Liver biopsy evaluation in chronic hepatitis b infection: 'Back to basics' - re-visiting semi-quantitative scoring systems

Aneeta Jassar[1],* V K Kumar[2]

[1]Senior Advisor, Dept. of Pathology, Military Hospital, Jalandhar Cantt. Jalandhar, Punjab, [2]Professor and Head, Dept. of Pathology, Mediciti Institute of Medical Sciences, Hyderabad, Telangana, India

*Corresponding Author: Aneeta Jassar
Email: draneetajassar@gmail.com

Abstract

Context: Despite advances in understanding into the pathophysiology of chronic hepatitis B, liver biopsy evaluation on H&E stained sections has stood the test of time. There is therefore, a need to re-visit the most commonly used scoring systems with an aim to look for an ideal semi-quantitative system which is objective, simple to apply and versatile enough to incorporate newer advances.

Aims: To compare various semi-quantitative scoring systems with a view to (a) look for consistency in reporting, (b) assess usefulness in follow-up and (c) recommend a scoring system that is practical and easy to apply.

Settings and Design: Chronic hepatitis B was defined as HBsAg positive status for at least six months. All liver biopsies were evaluated using semi-quantitative scoring systems viz the Knodell, Ishak's modified HAI and Scheuer systems.

Materials and Methods: Baseline biopsies were assessed for intra- and inter-observer variation in order to find the system best suited for routine use. The observer variation was also calculated separately for portal, peri-portal and lobular inflammation as well as for fibrosis.

Statistical Analysis used: A Kappa analysis up to 95% confidence interval was done to look for statistical significance of inter- and intra-observer agreement between the three scoring systems.

Results: On multivariate Kappa analysis, it was found that both inter- and intra-observer agreement were comparable in the Knodell and Ishak systems, and was significantly better with the Scheuer system. Further, agreement was better with fibrosis than with necro-inflammatory scores.

Conclusions: The authors recommend Ishak's modified system as it conveyed the most relevant and exhaustive information.

Keywords: Chronic hepatitis B, Ishak's system, Knodell's system, Liver biopsy, Scheuer system, Semi-quantitative scoring systems.

Introduction

India has an intermediate range of hepatitis B virus (HBV) endemicity with a carrier rate of approximately 2-5% and contributes 9% to the total HBV carriers worldwide.[1] Many carriers of hepatitis B surface antigen (HBsAg) are asymptomatic when examined clinically and may even have normal serum transaminase levels.[2] However, liver biopsies in these patients have revealed a range of histological appearances from normal through chronic active hepatitis to cirrhosis.[3] [Further, recent research has shown that the term 'carrier' is inappropriate and misleading as even apparently healthy 'carriers' have underlying liver pathology and may eventually develop hepatocellular carcinoma.[4] It has thus been proposed in recent literature to group all cases of chronic viral hepatitis as (a) those with normal liver histology and (b) those with abnormal liver histology.[5] Thus, the medical implications of liver biopsy in these patients are significant.

With the availability of Interferon and other anti-viral agents, specific therapy for HBV infection is no longer a dream. Liver biopsies are therefore becoming routine in these cases of chronic hepatitis B who are offered specific treatment and are followed-up regularly. Some of the practical problems encountered during liver biopsy reporting include

1. Lack of observer agreement,
2. Patient selection for anti-viral therapy,
3. Assessment of prognostic markers to guide therapy and
4. Objective follow-up of patients.

In the past, numerous semi-quantitative scoring systems have been put forth to aid interpretation of liver histology and overcome subjective bias.[6, 7] These objective scoring systems have claimed to improve standardization of liver biopsy reporting and have allowed comparison of data between centres. At least three major scoring systems are in vogue in various parts of the world, each having its own advantages. With increasing awareness about hepatitis B pathophysiology and the reality of specific antiviral therapy, there is a need to evaluate these scoring systems. In an increasingly interconnected world where patients are routinely transferred between centres in different cities and even countries, it becomes paramount that a uniform system of liver biopsy reporting be followed by all pathologists.

A study was therefore designed with a view to compare various scoring systems in a cohort of patients with chronic hepatitis B infection and to recommend the most appropriate one for reporting.

Aims

To compare various semi-quantitative scoring systems for evaluation of liver biopsies in chronic hepatitis B primarily with a view to

1. Look for consistency in reporting
2. To assess usefulness in follow-up
3. To recommend a scoring system that is practical and easy to apply

Materials and Methods

The material for this study was collected at a large tertiary hospital. Patients found to have chronic hepatitis B were accrued for study. Chronic hepatitis B was defined as HBsAg positive status for at least six months. These patients either presented with symptoms of liver disease (jaundice, impaired appetite, discomfort or pain in right upper quadrant of the abdomen) or were detected to be HBsAg positive incidentally during routine testing prior to blood donation.

