

LIVER TISSUE EXAMINATION

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Abstract

Histopathological study of the liver biopsy in viral hepatitis B allows to diagnose acute hepatitis, its severity and stage of evolution. Prediction of chronicity is feasible after two months. In chronic disease, histopathology allows to diagnose chronic hepatitis, its aetiology, grade of severity and stage of progression. Interface hepatitis and bridging confluent necrosis are important prognostic features.

Severity is graded according to extent of necro-inflammatory lesions, which include portal inflammation, interface hepatitis, intralobular liver cell damage and death, and confluent (bridging) necrosis. Staging of progression is based on extent of fibrosis and lobular architectural changes (cirrhosis).

Semiquantitative scoring of grade and stage is useful in trials of new drugs, in clinical research, and for comparison of pre- and post-treatment biopsies. It is not recommendable in routine diagnostic practice.

In situ hybridization and (immuno-)electron microscopy are less practical and mostly used in research.

Immunohistochemical staining for HBV antigens, especially for HBcAg and HBsAg, is useful for specifying the aetiology and the viral phase of the disease.

The viral replicative phase is characterized by mild activity, nuclear localization of HBcAg, cytoplasmic HBeAg and membranous expression of HBsAg; the viral clearance phase features more severe inflammation and necrosis, possibly including the bridging type, whereas immunostaining reveals nuclear, cytoplasmic and membranous HBcAg; HBsAg stains in the cytoplasm of some cells, and in cellular membranes; the residual integration phase reveals no or only mild activity and cytoplasmic HBsAg, without demonstrable HBcAg.

Attention for and reporting of premalignant lesions is important in improving adequate patient surveillance for possible development of hepatocellular carcinoma. Recognizable lesions include large cell and small cell liver cell dysplasia in dysplastic foci and nodules.

Introduction

Examination of liver tissue from living patients is performed on surgical and needle biopsies. It comprises mainly histopathological study, and may serve diagnostic and/or research purposes. Diagnostic investigations require close collaboration with the clinician to assure optimal reliability. Of paramount importance are adequate biopsies (1) and impeccable histopathological and histochemical techniques.(2)

I. Diagnostic Investigations

1. Histopathological study

Histopathology of the liver biopsy allows diagnosis of acute hepatitis, its degree of severity, and in most cases differentiation from chronic hepatitis.

Since histopathologically acute viral hepatitis B appears essentially similar to other forms of acute hepatitis, an aetiological diagnosis is less reliable. Acute viral hepatitis B may show different patterns; shortly described below.(3-5)

Acute hepatitis with spotty necrosis is the classical picture of self-limiting acute hepatitis. Cell damage tends to predominate in the centrilobular areas.(6)

Distinction can be made between an early, fully developed, late, and residual stage.(7)

Acute hepatitis with bridging necrosis represents more severe hepatitis with confluent areas of necrosis of the lytic type. The necrosis may link afferent and efferent vascular landmarks (portal-central bridging necrosis).(8)

Extensive confluent necrosis is often followed by collapse of the denuded reticulin framework, resulting in scarring fibrous septa. Older scars can be identified by their elastin content. (9, 10)

Acute hepatitis with panlobular or multilobular necrosis is more severe, seen in fulminant hepatitis.

Acute hepatitis with periportal necrosis features periportal interface hepatitis (piecemeal necrosis). The occurrence of this lesion in acute hepatitis B was considered an indicator of possible transition to chronicity (11); as was also bridging hepatic necrosis.(8) It appears that in the later stage of acute hepatitis (after 2 months) both lesions are unfavorable prognostic signs. (12, 13) The most reliable predictor of chronicity is the demonstrable presence of HBV antigens in scattered hepatocytes. (14)

The histological differentiation of acute hepatitis B from viral-like drug-induced and auto-immune hepatitis may sometimes be difficult. Chronic hepatitis is recognizable by predominance of portal and periportal changes, possible presence of elastin-containing septa, and detectable viral antigens (HBsAg, HBcAg) on immunostaining. Differentiation from bile duct obstruction and acute alcoholic hepatitis is feasible.

The histopathology of chronic viral hepatitis B comprises the elementary lesions of any form of chronic hepatitis; and some specific features. (15)

Common features in chronic hepatitis.

Portal infiltration. Most portal tracts are infiltrated by inflammatory cells, predominantly lymphocytes. (16)

Interface hepatitis (previously termed piecemeal necrosis) is typical of more active disease. It corresponds to lymphocytic infiltration at the interface between connective tissue (portal tracts and septa) and parenchyma, associated with apoptotic death of local hepatocytes. (15, 17) The lesion may be minimal, mild or severe.

