Chapter 1

Pathology of chronic hepatitis B and chronic hepatitis C

Faten Limaiem; Asma Sassi; Saadia Bouraoui; Sabeh Mzabi

University of Tunis El Manar, Tunis Faculty of Medicine, 1007. Tunisia
University Hospital Mongi Slim, La Marsa, Sidi Daoued, 2046. Tunisia

*Correspondence to: Faten Limaiem, University of Tunis El Manar, Tunis Faculty of Medicine, 1007. Tunisia

Email: fatenlimaiem@yahoo.fr

Abstract

Chronic viral hepatitis is a syndrome of persisting hepatotropic viral infection usually associated with chronic inflammation, hepatocyte injury and progressive fibrosis. Chronic viral hepatitis is typically classified by the responsible infecting virus and modified by the extent of pathological injury and clinical compensation. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections represent the two major causes of chronic liver disease and hepatocellular carcinoma. Despite inducing shared pathological events leading to oncogenic transformation, these two viruses present profound differences in their molecular features, life cycle and interplay with host factors, which significantly differentiate the prognostic and therapeutic approach to the related diseases. The liver injury present in chronic hepatitis may be variable, but the basic morphologic changes in all types of chronic viral hepatitis are similar. In this regard, the liver biopsy remains the gold standard and is an important tool in the evaluation of patients with liver disease.

Keywords: chronic viral hepatitis; hepatitis B virus; hepatitis C virus; liver biopsy; pathology

1. Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are major etiological agents of chronic liver disease and hepatocellular carcinoma (HCC). The pathology of hepatitis B and C is diverse and reflects the clinical course of the disease [1]. Following acute infection, most subjects clear the virus whereas others develop chronic hepatitis [1]. Liver biopsy is the traditional gold standard to ascertain the degree of liver injury, including both inflammatory
activity and fibrosis stage. In addition, liver biopsy is useful to identify precursor lesions of hepatocellular carcinoma and help identify and evaluate confounding diseases such as steatohepatitis, autoimmune hepatitis, and drug-induced liver disease [1]. Current guidelines for management of hepatitis B, however, do not recommend universal liver biopsy in all patients with chronic hepatitis B, but base this decision on several clinical, virological, and biochemical parameters [1].

2. Macroscopic Changes

The macroscopic changes in chronic hepatitis vary from a normal gross appearance to scarred multinodular hepatic cirrhosis. In chronic hepatitis without significant fibrosis, the changes are generally unremarkable [2]. As the stage progresses to cirrhosis widespread fibrous scarring and diffuse macro-nodular or mixed macro- and micronodular cirrhosis can be identified.

3. Histological Features

Morphological changes in chronic hepatitis B and C comprise lesions that are common to all etiologies of chronic hepatitis and other lesions (or patterns) that are characteristic, but not pathognomonic, of chronic HBV or HCV infection [2]. By definition, chronic hepatitis is a necroinflammatory process that may be complicated by fibrosis. A hallmark of chronic hepatitis is portal inflammation (portal hepatitis), mainly consisting of lymphocytes [1-4].

3.1. Chronic hepatitis B

Common histological changes in chronic hepatitis B include:

1. Hepatocellular injury – characterized by apoptotic bodies and liver cell dropout.

2. Inflammation – predominantly lymphocytic: in the portal tracts (Portal triaditis) at the interface between the parenchyma and portal connective tissue (Interface hepatitis / Piece-meal necrosis) in the parenchyma, at the site of the liver cell dropout (Lobular hepatitis).


4. Fibrosis – scarring with a fibrous expansion of portal areas that can extend to link adjacent vascular structures (bridging fibrosis). As the disease progresses, the scarring can completely surround groups of liver cells, and, along with hepatocellular regeneration, produce the nodules of cirrhosis [2,3].