In order to avoid confounding factors in evaluation of data, certain exclusion criteria were drawn up. These were:

1. Chronic alcohol consumption (more than 6 U/week)
2. HIV positivity
3. HCV positivity and
4. History of chronic drug intake

Cases thus selected were subjected to a baseline liver biopsy. All biopsies were evaluated using the Semi-quantitative Scoring Systems viz the Knodell, Ishak's modified HAI and Scheuer systems (Tables 1-3, Fig. 1-4).

Using semi-quantitative systems, baseline biopsies were assessed for intra- and inter-observer variation in order to find the system best suited for routine use. To assess intra-observer variation, the same person re-evaluated and re-scored the 50 biopsies after an interval of 4-6 weeks in a blinded manner. Inter-observer variation was calculated following scoring of biopsies independently by two observers. Total mean variation was calculated for HAI scores by dividing the total variation in scores by the number of biopsies (50). This is shown in Table 4.
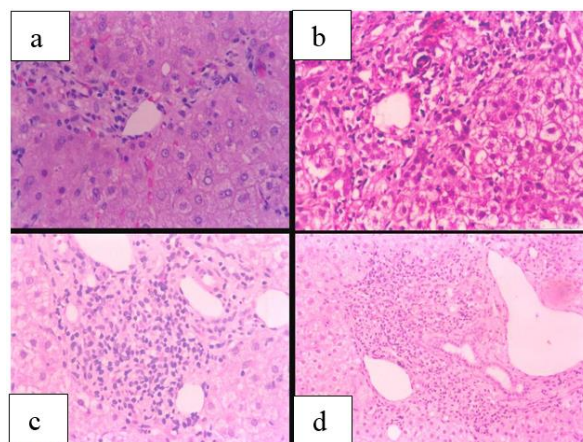

Fig. 1: Scoring for portal inflammation (a) Mild sprinkling of inflammatory cells – Knodell 1, Ishak HAI 1, Scheuer 1; (b) Moderate degree of portal inflammation – Knodell 3, Ishak HAI 2, Scheuer 1; (c) Moderate-marked inflammation in all portal areas – Knodell 4, Ishak HAI 3, Scheuer 1; (d) Marked inflammation involving all portal tracts – Knodell 4, Ishak HAI 4, Scheuer 1.
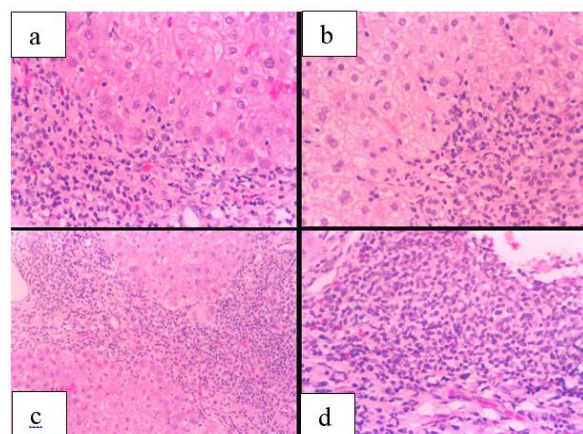