True interface hepatitis must be differentiated from periportal necro-inflammation in hepatitis A, from “biliary piecemeal necrosis” in chronic biliary diseases, and from spill-over of inflammatory cells unassociated with liver cell damage. (16)

Intralobular focal necro-inflammation (spotty necrosis) varies in extent with the severity of disease. Confluent lytic necrosis (bridging, panlobular, multilobular) characterizes severe disease, and clinical exacerbations of chronic disease.(18)

Hepatic rosettes correspond to clusters of surviving hepatocytes in areas of extensive necro-inflammation.(5)

Most authors consider bridging necrosis an ominous prognostic finding (19, 20) although the lesion may also heal. (21) Apparently bridging necrosis carries a sinister long-term prognosis when associated with interface hepatitis, whereas the cirrhotic evolution of chronic disease marked by interface hepatitis (“chronic active hepatitis”) is accelerated by bridging hepatic necrosis. (7)

Hepatic fibrosis in chronic hepatitis B occurs mostly in a septal pattern, comprising so-called active and passive septa. (11) Active septa are rich in cells; they represent extensive interface hepatitis eventually leading to portal-portal septal fibrosis. (7)

Passive septa carry few or no inflammatory cells, are sharply delineated, and derive from postnecrotic collapse after confluent necrosis.

The mesenchymal cells responsible for fibrosis comprise portal/septal myofibroblasts, interface myofibroblasts and activated intralobular hepatic stellate cells. (22)

Parenchymal regeneration is evidenced by thickening of liver cell plates (2-3 cells wide), and increased numbers of bi- and tri-nucleated hepatocytes.(15) Activation of liver progenitor cells also participates in regeneration in chronic hepatitis.(23, 24)

Parenchymal regeneration in between a restructuring fibrous scaffold, leads to progressive disturbance of the lobular architecture and results in cirrhosis, usually of the macronodular type. (25)

Necro-inflammation may continue in the cirrhotic stage (active cirrhosis) or burn out (inactive cirrhosis). (25)

The common elementary lesions of chronic hepatitis constitute the base for grading and staging in chronic liver disease (see below).

Specific features of chronic hepatitis B

The most distinctive histological feature for identifying HBV aetiology is the “ground-glass hepatocyte”.(26) It has a finely granular, and faintly eosinophilic cytoplasm due to proliferation of the smooth endoplasmic reticulum containing accumulated Hepatitis B surface Antigen (HBsAg). (27, 28) Ground-glass cells are highlighted by special stains, including Shikata’s orcein or aldehyde fuchsin (29) and Victoria blue.(30)

Orcein staining needs to be critically performed. (31) Ground-glass hepatocytes can be specifically stained by more sensitive immunohistochemistry (32) (see below).

Ground-glass cells must be differentiated from oncocytic change, due to densely packed mitochondria (mitochondriosis) of unclear significance. (33, 34) The differential diagnosis of HBsAg positive ground-glass cells further includes a similar appearance due to drug-induction, to cyanamide toxicity, to Lafora’s disease, and to fibrinogen storage disease. (35)

Ground-glass inclusions from several causes may co-exist in the same patient and in the same hepatocyte. (36)

“Sanded nuclei” are another, though not obvious change in some cases of chronic hepatitis B. These are pale finely granular inclusions in nuclei containing huge amounts of HBcAg particles (37), staining reddish violet with chromotrope aniline blue.(38)

A study on the frequency of characteristic features for chronic hepatitis B, C, autoimmune and cryptogenic hepatitis concluded that the respective histological patterns have low sensitivity, but high specificity and predictability. (39)

Chronic hepatitis B can be distinguished from acute hepatitis by older fibrosis (positive elastin stain) and presence of orcein positive (HBsAg positive) ground-glass cells or inclusions. (6) Bilirubinostasis is rare in chronic and frequent in acute hepatitis.

Chronic biliary diseases (e.g. PBC, PSC) are differentiated by their features of ductopenia, cholate stasis, cholestatic liver cell rosettes and biliary type fibrosis. Chronic alcoholic liver disease has its own characteristics of steatosis, pericellular fibrosis, Mallory bodies, satellitosis and micronocular pattern of cirrhosis.