The most distinctive histological feature that readily distinguishes chronic viral hepatitis B infection is the ‘ground-glass hepatocyte’. These cells have a finely granular cytoplasmic inclusion which consists of proliferated endoplasmic reticulum containing abundant HBs
antigen that pushes the cell contents, including the nucleus, to the side, usually leaving a visible halo separating the inclusion from the cell membrane (Figure 1). Ground-glass cells can be highlighted by histochemical stains such as Shikata’s orcein and Victoria blue stains [5,6].

Two major types of ground glass hepatocytes have been identified based on morphology and distribution [7]. The two types have different biologic significance, each one having specific pre-S deletion mutations [8]. Type I ground glass hepatocytes are usually scattered randomly throughout the lobules and are found throughout the replicative phase. Type I ground glass hepatocytes have eccentric nuclei, with ground-glass inclusions within the cytoplasm [8]. Type II ground glass hepatocytes are distributed in large groups called clonal patterns and are usually seen in the late nonreplicative stage or in the cirrhotic liver [7].

3.2. Chronic hepatitis C

The histologic features of chronic hepatitis C include changes that are common in all etiologies of chronic hepatitis and changes that are characteristic, although not pathognomonic, of chronic HCV infection. Histologically, most cases of chronic hepatitis caused by HCV tend to be mild in degree. Three characteristic features of chronic HCV, although not pathognomonic, were originally described as [1] epithelial damage of small bile ducts, [2] formation of lymphoid aggregates and sometimes lymphoid follicles with germinal centers in portal tracts and, [3] steatosis (Figure 2). This histological triad is rarely seen in chronic HBV
or autoimmune hepatitis and therefore strongly suggests the diagnosis of chronic HCV [9]. The pathogenesis of these characteristic changes has not been elucidated. In addition to these three features, some reports have been made regarding Kupffer cell hyperplasia as a feature of HCV infection [10]. Also, ductular reaction and an increased number of hepatic progenitor cells are associated with a greater degree of fibrosis [11]. Steatosis in hepatitis C is usually macrovesicular; it is associated both with infection by HCV genotype 3, in which the virus is thought to be directly steatogenic, and with metabolic aberrations including insulin resistance in patients with chronic hepatitis C [12]. Steatosis and insulin resistance are associated with more severe fibrosis, poor treatment response, and increased risk of hepatocellular carcinoma in patients with chronic hepatitis C [13-16]. Non-necrotizing granulomas occur in a small percentage of cases, but other concurrent causes of granulomas should always be excluded [17]. The presence of scattered lobular acidophil bodies is a common feature of chronic hepatitis C. Mallory hyaline-like cytoplasmic inclusions have also been reported and have been associated with progression of fibrosis [18].

![Figure 2](image)

**Figure 2**: Chronic hepatitis C: portal tract containing a lymphoid aggregate associated with steatosis. (Haematoxylin and eosin, magnification × 400).

4. **Grading and Staging**

It has to be understood that hepatitis B virus does not cause direct cytopathic injury, but initiates an immune response that results in liver damage. The histological changes in the liver of patients with hepatitis B virus infection is a reflection of the interaction between viral replication and host immune response that attempts to eradicate the virus. The necrosis and inflammation can be ‘graded’ and the end result of the healing and repair by way of fibrosis
or the chronicity of these changes ('stage') can be readily assessed in a liver biopsy [5,19,20]. Establishing grade and stage are currently the main indications for liver biopsy in patients with chronic hepatitis [21-24]. This information is useful in predicting short- and long-term prognosis, deciding treatment options and their timing, and assessing changes occurring during or after any treatment. Including grade and stage in the final pathology report on cases of chronic hepatitis is therefore considered mandatory. Adequate tissue for evaluation is requisite for grading and staging of liver disease, because small biopsy specimen size can affect the accuracy of histologic interpretation [25]. Specimens obtained with cutting biopsy needles are superior to those obtained with suction needles [26]. Larger-gauge needles (14- to 16-gauge) and biopsy specimens at least 2 cm long are more likely to be adequate for assessing histologic changes [25]. The number of portal tracts required for an adequate liver needle biopsy is between 6 and 11, although 11 portal tracts are generally considered an optimal specimen [25]. A comprehensive histopathological evaluation of liver biopsy in chronic hepatitis B infection utilizes a standard panel of special stains as well as selective immunohistochemistry. These methods are especially useful in grading and staging of chronic hepatitis and establishing a diagnosis of cirrhosis. In most of the institutions the standard initial set of special stains includes reticulin, Masson trichrome, and diastase-pre-treated periodic acid Schiff. Periodic acid-Schiff, Shikata's orcein, and iron stain are used only rarely. Over the last few decades, several grading and staging schemes for chronic hepatitis have been proposed and developed. The simplest method, familiar to most pathologists, consists of classifying both grade and stage in descriptive terms such as mild, moderate, and severe [27]. The overall severity of necroinflammation and fibrosis is considered, but there are no specific rules to guide this evaluation, which remains highly subjective. In the more complex methods, the final grade and stage emerge from combining numerical scores attributed to each histologic lesion; all methods are based on similar principles, regardless of the specific criteria used (tables 1 and 2).