Fig. 2: Scoring for periportal inflammation (a) Mild/focal piecemeal necrosis (PN) in few portal tracts – Knodell 1, Ishak HAI 1, Scheuer 2; (b) Mild-moderate but focal PN in most portal tracts – Knodell 3, Ishak HAI 2, Scheuer 3; (c) Moderate PN continuous around <50% of tracts – Knodell 3, Ishak HAI 3, Scheuer 3; (d) Severe PN continuous around >50% of tracts – Knodell 4/5/6 depending upon presence or absence of bridging necrosis, Ishak HAI 4, Scheuer 4.
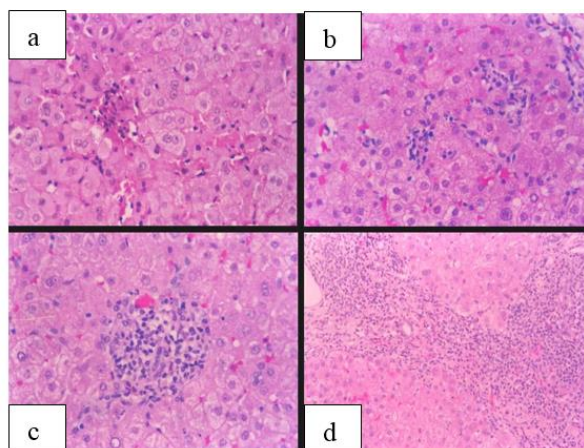
Fig. 3: Scoring for lobular inflammation ('spotty necrosis') (a) Mild or scattered inflammation – Knodell 1, Ishak HAI 1; Scheuer 1; (b) Moderate inflammation – Knodell 3, Ishak HAI 2, Scheuer 2; (c) Marked inflammation in >2/3 of lobules – Knodell 4, Ishak HAI 3, Scheuer 3; (d) Bridging necrosis – Knodell 5/6/10 depending upon presence or absence of PN, Ishak HAI 4/5/6, Scheuer 4.
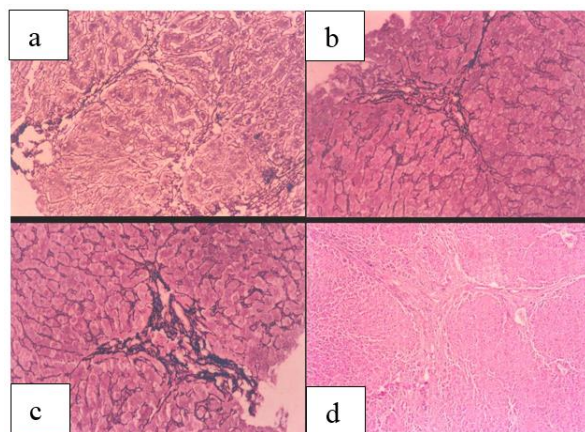

Fig. 4: Scoring of fibrosis. (a) Normal reticulin framework (no fibrosis) – All systems 0; (b) Fibrous portal expansion – Knodell 1, Ishak 1/2 depending upon number of portal tracts involved, Scheuer 1; (c) Fibrosis with architectural distortion – Knodell 2, Ishak 3/4/5 depending upon type of bridging fibrosis, Scheuer 3; (d) Cirrhosis – Knodell 4, Ishak 6, Scheuer 4.

Table 1: The knodell histologic activity index (HAI)

I. Periportal +/− B Bridging Necrosis		II. Intralobular Degeneration and Focal Necrosis		III. Portal Inflammation		IV. Fibrosis	
A. None	0	A. None	0	A. No portal inflammation	0	A. No fibrosis	0
B. Mild piecemeal necrosis	1	B. Mild (scattered foci of necrosis in <1/3 of lobules or nodulles)	1	B. Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	1	B. Fibrous portal expansion	1
C. Moderate piecemeal necrosis (< 50% of the circumference of most portal tracts)	3	C. Moderate (involvement of 1/3-2/3 of lobules or nodules)	3	C. Moderate (increased inflammatory cells in 1/3-2/3 of portal tracts)	3	C. Bridging Fibrosis (portal-portal or portal-central linkage)	2
D. Marked piecemeal necrosis (> 50% of the circumference of most portal tracts)	4	D. Marked (involvement of >2/3 of lobules or nodules)	4	D. Marked (dense packing of inflammatory cells in >2/3 of portal tracts)	4	D. Cirrhosis	4
E. Moderate piecemeal necrosis *plus* bridging necrosis	5						
F. Marked piecemeal necrosis *plus* bridging necrosis	6						
G. Multilobular necrosis	10						

Maximum possible score in Knodell System: 22

Table 2: Ishak's modified HAI

Grading: Necroinflammatory Scores	
Change	**Score**
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	
• Absent	0

• Mild (focal, few portal areas)	1
• Mild / moderate (focal, most portal areas)	2
• Moderate (continuous around <50% of tracts/septa)	3
• Severe (continuous around >50% of tracts/septa)	4
B. Confluent necrosis	
• Absent	0
• Focal confluent necrosis	1
• Zone 3 necrosis in some areas	2
• Zone 3 necrosis in most areas	3
• Zone 3 necrosis + occasional portal-central bridging	4
• Zone 3 necrosis + multiple portal-central bridging	5
• Panacinar or multiacinar necrosis	6
C. Focal (spotty) lytic necrosis, apoptsis and focal inflammation	
• Absent	0
• One focus or less per 10x objective	1
• Two to four foci per 10x objective	2
• Five to ten foci per 10x objective	3
• More than ten foci per 10x objective	4
D. Portal inflammation	
• None	0
• Mild, some or all portal areas	1
• Moderate, some or all portal areas	2
• Moderate/ marked, all portal areas	3
• Marked, all portal areas	4
Maximum possible score for grading	18
Staging: architectural changes, fibrosis and cirrhosis	
Change	**Score**
• No fibrosis	0
• Fibrous expansion of some portal areas, with or without short fibrous septa	1
• Fibrous expansion of most portal areas, with or without short fibrous septa	2
• Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
• Fibrous expansion of most portal areas with marked bridging [portal to portal (P-P) and portal to central(P-C)]	4
• Marked bridging [P-P and/or P-C] with occasional nodules (incomplete cirrhosis)	5
• Cirrhosis, probable or definite	6
Maximum possible score for staging	6