2. Immunohistochemistry, in situ hybridization, electron and immuno-electron microscopy

Immunohistochemical staining with specific antibodies for HBV antigens allows to specify the HBV aetiology of chronic hepatitis and the viral phase in the disease. (3, 40)

In situ hybridization (ISH) of viral DNA is helpful in detecting HBV infection and its topography in the infected cells.

Various direct and indirect immunofluorescence and immunoperoxidase procedures can be applied on fresh frozen and

paraffin embedded tissue. The sensitivity of immunoperoxidase methods can be enhanced by techniques of antigen retrieval and signal amplification like the Immunomax (41) and the EnvisionTM+ system.(42)

In acute hepatitis B, very little or no viral antigens are demonstrable (4, 43, 44), except in the very early phase (45, 46) and according to one study in patients infected with a mutant HBV (“silent HBV”). (47)

In chronic hepatitis B, antigen localization patterns vary. The course of chronic hepatitis B comprises three successive phases: virus tolerance (viral replication) phase, virus clearance and residual integration phase. (48-50)

During the immunotolerant, viral replicative phase there is only mild hepatocellular damage and inflammation. Immunostaining reveals predominant nuclear localization of HBcAg. (51, 52) HBcAg and HBeAg generally have a coincident cellular expression (53-55), but the ratio of HBcAg to HBeAg may differ in subcellular locations. Strong cytoplasmic HBeAg is a marker of high viral replication. (52, 54) HBsAg is found in the cytoplasm of some hepatocytes and in the membrane of numerous cells in a honeycomb-like pattern. (56, 57)

The viral clearance phase is characterized by immune elimination of virus-infected hepatocytes, seroconversion from HBeAg to HBe antibody, and reduction of viral replication. (58) Liver histopathology consists of more severe lesions, including confluent necrosis of variable extent. (59) Immunostaining reveals nuclear, cytoplasmic and membranous HBcAg. Cytoplasmic HBcAg correlates predominantly with liver damage (51, 52, 60) and proliferation (61) HBsAg shows weak cytoplasmic positivity in some hepatocytes and a membranous staining pattern. (49, 57, 62, 63) HBeAg has been found localized in nucleus and cytoplasm of hepatocytes. (52, 54, 64, 65) and in liver cell membranes (66)

Some virus-infected hepatocytes apparently escape immune elimination, resulting in persistence of viral infection and integration of viral DNA into the host genome (viral integration phase). (67, 68) If damage during the virus clearance phase was not extensive, the liver may recover to only minimal abnormalities.(69) In case several exacerbations occurred (70) the liver may have developed cirrhosis.(49)

Active replication of HBV has ceased, but HBsAg is produced by hepatocytes that contain integrated HBV-DNA. (71) HBsAg accumulates in clusters of hepatocytes. HBcAg is usually undetectable. (72) Mild inflammation may persist for some time, but substantial necro-inflammation should alert for a possible superinfection with another virus. (73)

Some patients show ongoing active disease after HBeAg seroconversion, due to persistence of a precore stop mutant HBV with deficient HBeAg synthesis (74, 75) or other mutations. (76) Immunostaining in such cases reveals cytoplasmic HBcAg (75, 77, 78) and precore peptide.(79)

HBxAg has been visualized in the nucleus and cytoplasm of hepatocytes, more widely present than HBsAg or HBcAg, perhaps representing a more sensitive immunohistochemical test for HBV infection. (80) Its presence in hepatocellular carcinoma cells in HBV+ patients supports its involvement in hepatocarcinogenesis. (81)

In situ hybridization (ISH) for detection of HBV sequences has been performed with radioactively and with chemically labeled probes, the former yielding higher sensitivity, the latter better resolution. ISH identified the hepatocyte cytoplasm as the site of HBV replication. (82-86) Combination of ISH with immunostains for HBV antigens revealed that HBV DNA is present in numerous antigen negative cells, but co-localizes mainly with cytoplasmic and less with nuclear HBcAg. (85, 87, 88) Hepatocytes with cytoplasmic HBsAg accumulation are not virus replicating. (89)

HBV specific sequences were also found in bile duct epithelium, endothelial and vascular smooth muscle cells. (84) In hepatitis B, immunohistochemistry is more sensitive than ISH. (90)

Electron and immuno-electron microscopy allow to visualize and identify structural components of HBV in liver cells.

