott Architect i2000 (Abbott, Wiesbaden, Germany).

STATISTICAL ANALYSIS

Continuous variables are expressed in median (IQR) or mean ± SD. Categorical variables were summarized by counts and percentages. One-way analysis of variance and Kruskal–Wallis test were used to compare the P-I-R score in specific conditions. Chi-squared test and Fisher's exact test were used for categorical variables. McNemar's test was used to assess the changes of histological scores pretreatment and posttreatment. Paired-samples *t* test and Wilcoxon signed-rank test were used to compare the changes of clinical data pretreatment and posttreatment. Fleiss's kappa statistics were used to assess the interobserver agreement. All statistical tests were two-sided. Statistical significance was considered when $P < 0.05$. Data were analyzed using SPSS software (version 22.0; SPSS, Inc., Chicago, IL).

Results

PATIENT ENROLLMENT AND CHARACTERISTICS

There were 112 anti-HBV treatment-naïve patients with LBx for assessment of pretreatment grading of necroinflammation and staging of fibrosis enrolled in this study. After entecavir-based therapy for 1.5 years, 96 with paired LBx and sufficient tissue for assessment were evaluated as part of the clinical study. Of these, 71 patients who had pretreatment or posttreatment LBx with significant fibrosis or cirrhosis (Ishak ≥ 3) were analyzed for the purposes of this follow-up study. All cases were followed up with longitudinal clinical data and liver stiffness every 6 months. The flow chart of the patient enrollment is shown in Fig. 1.

The enrolled patients were predominantly (80%) male with a mean age of 40 years. At baseline, 79% were HBeAg positive, with a median level of HBV DNA, alanine aminotransferase (ALT) and LSM being 7.0 log IU/mL, 83.0 IU/mL, and 12.0 kPa, respectively. The demographic features of the patients are shown in Supporting Table S1.

HISTOLOGICAL ASSESSMENT OF LBx

Necroinflammatory Activity

Full data for necroinflammatory activity are summarized in Supporting Table S2. After 78 weeks of treatment, necroinflammation improved remarkably, with the median Ishak HAI score being decreased from 7 to 4 ($P < 0.001$). The proportion of patients with mild or no necroinflammation (necroinflammatory scores ≤ 3) increased from 3% (2 of 71) at baseline to 28% (20 of 71) at week 78. In all of the 17 patients with highly active disease (HAI > 10) at baseline, HAIs were reduced to mildly active or inactive scores after treatment (Supporting Table S2).

Fibrotic Staging (Ishak and Laennec)

Data for fibrotic staging are shown in Table 1 and in Supporting Table S2. As evaluated by Ishak score changes, fibrosis was reversed in 33 of 71 patients (46%), no change in 35 of 71 patients (49%), and progressed (increased one stage) in 3 of 71 (4%; Table 2). Of note is that the proportion of patients with cirrhosis decreased from 72% (51 of 71) at baseline to 52% (37 of 71) at week 78 (Supporting Table S2). Of the 3 patients with increasing stage, 1 had overt hepatic steatosis attributed to clinically confirmed frequent alcohol drinking after starting the treatment; the other 2 patients had no identifiable concomitant causes, which could explain the progression of liver disease.

If further subclassified by the Laennec fibrosis scoring system, the proportion of cirrhosis 4A increased from 30% (21 of 71) at baseline to 44% (31 of 71) at week 78, whereas the proportion of 4C decreased from 14% (10 of 71) to 4% (3 of 37; Supporting Table S2).

P-I-R Classification of Liver Fibrosis Quality

Then, we used the P-I-R classification to evaluate 71 paired liver biopsies of CHB patients before and after entecavir-based therapy for 78 weeks.