Maximum possible score in Modified Ishak System 24

Table 3: The scheuer system

Grade	Portal/Periportal Activity	Lobular Activity
0	None or minimal	None
1	Portal inflammation (CPH)	Inflammation but no necrosis
2	Mild piecemeal necrosis (mild CAH)	Focal necrosis or acidophil bodies
3	Moderate piecemeal necrosis (moderate CAH)	Severe focal cell damage
4	Severe piecemeal necrosis (severe CAH)	Damage includes bridging necrosis

Stage	Fibrosis
0	None
1	Enlarged, fibrotic portal tracts
2	Periportal or portal-portal septa but intact architecture
3	Fibrosis with architectural distortion but no obvious cirrhosis
4	Probable or definite cirrhosis

Maximum possible score in Scheuer System: 12

Table 4: Mean observer variation using semi-quantitative systems

Mean Variation	Knodell Score	Ishak score	Scheuer Score
Inter-observer	4.0	5.0	1.0
Intra-observer	3.0	3.5	0.8

Table 5: Observer Variation

Histological Parameter	Knodell Score [mean variation/ maximum score]	Ishak Score [mean variation/ maximum score]	Scheuer Score [mean variation/ maximum score]
Inter-Observer Variation			
Portal inflammation	0.7 / 4	0.5 / 4	
Periportal inflammation	2.5 / 10	1.0 / 4	0.2 / 4
Lobular inflammation	0.5 / 4	3.0 / 10	0.5 / 4
Fibrosis	0.3 / 4	0.5 / 6	0.4 / 4
Intra-Observer Variation			
Portal inflammation	0.5 / 4	0.5 / 4	
Periportal inflammation	2.0 / 10	0.5 / 4	0.2 / 4
Lobular inflammation	0.4 / 4	2.0 / 10	0.3 / 4
Fibrosis	0.2 / 4	0.5 / 6	0.3 / 4

Table 6: Kappa analysis of observer variation

	Knodell Score	Ishak Score	Scheuer Score
Inter-Observer Variation			
Inflammation scores (portal+periportal+lobular inflammation)	0.349	0.481	0.792
Fibrosis scores	0.712	0.752	0.913
Total scores	0.456	0.561	0.862
Intra-Observer Variation			
Inflammation scores (portal+periportal+lobular inflammation)	0.644	0.721	0.821
Fibrosis scores	0.921	0.963	0.982
Total scores	0.756	0.862	0.896

Statistical Analysis and Results

The observer variation was also calculated separately for portal, peri-portal and lobular inflammation as well as for fibrosis. The mean intra- and inter-observer variations were calculated for each histologic parameter. These results are tabulated in table 5. A Kappa analysis up to 95% confidence interval was done to look for statistical significance of inter- and intra-observer agreement between the three scoring systems. Using this method of analysis, values greater than 0.70 represent very good agreement, 0.51-0.70 good agreement, and 0.3l- 0.50 moderate agreement. The analysis was done separately for necro-inflammatory and fibrosis scores. The results of the Kappa analysis of inter- and intra-observer agreement for the Knodell, Ishak and Scheuer systems are summarised in table 6.

Discussion

Several studies have now proved that there is no correlation between the severity and type of biochemical abnormality with the histologic activity in the liver in chronic hepatitis B infection.[4] Further, a normal ALT or AST does not guarantee that liver disease is inactive or absent.[8] Necrosis of liver cells is not required for the release of aminotransferases. In fact, there is poor correlation between the degree of liver cell damage and the level of the aminotransferases. Cases with normal transaminases may show a range of histologic appearances on liver biopsy.[9]

Liver biopsy evaluation in chronic hepatitis B remains the 'gold standard' and serves multiple purposes including confirmation of clinical diagnosis, assessment of severity of necroinflammation and fibrosis, evaluation of possible concomitant disease processes, assessment for deciding optimal therapeutic intervention and long-term follow-up thereafter. The

recommended practice for histopathological evaluation of chronic hepatitis involves separate statements for the cause of disease, if known, for severity of necroinflammatory lesions, and for the extent of parenchymal fibrosis.[10] It has been increasingly appreciated that an objective, semi-quantitative system of reporting necro-inflammatory and fibrotic changes separately must be used by pathologists. A related question is how to decide on a semi-quantitative system that permits better statistical comparisons between pathologists but at the same time conveys enough information to be clinically useful in deciding therapy and for follow-up.