HBc particles appear as spherical structures, 24-27nm in diameter, in the nucleoplasm, and in the hyaloplasm between hepatocellular organelles. (89, 91) The finding that cells with nuclear HBcAg are mostly free from HBV-DNA by ISH (87, 88) suggests an accumulation of empty or non-replicating core particles. (89)

Encapsulation of core particles within the endoplasmic reticulum (Dane particle formation) has been documented. (92-94) Membrane localization of HBcAg in the hepatocellular plasmalemma was confirmed by immuno-electron microscopy. (95)

HBsAg appears as filaments within the cisternae of the endoplasmic reticulum. (96-98) Ground-glass hepatocytes exhibit a marked hyperplasia of the endoplasmic reticulum which dislocates the cytoplasmic organelles to the cell periphery. Within the cisternae, there are typical filaments giving a positive reaction for HBsAg and pre-S by immuno-electron microscopy. (27, 28, 89, 98)

3. Grading and staging of chronic viral hepatitis B. Semiquantitative scoring

The old classification of chronic hepatitis (99) was essentially a rough grading system, distinguishing milder from more severe variants of disease.

Progress in aetiological insight and treatment options necessitated a revised classification.

The new classification of chronic hepatitis is based on aetiology (viral, autoimmune, drug-induced and cryptogenic), also taking into account the histological grade of disease activity and the stage of evolution in terms of fibrosis and architectural derangement (cirrhosis).(100, 101)

Grading and staging is done in (preferably standardized) verbal descriptions, for instance: mild, moderate and severe chronic hepatitis as grades; and mild, moderate, severe fibrosis and cirrhosis as stages.(100)

Several semiquantitative scoring systems have been proposed, in which numerical scores are assigned to different grades and stages of chronic hepatitis. These methods are primarily indicated in the context of therapeutic trials or research projects; they are not intended to replace verbal reports in routine diagnostic practice. (100, 102-107)

The first successful scoring system specifically designed for chronic hepatitis is widely known as the Knodell Histological Activity Index (HAI).(108) Scores for the individual lesion categories are added to obtain an overall "histological activity index". This broadly used system is subject to a number of criticisms, the most important being that scores for necro-inflammation (grading) are added to those for fibrosis (staging). (100, 109)

Subsequent scoring systems have taken these deficiencies into account and comprise more simple and more complex prescriptions. Several simple and easily applicable systems are available. (110-112) The French METAVIR group devised a simple algorithm for standardized grading (113), and an assessment of fibrosis (staging) in five categories. (114) Simpler scoring systems provide less information but tend to be more reproducible than complex ones. (115)

An updated version of the Knodell HAI16 separates grading from staging and is more detailed to provide more information. It assures adequate inter-observer reliability. (117)

Several problems are inherent to all scoring systems available: use of arbitrary scores that are not mathematically valid, observer variation, sampling variability and aetiological diversity. (103)

Accuracy and consistency of scoring procedures can be improved by previous planning and agreement between clinician(s) and pathologist(s), by excluding biopsy specimens of inadequate size and quality, by agreement on definition of histological criteria at the start of the study, by preferable use of dual observers, by avoiding long time intervals between scoring of biopsies, and by checking intra- and inter-observer variation. (116)

In general, staging of fibrosis and architectural changes has proved to be more reproducible than grading of necro-inflammatory lesions. (115, 118)

Semiquantitative scoring of liver biopsies has been extensively applied in clinical trials for new treatments of chronic hepatitis B, C and D.(119)

Morphometry has been used as well for quantitation, mostly for assessment of fibrosis. (120, 121) Image analysis based

automated quantification of liver fibrosis was claimed to be a sensitive, precise, objective and reproducible method for quantification, thus supplementing scoring systems that are more based on distribution patterns of fibrosis. (122)

The potential usefulness of a mathematical scoring system based on fractal geometry for quantifying irregular patterns of fibrosis as observed in chronic hepatitis was recently addressed. (123)

4. Precancerous lesions

Both HBV and HCV infections may lead to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). (124, 125) Some cellular changes and nodular lesions are considered premalignant or precursors of HCC. Recognition and reporting of such lesions is important for adequate patient surveillance. Liver cell dysplasia (LCD), which may be of the large cell type (126) or of the small cell type (127) belongs to this type of change.

Large cell LCD in biopsies of patients with chronic hepatitis B (or C) is an independent risk factor for the development of HCC. (128, 129) Small cell LCD might originate from hepatic progenitor cells (130) and may represent an early step in carcinogenesis. (129)

Clusters of LCD measuring less than 1mm in diameter are termed “dysplastic foci”. Lesions measuring more than 1mm in diameter are designated as nodules. (131)

A macroregenerative nodule (measuring 0,8cm or more) is particularly common in macronodular cirrhosis. Histologically it shows hyperplastic liver parenchyma but no cellular atypia nor disorder in muralia arrangement. Dysplastic nodules do display atypical features, but not severe enough to qualify for frank HCC. Dysplastic nodules are subclassified as low or high grade, and considered distinct stages in the multistep process of hepatocarcinogenesis. (132) Foci and nodules should be carefully identified and reported. (5, 133)

5. Special cases of chronic viral hepatitis B

Short reference is made to those conditions in which liver tissue examination may be contributory.