Before treatment, there were 58%, 29%, and 13% of biopsies in the progressive, indeterminate, and regressive groups, respectively (Table 2). There were significant differences of serum ALT, aspartate aminotransferase (AST), albumin (ALB), and HBeAg positivity percentage among the three categories. The progressive group showed the higher virus load and more-severe inflammation as well, followed, in turn,

TABLE 1. Posttreatment P-I-R Score Versus Changes of Ishak Stage to Evaluate Disease Progress or Reverse

	Posttreatment P-I-R Score (n = 71)		
Ishak (Pre-Post)	Progressive (n = 8)	Indeterminate (n = 8)	Regressive (n = 55)
Increase, n = 3	Absolutely advancing 67% (2 of 3)	0	33% (1 of 3)
Stable, n = 35	Probably advancing 17% (6 of 35)	11% (4 of 35)	Probably reversing 72% (25 of 35)
Decrease,n = 33	0	12% (4 of 33)	Absolutely reversing 88% (29 of 33)

TABLE 2. Patient Characteristics According to P-I-R Classification Pretreatment and Posttreatment

	Progressive	Indeterminate	Regressive	*P* Value
Pretreatment				
N (%)	41 (57.7)	21 (29.6)	9 (12.7)	—
Age, years	38 ± 10	37 ± 9	37 ± 8	0.879
Sex (male), n (%)	34 (82.9)	15 (71.4)	8 (88.9)	0.523
PLT, $\times 10^9$/L	160.0 ± 55.9	168.5 ± 68.7	160.6 ± 45.8	0.859
ALT, U/L	94.7 (54.8, 167.5)	75.0 (40.2, 116.5)	35.0 (31.6, 45.1)	0.001
AST, U/L	66.0 (44.3, 140.5)	46.0 (34.5, 72.1)	33.6 (27.5, 37.6)	<0.001
ALB, g/L	41.6 (38.8, 44.1)	44.0 (41.9, 46.3)	47.2 (44.9, 50.9)	0.004
HBeAg (+), n (%)	37 (90.2)	14 (66.7)	5 (55.6)	0.013
HBV DNA, log IU/mL	7.1 ± 1.3	6.9 ± 1.2	5.4 ± 1.6	0.003
LSM, kPa	14.1 (11.5, 18.0)	8.9 (6.4, 11.8)	7.3 (6.8, 11.6)	<0.001
CPA	5.3 (3.3, 8.8)	3.3 (2.4, 4.5)	2.6 (1.9, 4.5)	0.001
Necroinflammation score, n (%)				<0.001
0-3, n = 2	0	1 (4.8)	1 (11.1)	
4-6, n = 29	9 (22.0)	13 (61.9)	7 (77.8)	
7-9, n = 23	17 (41.5)	5 (23.8)	1 (11.1)	
≥10, n = 17	15 (36.6)	2 (9.5)	0	
Ishak score, n (%)				0.134
3, n = 11	4 (9.8)	5 (23.8)	2 (22.2)	
4, n = 9	3 (7.3)	3 (14.3)	3 (33.3)	
5, n = 23	14 (34.1)	6 (28.6)	3 (33.3)	
6, n = 28	20 (48.8)	7 (33.3)	1 (11.1)	
Posttreatment				
N (%)	8 (11.3)	8 (11.3)	55 (77.5)	—
Age, years	38 ± 11	36 ± 9	38 ± 10	0.888
Sex (male), n (%)	6 (75.0)	8 (100.0)	43 (78.2)	0.457
PLT, $\times 10^9$/L	147.6 ± 56.9	167.3 ± 56.9	153.4 ± 52.6	0.739
ALT, U/L	29.2 (14.0, 36.8)	40.4 (24.8, 50.8)	22.0 (16.0, 31.0)	0.021
AST, U/L	25.1 (17.5, 33.0)	32.6 (30.7, 40.5)	22.0 (17.0, 27.0)	0.002
ALB, g/L	42.5 ± 3.9	46.1 ± 2.6	44.8 ± 3.3	0.092
HBV DNA, Log IU/mL	0.7 (0, 1.5)	0 (0, 1.6)	0 (0, 1.4)	0.552
HBV DNA undetectable rate, n (%)	4 (50.0)	5 (62.5)	41 (74.5)	0.316
LSM, Kpa	7.7 (6.0, 13.9)	6.9 (4.9, 10.8)	6.8 (5.3, 8.6)	0.441
CPA	4.3 (2.2, 8.8)	4.2 (1.9, 6.2)	3.0 (2.0, 4.4)	0.324
Necroinflammation score, n (%)				1.000
0-3, n = 20	2 (25.0)	2 (25.0)	16 (29.1)	
4-6, n = 48	6 (75.0)	6 (75.0)	36 (65.5)	
7-9, n = 3	0	0	3 (5.5)	
≥10, n = 0	0	0	0	
Ishak score, n (%)				0.469
3, n = 19	1 (12.5)	2 (25.0)	16 (29.1)	
4, n = 15	0	2 (25.0)	13 (23.6)	
5, n = 25	5 (62.5)	2 (25.0)	18 (32.7)	
6, n = 12	2 (25.0)	2 (25.0)	8 (14.5)	