**Grading** is performed semiquantitatively by assessing necroinflammatory lesions, both in the portal-periportal area (i.e., portal inflammation and interface hepatitis) (Figure 3) and in the lobular parenchyma (i.e., lobular necrosis/apoptosis and inflammation) (Figure 4). Each lesion is scored, with higher numbers coinciding with more severe lesions. The sum of the scores gives the grade of hepatitis.
Figure 3: Interface hepatitis. This portal tract shows inflammatory cells spilling over into the periportal area, creating an irregular outline of the portal tract. (Haematoxylin and eosin, magnification × 200).

Figure 4: Lobular necroinflammation. An aggregate of inflammatory cells (arrowhead) is shown. (Haematoxylin and eosin, magnification × 200).

Staging is an assessment of the extent and location of fibrosis and of accompanying changes in parenchymal architecture. All systems express stage on a linear numerical scale, with stage 0 (zero) corresponding to no fibrosis and the highest stage to a diagnosis of cirrhosis (Figure 5); definitions of the intermediate stages differ with the staging systems.
There is no general consensus about which is the best system for grading and staging chronic hepatitis in routine practice [28]. However, a uniform, universally applied system is highly desirable because it would facilitate comparison of different clinicopathologic studies and improve interobserver reproducibility. However, in the absence of agreement, we share the opinion of most authoritative liver pathologists that it does not matter which system is used in daily practice [28,29]. Comparison of grading and staging systems is summarized in table 1 and table 2 respectively.

**Figure 5**: Architectural distortion of the liver and fully developed cirrhosis. Large irregular regenerative nodules are separated by thick fibrous bands. (Haematoxylin and eosin, magnification × 40).
Table 1. Comparison of Grading Systems [30-33].

<table>
<thead>
<tr>
<th>Grading Scheme</th>
<th>Parameters Scored</th>
<th>Scale Used</th>
<th>Overall Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheuer (1991) [20]</td>
<td>Portal/periportal activity</td>
<td>0–4</td>
<td>Reported as a sum of individual scores with a range of 0–8</td>
</tr>
<tr>
<td>Batts and Ludwig (1995) [21]</td>
<td>Lymphocytic piecemeal necrosis, Lobular inflammation and necrosis</td>
<td>No activity, minimal, mild, moderate, severe</td>
<td>Severity of lesion (piecemeal or lobular) determines grade</td>
</tr>
<tr>
<td>Ishak (1995) [22]</td>
<td>Periportal or perisepal interface hepatitis (piecemeal necrosis), Confluent necrosis, Focal lytic necrosis, apoptosis, focal inflammation, Portal inflammation</td>
<td>0–4, 0–6, 0–4</td>
<td>Reported as a sum of individual scores with a range of 0–18</td>
</tr>
<tr>
<td>Bedossa and Poynard (METAVIR, 1996) [23]</td>
<td>Piecemeal necrosis, Lobular necrosis</td>
<td>0–3 (none, mild, moderate, severe), 0–2 (none, mild, moderate, severe)</td>
<td>Overall histologic activity determined by algorithm combining piecemeal and Lobular necrosis: A0 = none, A1 = mild, A2 = moderate, A3 = severe</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Staging Systems [30-33].