5.1. Coinfection with Hepatitis D virus

Coinfection with Hepatitis D virus (HDV, delta agent) alters the course of acute hepatitis B, favours chronic evolution and enhances severity of disease. (110, 134, 135) Microvesicular steatosis of hepatocytes (morula cells) was a notable feature in an outbreak of HDV infection in Venezuelan Indians. (136-138)

Specific immunostaining allows to confirm HDV aetiology. HDAg is detected in hepatocyte nuclei (139, 140) and in the cytoplasm and plasmalemma of hepatocytes. (141) Large amounts of nuclear HDAg may cause a “sanded nuclei” appearance. (142, 143)

Double immunostaining reveals separate expression of HDAg versus HBsAg and HBcAg; co-expression in the same cell is found though rarely with HBcAg. (141, 144)

ISH for HDV RNA may be more sensitive than immunostaining for HDAg. (84, 145, 146)
HDV RNA localizes in nuclei, usually together with HDAg, but occasionally alone. (146)

5.2. Recurrent HBV infection post transplantation

Recurrent HBV infection post transplantation may either show the typical features observed in non-transplanted liver, or, more rarely, atypical patterns of liver damage.

Atypical patterns include hepatocyte ballooning, fatty change and a distinctive cholestatic syndrome, which also may occur in combination. (147)

Hepatocyte ballooning typically occurs without significant inflammation, but shows high level of viral replication reflected in diffuse nuclear and cytoplasmic immunostaining for HBcAg and HBeAg. (148) Ballooning associated with steatosis may explain the term “steatoviral hepatitis”. (149)

The terms “fibrosing cholestatic hepatitis” (fibrosing cytolytic hepatitis, fibroviral hepatitis) refer to a distinctive pattern of injury associated with rapidly progressive graft failure. (147-153) The histological pattern comprises periportal fibrosis surrounding ductular structures, prominent hepatocyte ballooning, bilirubinostasis, and mild or absent inflammation. Immunostaining reveals markedly positive cytoplasmic HBcAg. This stage may be followed by extensive postnecrotic collapse, fibrosis and ductular reaction.

This lesional pattern was also reported in other immunodeficiency states, including HIV infection (154) and following renal transplantation.(155)

5.3. Association of HBV with other viral infection

Dual and triple infections with HBV, HCV and HDV occur. Histopathologically, there are no specific findings to suggest the possibility of multiple infections. (156) Immunohistochemical analysis may reveal suppression of HBsAg and HBcAg by simultaneous HCV infection. (157)

HCV infection is suspected on the base of characteristic histological features¹⁵⁸ and positive immunostaining with specific antibody. (159)

Co-infection of HBV with cytomegalovirus (CMV) infection can be identified by typical nuclear inclusions, abnormal basophilic granular cytoplasm, microabscesses, and immunohistochemical staining for CMV antigen. (5)

Co-infection of HBV with HIV usually results in diminished histological activity (160), although more severe activity was also reported. (161) Immunostaining revealed more diffuse staining for HBV and HDV antigens in tissue specimens of HIV coinfecting patients.(160)

5.4. Association of HBV with concomitant liver disease

Histopathological study often reveals clinically unsuspected concomitant liver disease. Examples include: alpha-1-antitrypsin deficiency, alcoholic liver disease, primary sclerosing cholangitis, haemochromatosis, amongst others.(2)

II. Special Studies (Research)

Inventive investigations in different ways of tissue examination are applied to broaden the insight in pathophysiology. Examples include: semiquantitative evaluation of activated hepatic stellate cells (162-164); immunohistochemical studies on lymphocyte subsets, antigen presenting cells, adhesion molecules and apoptosis markers (165, 166); study of cytokines (167); elution of liver infiltrating lymphocytes (168); microdissection of sublobular regions of high and low degree of liver damage and their relationship to HBV variants (169); and cDNA microarray based analysis of differences in gene expression between chronic hepatitis B and C. (170)

To meet future requirements, pathology evaluates appropriate techniques of tissue preparation for high throughput molecular analysis.(171)

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