by the indeterminate and regressive groups. Liver stiffness was significantly higher ($P < 0.001$) in progressive (14.1 kPa) than in indeterminate (8.9 kPa) and regressive (7.3 kPa).

After treatment, there were 11%, 11%, and 78% of biopsies in the progressive, indeterminate, and regressive groups, respectively (Table 2). ALT and AST levels were lower in the regressive group than in the progressive and indeterminate groups ($P < 0.05$). A total of 75% of patients in the regressive group were HBV-DNA undetectable, which was more than that of patients in the progressive (50%) and indeterminate groups (63%). Liver stiffness was not significantly different ($P = 0.514$) in the progressive (7.7 kPa), indeterminate (6.9 kPa), and regressive groups (6.8 kPa) after treatment.

Pretreatment and posttreatment clinical data were compared among each category. In the regressive group, ALT and ALB had significant improvement after 78 weeks of therapy (Supporting Table S3). ALT decreased from 83.1 to 22.0 U/L ($P < 0.05$) and ALB increased from 43.1 to 44.8 ($P < 0.05$); both were better than the progressive group. Although platelet counts (PLT) were decreased in all groups, the changes were better in regressive patients (160.7-153.4 $\times 10^9$/L) than that in progressive patients (174.6 to 147.6 $\times 10^9$/L; Supporting Table S3).

P-I-R score produced a substantial interobserver agreement (kappa value of 0.71), whether participants were specialists (hepatopathologists), nonspecialist physicians (general surgical pathologists, hepatologists), or nonphysican (scientist/engineer; Supporting Table S4).

P-I-R FIBROSIS QUALITY VERSUS NECROINFLAMMATORY ACTIVITY

On baseline LBx, patients with a progressive form of fibrosis showed higher total HAI scores, portal inflammation, and interface hepatitis than those with a regressive form of fibrosis; those with an indeterminate form of fibrosis showed intermediate values of the Ishak HAI. However, these differences were not observed in the posttreatment LBx, suggesting that differences between P-I-R categories in pretreatment were related to viral hepatitic activities, whereas viral hepatitic injury was largely absent in all posttreatment LBx (Table 1 and Supporting Table S5).

POSTTREATMENT P-I-R FIBROSIS QUALITY VERSUS CHANGES IN ISHAK FIBROSIS STAGE PRETREATMENT AND POSTTREATMENT

The comparison of posttreatment P-I-R fibrosis quality with changes in Ishak fibrosis stage pretreatment and posttreatment are shown in Table 1. In the 33 of 71 (46%) patients whose Ishak score decreased by ≥ 1 stage, none showed progressive injury on posttreatment LBx, with 88% and 12% showing regressive and indeterminate, respectively. In those with stable Ishak stages, progressive injury in the posttreatment LBx was seen in 17%, while regressive changes were seen in 72%, and 11% were classified as indeterminate. Two of the 3 patients with an increased Ishak stage in the posttreatment biopsy showed progressive changes (67%).

The combination of change in Ishak stage and the P-I-R score could then be used to suggest whether a patient's posttreatment liver was displaying "absolutely or probably advancing" liver disease despite viral suppression "absolutely or probably reversing" liver disease after viral suppression (Table 1). With the same directionality implied by changes in both Ishak and P-I-R score, the overall change in liver disease could be considered "absolute" (i.e., reflecting likelihood of clinical outcome). If only one or the other parameter showed directionality, then the change would be considered to be clinically "probable." Those with predominantly progressive changes had more fluctuating liver stiffness values, compared to patients with "absolutely reversing" clinical liver disease who were more stable and gradually improved (Supporting Fig. S1).