<table>
<thead>
<tr>
<th>Staging Scheme</th>
<th>Stage 0</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheuer (1991) [20]</td>
<td>No fibrosis</td>
<td>Enlarged, fibrotic portal tracts</td>
<td>Periportal fibrosis or portal-portal septa but intact architecture</td>
<td>Fibrosis with architectural distortion but no obvious cirrhosis</td>
<td>Cirrhosis, probable or definite</td>
</tr>
<tr>
<td>Bedossa and Poynard (METAVIR, 1996) [22]</td>
<td>No fibrosis</td>
<td>Portal fibrosis without septa</td>
<td>Portal fibrosis with rare septa</td>
<td>Numerous septa without cirrhosis</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>
5. Severity Of Histological Changes In Different Phases Of Chronic Hepatitis B

5.1. Immune-tolerant phase

The immune-tolerant phase can last from a few years to more than 30 [34,35]. During this phase, liver inflammation and fibrosis are absent or minimal. Patients with chronic hepatitis B virus who have remained in this phase have mild disease, with disease progression being minimal; whereas patients who progress to the immune-active phase often have disease progression [35]. In this setting, ground glass hepatocytes are numerous.

5.2. Immune-active (Clearance) phase

In this phase, lymphocytic portal inflammation with interface hepatitis and spotty lobular inflammation are prominent. Sometimes, bridging and confluent necrosis may be identified. Ongoing necroinflammatory damage may lead to variable degrees of fibrosis or cirrhosis.

5.3. Immune-inactive phase

Liver histology in this and the immune-tolerant phase is similar, inflammation being minimal [36,37]. There is usually improvement in liver fibrosis and inflammation over time. In those who remain in the inactive phase, the liver fibrosis is absent or minimal in degree and shows no evidence of progression over time.

6. Dysplasia And Cancer

Hepatitis B virus can promote hepatocellular carcinoma in many ways, including through its integration in the host genome. Hepatitis B virus -DNA integration into human chromosomes has been detected in 80-90% of hepatitis B virus -related hepatocellular carcinomas and insertions have been associated with genetic alterations within the cell genome, including generalized genomic instability, gene and chromosomal deletions, translocations, amplifications of cellular DNA, and generation of fusion transcripts [38]. Genome-wide next-generation sequencing approaches have identified recurrent sites for hepatitis B virus -DNA integration [39]. Genes reported to be frequently altered are TERT, MLL4, CCNE1, NTK2, IRAK2 and p42MAPK1 [40]. Although cirrhotic livers are at greater risk for hepatocellular carcinoma,
(Figure 6), patients with chronic hepatitis B can develop hepatocellular carcinoma in the absence of cirrhosis, supposedly because of direct viral integration in the host genome and the direct oncogenesis effects of hepatitis B virus [41]. The role of liver biopsy is to identify precursor lesions including cirrhosis and liver cell dysplasia and to differentiate macroregenerative nodules from well-differentiated hepatocellular carcinoma. Liver inflammation may contribute to the accumulation of critical mutations in the host genome that contribute to carcinogenesis [42]. Non tumorous liver parenchyma in livers affected by hepatocellular carcinoma have been reported to have higher inflammatory grades when compared to livers without tumors [43]. In the same study, livers with hepatocellular carcinoma had a higher incidence of hepatitis B core antigen (HBCAg) positivity, suggesting a significant role of ongoing persistent chronic inflammation and actively replicating hepatitis B virus in carcinogenesis. Precancerous lesions of hepatocellular carcinoma include: (1) cytological changes indicative of hepatocellular dysplasia often occurring as expansile foci, termed “dysplastic foci”; and (2) nodular lesions detectable on gross and, often, on radiological examination, characterized by cytological or structural atypia and termed “dysplastic nodules”. Hepatocellular changes that have mostly been associated with carcinogenesis in chronic liver disease include small cell change, large cell change, and iron-free foci [43]. Large cell change and especially small cell change are considered predictors of hepatocellular carcinoma, and their presence should be reported in a liver biopsy in patients with chronic viral hepatitis [44].