While applying the three semi-quantitative systems for evaluation of baseline biopsies, certain practical problems were encountered. The Knodell HAI scoring system is a discontinuous scale. This means, for instance, that the difference in scores between piecemeal plus bridging necrosis on one hand (score of 6), and multilobular necrosis on the other (score of 10) is four, even though these changes form a continuum and are quite similar. This is likely to produce greater observer variation. Further, this system has more sub-categories (seven) for periportal changes than for the other three parameters (four each). This is probably because Knodell considered periportal changes to be of greater prognostic significance than the other parameters.[11] The Ishak system separates necro-inflammatory and fibrosis scores and has five sub-categories for the former (viz. piecemeal necrosis, confluent necrosis, focal or spotty necrosis and portal inflammation) and six for the latter.[7] This system separates focal necrosis and confluent necrosis. Further, Ishak uses the criteria of number of foci per 10X objective to grade necrosis; this is cumbersome to apply, but increases objectivity. Ishak's system has six scores for fibrosis unlike the other two systems; this is an advantage when subtle changes of fibrosis have to be scored. The Scheuer system is easy to memorize and apply routinely as it has only four subcategories for each parameter.[12,13] However, a drawback of this system is that it clubs together portal and peri-portal changes. Portal inflammation, irrespective of its extent and severity is given a score of one. This undermines the significance of portal inflammation and does not distinguish between different grades of severity. It is reasonable to expect better observer agreement with this system due to the low total HAI score, but it does not take into account the entire spectrum of morphological changes.

On evaluating the three semi-quantitative systems for intra- and inter-observer variation, it was found that the mean variation was the least with the Scheuer system than the Knodell and Ishak systems. The observer variations were also calculated for four histologic parameters separately, namely portal, periportal and lobular inflammation and fibrosis. It was found that the observer variation was the greatest with

necro-inflammatory scores and the least with fibrosis. On multivariate Kappa analysis, it was found that both inter- and intra-observer agreement was comparable in the Knodell and Ishak systems, and was significantly better with the Scheuer system. Further, agreement was better with fibrosis than with necro-inflammatory scores. Although the slide-by-slide analysis is less mathematically rigorous, it gives another measure of overall agreement. The different methods of analysis produced very similar results, and this helps to strengthen the conclusions reached. On each occasion that the slides were circulated, very similar results were seen with either scoring system. This is in concordance with the findings of Goldin et al who compared Knodell and Scheuer systems and found better agreement with the Scheuer than Knodell scores. Their study also showed better agreement for fibrosis scores than for inflammation scores.[14]

However, better agreement is not the only criterion by which a scoring system should be judged, and this is especially true if better agreement is obtained by simplifying the scoring system to such an extent that important morphological information is lost. In this regard, Ishak's modified system conveys the relevant information about the morphological changes and is objective as well as easy to apply by pathologists. It takes into account all the important changes in the liver in hepatitis B and gives the necroinflammatory and fibrosis score separately. The fibrosis scoring is more exhaustive than in other systems and this may be especially useful in the future as new "fibrosis-reversing" strategies are being put into clinical use.[15]

Of the fifty cases, twenty-eight qualified for interferon therapy. These patients were prescribed 3 million units (MU) of interferon-alpha (IFN-α) thrice a week for six months. Follow-up biopsies were available for twenty-two cases. All these patients had completed the course of interferon and biopsies had been done after approximately six months of the initial biopsy. All biopsies were scored by the Ishak's modified HAI Scoring System and the changes in necro-inflammation and fibrosis were recorded and statistically analyzed. All cases showed a reduction in histologic activity (mean reduction 2.5; range 1-3). The reduction was maximal with respect to lobular and peri-portal inflammation and least with fibrosis. Thus valuable information regarding response to therapy could be quantified by using the modified Ishak system.

It is therefore recommended that Ishak's modified HAI Scoring System be applied to liver biopsies across all centres in order to ensure uniformity of reporting and objective follow-up after therapeutic intervention.

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