The detailed information of the 25 "probably reversing" patients is shown in Table 3; 2 representative cases with paired liver biopsies are shown in Fig. 4. In addition to P-I-R fibrosis quality and Ishak stage, Laennec score in cirrhosis, collagen percentage area, and LSM were also listed. It showed that most cases had significant decrease with all measures. Although the same stage of Ishak (Laennec 4B to 4A), the median value of collagen was decreased from 4.2 to 3.2, and LSM decreased from13.8 to 6.8 kPa, respectively.

Discussion

Liver biopsy and different grading/staging systems have made great contributions to evaluation of chronic

TABLE 3. The Five Fibrosis Scoring or Staging Systems of the 25 "Probably Reversing" Cases

	P-I-R		Ishak Score		Laennec Score		CPA		LSM (kPa)	
Case No.	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	P	R	3	3	NA	NA	2.6	1.3	13.8	6.3
2	P	R	3	3	NA	NA	2.6	1.7	12.2	9.1
3	P	R	3	3	NA	NA	3.2	3.4	—	—
4	P	R	3	3	NA	NA	3.3	2.6	18.0	4.5
5	I	R	3	3	NA	NA	2.3	1.7	10.5	9.5
6	I	R	3	3	NA	NA	5.0	4.3	8.3	7.7
7	I	R	3	3	NA	NA	2.2	1.9	11.8	5.7
8	R	R	3	3	NA	NA	2.4	2.8	—	5.3
9	R	R	3	3	NA	NA	2.1	2.4	7.3	4.6
10	P	R	4	4	NA	NA	3.0	3.2	14.3	6.1
11	I	R	4	4	NA	NA	2.9	2.0	11.0	6.2
12	R	R	4	4	NA	NA	4.8	5.8	7.6	12.1
13	P	R	5	5	4B	4A	5.9	3.0	14.4	4.4
14	P	R	5	5	4A	4A	4.2	—	8.6	6.3
15	P	R	5	5	4B	4A	3.7	3.2	15.2	7.3
16	I	R	5	5	4B	4A	4.3	4.0	7.9	4.8
17	I	R	5	5	4A	4A	4.3	5.6	5.3	5.4
18	P	R	6	6	4C	4A	5.3	4.0	35.3	10.0
19	P	R	6	6	4B	4A	8.8	4.4	17.2	8.2
20	P	R	6	6	4B	4A	7.4	3.2	27.9	10.5
21	P	R	6	6	4C	4A	9.4	4.1	42.2	8.8
22	P	R	6	6	4B	4B	7.7	4.6	14.3	9.1
23	P	R	6	6	4B	4A	8.8	5.0	25.4	9.2
24	I	R	6	6	4A	4A	3.8	4.5	26.7	25.8
25	I	R	6	6	4A	4A	4.5	3.0	6.4	6.1
Total median	P	R	5	5	4B	4A	4.2	3.2	13.8	6.8

Dash ("—") indicates data missing, Laennec score was only applicable for patients with Ishak 5 and 6.
Abbreviation: NA, not applicable.

viral hepatitis prognosis and, eventually, in the making of treatment decisions.[8,19,20] However, in the era of effective antiviral therapies, the clinical needs of treatment evaluation require an evolution of pathological LBx assessments.[21] Although, generally speaking, advanced stages of scarring would suggest an