![Figure 6: Hepatocellular carcinoma arising in a cirrhotic liver. (Haematoxylin and eosin, magnification × 100).](image)

**7. Immunohistochemistry**

Immunostaining using primary antibodies directed against the hepatitis B virus surface
and core antigens is routinely used in clinical practice [5,46]. Positive findings confirm hepatitis B virus infection. Also, the pattern of expression may help determine the phase of infection. Immunostains for HBs (Ag) and HBc Ag allow identification of hepatitis B as the etiologic agent of chronic hepatitis. The immunoexpression patterns of HBs Ag and HBc Ag may help determine the phase of infection. HBs Ag is usually not expressed in acute hepatitis. In chronic hepatitis B, HBs Ag expression may be cytoplasmic and/or membranous, whereas HBc Ag expression may be nuclear and/or cytoplasmic. Diffuse membranous staining for HBs Ag suggests active viral replication. In the immune-tolerant phase (or in immunosuppressed hosts), there is diffuse nuclear HBc Ag and membranous HBs Ag positivity, without marked inflammation, with an inverse relationship between the degree of diffuse membranous expression and inflammatory activity [47]. Presence of HBs Ag in clusters of hepatocytes along with a negative result for HBc Ag may represent an inactive carrier state, without ongoing viral replication.

8. Writing The Histology Report

The following guidelines summarize what needs to be done to produce a clinically useful liver histology report in cases of chronic viral hepatitis [44].

1. Assess the adequacy of the biopsy by measuring the length of the specimen and counting the number of portal tracts [44]. Write these data in the final report to make clinicians aware of any potential sampling error in the grading and staging [44].

2. Describe the type and severity of necroinflammation and fibrosis in words: by using numbers alone, some clinically useful information, e.g. any presence of bridging necrosis, may be lost [44].

3. Describe any presence and severity of adjunctive lesions:
   • steatosis (strongly recommended): graded on a scale from 0 to 3
   • siderosis (recommended): graded on a scale from 0 to 4 (use more detailed scores for special purposes)
   • dysplasia (recommended): separately report the presence of large cell changes and small cell changes

4. use immunostaining as appropriate (HBV antigens)

5. search for any concomitant diseases

6. use a validated (not home-made) scoring system for grade of activity and stage of fibrosis

7. write a conclusion and:

   (a) state whether the pathological findings are consistent with chronic hepatitis
(b) state the findings that are consistent with a viral etiology
(c) state whether there are changes consistent with concomitant diseases (specify which) [44].

9. Conclusions

The liver injury present in chronic hepatitis may be variable, but the basic morphologic changes in all types of chronic viral hepatitis are similar. In this regard, the liver biopsy remains the gold standard and is an important tool in the evaluation of patients with liver disease [48]. Adequate clinical information must be provided by the clinician in order for a pathologist to render the most complete diagnosis. A statement regarding viral serologic markers, whether positive or negative, has to be indicated in the requisition slip. In cases of HCV and HBV, knowledge of HCV RNA viral load and HBV DNA is helpful [44]. Also, information that may not be directly related to HCV or HBV may be important, such as being overweight or morbidly obese, drinking alcohol, or family history of metabolic liver disease [44]. This information aids the pathologist in closely assessing certain features that may be subtle or even masked by the chronic hepatitis histology. For pathologists, the components to be covered in a report should include (1) a comment on the cause, (2) the grade of necro-inflammation, (3) the stage of fibrosis and staging system used [49].

10. References


42. Vaiphei K. Correlative analysis of histological profile of the adjoining liver parenchyma with liver enzyme levels in hepatocellular carcinoma and their comparison with chronic liver disease in autopsy cases. Indian J Pathol Microbiol 2007;50:711-717.