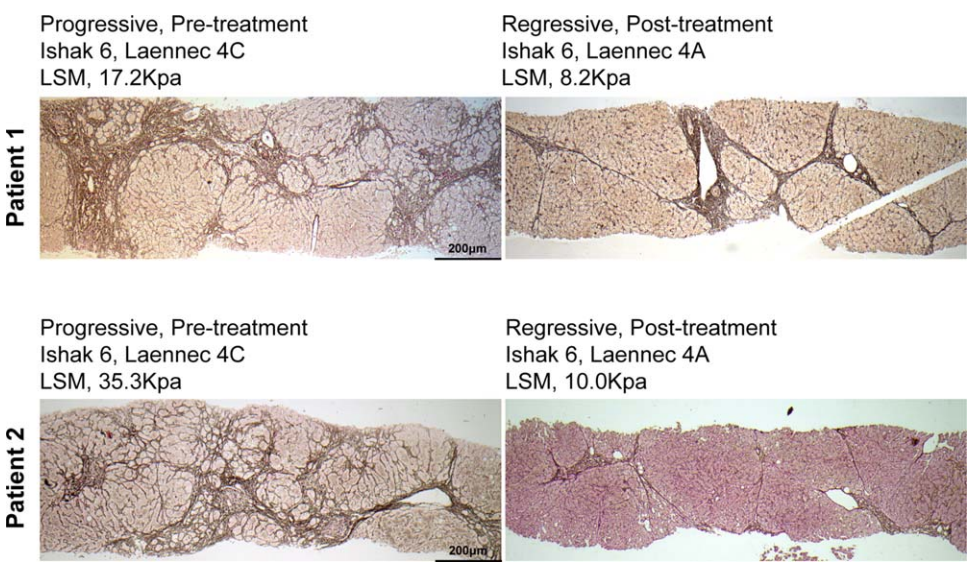

FIG. 4. Liver biopsy samples of patients with stable Ishak score. In both of the 2 cases, fibrosis activity index changed from progressive predominantly to regressive predominantly before and after treatment, whereas Ishak score remained in stage 6 (Reticulin).

imperative to treat, there is actually a wide array of qualities of "advanced" fibrosis, from inflamed, edematous "active" or progressive scarring to acellular, densely compacted "inactive" or regressive scarring, that has not been captured by current classifications.[12,22]

FIBROTIC STAGE OF DISEASE AND P-I-R ASSESSMENTS

Not surprisingly, many posttreatment LBx show changes in staging of fibrosis by the Ishak classification, but it is perhaps surprising that in our series this is true only in approximately half of the patients, with 35 of 71 showing stable stage assessment. This relates, to some degree, to the relatively coarse assessment provided by a six-point semiquantitative system. For example, as a reliable quantitative method in evaluation of liver fibrosis, collagen percentage area obtained from SHG analysis of tissue could show dynamic changes within single stages of Ishak classification that would therefore not register with Ishak assessments.[16]

Both of these techniques, however, only convey a static assessment of the tissue *at the time of biopsy* and do not suggest directionality of the injury/repair process. Directionality with these systems can only be inferred from comparison of two or more LBx at different time points. However, by introducing the concepts of active parenchymal extinction and the "hepatic repair complex" of Wanless et al.,[12,14] a single tissue sample can also give a sense of directionality: Are the lesions predominantly of the progressive (active) form or of the regressive (repairing) form? This stromal assessment is independent of Ishak stage or of SHG quantification, reflecting not rough or precise estimates of amount of stroma, but of qualities of stroma beyond those considered in these other methods.

This assessment then also sheds light on the Laennec staging system, which divides cirrhosis into subclasses of 4A, 4B, and 4C, which show significant correlations with clinical parameters, in particular, hepatic venous wedge pressure.[9,10] That system illustrates the three substages as having increasing stromal-to-parenchymal ratios with each successive step; however, looking at the images in those articles through the lens of our P-I-R assessment, 4A, as illustrated, is clearly a predominantly regressive lesion, that is, not a precursor to stages 4B and 4C, but a resolving phase of these. This "discrepancy" points to the need for a more nuanced and dynamic understanding of stage of disease beyond mere assessment of scar.

A more complete evaluation of the prognostic value of the P-I-R system will require analysis of subsequent clinical outcome. Indeed, these patients continue to be enrolled in follow-up assessments. The reasons identified for those progressive after treatment were slower HBV-DNA response and *de novo* alcoholic and nonalcoholic fatty liver disease.

SUGGESTIONS FOR A NEW CLASSIFICATION OF LBx ASSESSMENT OF VIRAL HEPATITIS

The utility of LBx grading and staging assessment is clearly changing in this era of successful hepatitis virus suppression or eradication. Although some need for pretreatment LBx assessment in hepatitis B remains, that need has been almost eliminated in hepatitis C. However, in both diseases, there are two new pressing questions for which LBx may yield helpful information. Our data shed no significant light on the first question: Is the patient still at significant risk for development of virus-associated malignancy? Our data do shed light on the second question: Will any individual patient resolve their liver disease after successful treatment?

We know that some patients, in fact, do not get better, but go on to progressive liver failure. Of course, some of these may have developed new diseases complicating their posttreatment course; such information is best evaluated by clinical correlation with posttreatment biopsy. Thus, there is already an indication of some utility to posttreatment LBx in this new era. However, the other potent worry is that some patients, for reasons that have yet to be fully elucidated, have self-sustaining persistent liver injury in the absence of new, concomitant disease or detectable virus. We believe that P-I-R assessment sheds light on this possibility.

In aggregate, then we can suggest a tentative new approach to LBx assessment, which we name the "Beijing classification" (Table 4). We developed this approach to be useful in both pretreatment and posttreatment LBx for hepatitis B and posttreatment LBx, alone, in hepatitis C. Although different approaches can be created for both, this would increase the complexity for the general diagnostic pathologist and the focused hepatopathologist alike. We aim for simplicity in three, rather than the traditional two categories of histological stages: (1) necroinflammatory activity; (2) stage of fibrosis; and (3) P-I-R stromal assessment of

TABLE 4. Beijing Classification for Histological Assessment of Chronic Viral Hepatitis

Hepatitis Assessment	Description	Previous Classifications
Inactive	Portal inflammation only or rare foci of interface or lobular hepatitis; no confluent necrosis	Chronic persistent hepatitis Ishak HAI 1-5 Metavir A1
Active, non-severe	Varying degrees of interface and lobular hepatitis easily identified at low power; no confluent necrosis	Chronic active (aggressive) hepatitis Ishak HAI 5-12 Metavir A1-A2
Active, severe*	Confluent necrosis (perivenular drop out or bridging necrosis or parenchymal collapse) Note: This definition of severe activity raises the question of possible concomitant diseases (e.g., AIH, DILI) or immunosuppression (e.g., untreated HIV).	Chronic active (aggressive) hepatitis Ishak HAI 13-18 Metavir A3
Fibrosis stage		
Early	No fibrosis or portal fibrosis	Ishak 0-2 Metavir F0-1
Intermediate	Fibrous septa, focal or frequent	Ishak 3-4 Metavir F2-F3
Advanced	Fibrous septa with focal or diffuse nodularity (developing or established "cirrhosis")	Ishak 5-6 Metavir F3-F4
P-I-R fibrosis quality		
Predominantly **Progressive** features	Most of specimen shows progressive forms of stroma.	Laennec 4A† or 4B or 4C
Indeterminate	Uncertain mix/balance between progressive and regressive stroma	Laennec 4B
Predominantly **Regressive** features	Most of specimens regressive forms of stroma	Laennec 4A
Not applicable	Not used in biopsies with "early stage" fibrosis (i.e., without fibrous septa)	

*When severe (confluent) necrosis is present in biopsy specimens from patients with viral hepatitis, it indicates the need for clinical exclusion of concomitant autoimmune hepatitis (AIH), drug/toxin-induced liver injury, coinfection with other hepatotropic viruses, or immunosuppression (e.g., HIV coinfection).
†Published illustration of Laennec 4A shows predominantly regressive features, but progressive 4A may also be identified.

relative degrees of progressive and regressive features. For the first two, we simplify from other systems for ease of clinical use, though, of course, more detailed assessments can still be applied for research applications using previous more finely grained systems like the Knodell or Ishak systems or the Metavir system, which was developed by demonstration of intraobserver reproducibility.

For necroinflammatory activity, we recognize two clinical issues. First, that the old terminology of "chronic persistent" and "chronic active (or aggressive) hepatitis" were and remain prognostically useful for hepatitis B. Thus, the first two degrees of this category of "inactive or minimally active" and "active, nonsevere," which correspond to the historical terminology. The division of active into "nonsevere" and "severe," based on the absence/presence of confluent necrosis, reflects the numerical weight given this form of injury in the Ishak grading as well as the clinical import of severe/confluent necrosis in assessment of viral hepatitis LBx. In chronic hepatitis, confluent necrosis is an important indicator of possible comorbidities clinical import, such as immunosuppression (e.g., otherwise unsuspected HIV disease), coinfection with other hepatotropic viruses (particularly hepatitis D virus in hepatitis B patients), or concomitant new/unsuspected autoimmune hepatitis or drug-induced liver injury (DILI).[23]

For fibrotic staging, we favor a three-point system for general clinical use. This reduced number of points is expected to increase intraobserver consistency while still supplying the full information necessary for clinical decision making. (As already noted, for clinical studies involving populations of patients, other previous systems can be easily substituted. Note that this three-point system maps very easily to the six points of Ishak staging and generally well with the four Metavir stages.) We emphasize presence/absence of fibrous septa between the first two stages given that in the absence of fibrous septa, the subsequent P-I-R stromal assessment is not applicable. We separate "advanced stage" (developing or established "cirrhosis") from LBx

without nodularity, only fibrous septa, because it is only the former that is likely to be significantly at risk for self-perpetuating, disease-independent progression; fibrous septa, whether frequent or focal, are unlikely to suggest a potentially worse prognosis. Also, Laennec staging, which may offer some utility, is only thereby applicable in the "advanced stage" LBx, sharpening intrasystem comparisons for research studies.

Finally, we add the P-I-R fibrosis quality assessment as part 3 of the new classification. This applies only to LBx with at least fibrous septa, given that merely fibrotic portal tracts do not clearly show the parenchymal injury and progressive or regressive scarring being assessed. Again, for simplicity and an expectation of good intraobserver agreement, we suggested only three categories. In our practical use of the system, it was always clear at low power (Fig. 3) how to categorize any LBx as long as there was sufficient tissue sampling, as per published assessments for adequacy.[24-27] Also, given that the causes of diverse liver diseases, including viral, insulin resistance/oxidative stress, toxins/alcohol, or immune attacks, would lead to similar forms of parenchymal extinction and subsequent fibrosis thereafter, this new assessment system may eventually show utility for evaluation of dynamic changes of fibrosis in other chronic liver diseases such as chronic hepatitis C, nonalcoholic/alcoholic steatohepatitis, and drug-induced or autoimmune liver diseases. This possibility requires further clinical studies.

This new *predominantly progressive, predominately regressive*, and *indeterminate* classification has two important clinical strengths. First, it can dynamically reflect the tendency of disease development, independent of inflammation activity, fibrosis severity, or treatment experience. Therefore, the information from one individual biopsy sample may not only give the previous cross-section evaluation for inflammation and fibrosis, but also the new stromal assessment for changes indicative of disease direction (i.e., prognosis). Second, it extends the conventional definition of "reversal of fibrosis" as reflecting a decrease of more than one stage from Ishak system.[19,28,29] In those patients with the same fibrosis stage from the paired biopsies before and after treatment, the new classification from *progressive/indeterminate* (before) to *regressive* (after) also indicates improvement.

In conclusion, our study shows the clinical utility of pretreatment and posttreatment LBx to provide clinically meaningful and actionable information independent of purely clinical parameters, such as serum tests and noninvasive LSMs. This Beijing classification, with a P-I-R fibrosis quality assessment, takes the first step to update the forms of information derivable from LBx examination in chronic viral hepatitis to reflect the most current concepts of liver parenchymal injury and repair. We recognize that refinements await, based on more robust clinical data, particularly longer-term follow-up after the initial posttreatment biopsy, as well as important studies to evaluate the intraobserver variability with this system. We hope that the Beijing classification may help to evaluate efficacy and, most important, optimize treatment strategies.

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