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## The Liver Biopsy in Viral Hepatitis C

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**The World Health Organization estimates the world prevalence of viral hepatitis C at 3%. The advent of therapeutic regimens for viral hepatitis has made the liver biopsy a routine specimen in the surgical pathology laboratory. The clinician will need to know the degree and type of necroinflammatory activity and the presence and level of fibrosis, before making the decision to start therapy. The biopsy is essential in determining the nature and extent of hepatic injury, the degree of inflammation, the type of inflammatory**

**reaction, the distribution of fibrosis and the presence of other findings affecting the patient's prognosis. A scoring system is essential in the therapeutic trials for treatment of viral hepatitis and can be helpful in making therapeutic decisions. The aim of this article is to summarize the terminology, histologic findings, the available and more commonly used scoring systems and the surgical report of chronic viral hepatitis C.**

*Key words: Viral hepatitis C, Liver biopsy, Histopathology, Pathology*

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**T**he world prevalence of viral hepatitis C is estimated at 3% by the World Health Organization. In Puerto Rico, the prevalence of this disease has been estimated at 6.3% in a population sample of adults living in San Juan (1). Chronic viral hepatitis C is a fibrosing disease with a variable progression to cirrhosis. The median expected time to cirrhosis is approximately 30 years, with thirty percent of the patients having a faster progression to cirrhosis in 20 years and another 30% not progressing to cirrhosis in over 50 years (2). The advent of therapeutic regimens for viral hepatitis has made the liver biopsy a routine specimen in the surgical pathology laboratory, because it can detect patients with clinically silent cirrhosis (3). While viral factors, such as genotype and quantity of RNA are the most significant in determining the response to interferon-based therapy, these do not independently correlate with the level of fibrosis (4). The rate of progression to cirrhosis depends also on several epidemiologic factors, such as age at infection, duration of infection, alcohol consumption, gender and immune status (5). The inflammatory activity of this disease has not been linearly correlated to cirrhosis, even though there is a significant correlation among the degree of

inflammatory activity and the level of fibrosis. In patients infected with genotype 1b, increases in inflammatory activity correlate with subsequent increases in fibrosis scores (6). Since the efficacy of therapy has not been 100% successful in obtaining a sustained response and therapy is toxic to the patients, who may feel worse with the medications than with this asymptomatic hepatitis, it is reasonable to restrict treatment to those patients who have a greater chance of success or to those with a higher risk of progression to cirrhosis. The decision to start treatment is made easier for the clinician and the patient if the degree and type of necroinflammatory activity and the presence and level of fibrosis are known. Currently, liver biopsy is the only way to obtain this information. The biopsy is essential in determining the nature and extent of hepatic injury, the degree of inflammation, the type of inflammatory reaction, the distribution of fibrosis and the presence of other findings affecting the patient's prognosis, such as dysplasia, steatosis and other concomitant pathologies.

Our aim is to describe the terminology and histologic findings, to discuss the available and more commonly used scoring systems and to present the elements of the surgical report of chronic viral hepatitis C for practicing anatomic pathologists and clinicians interested in hepatology.

Terminology and reporting practices must keep pace with the advent of greater insight into the etiology of chronic liver disease. Etiology is the most important consideration, because therapy and prognosis is different, depending on whether the etiology is autoimmune, drug induced, or viral hepatitis B or C. Patients with viral

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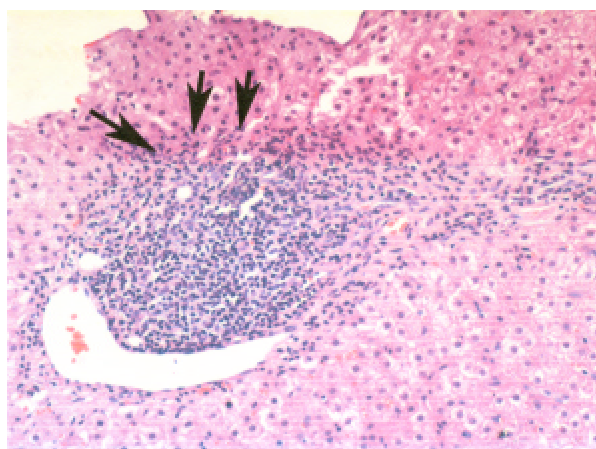
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hepatitis B with mild interface hepatitis will most likely resolve without ever developing cirrhosis, while the outcome is not so favorable when the etiologic agent is viral hepatitis C. The following elements should be considered in the evaluation of liver biopsies in patients with clinically suspected chronic viral hepatitis: adequacy of the biopsy, grade of inflammatory activity, stage of fibrosis, presence of dysplasia, presence and amount of steatosis and presence of other incidental findings. We will now proceed to describe each of these elements and their clinical relevance.

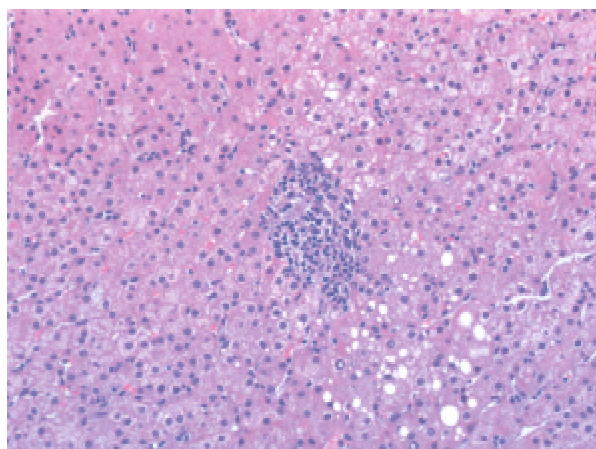
**I. Adequacy of the biopsy: size.** An adequate liver biopsy should measure 1-4 cm long, preferably at least 1.5 cm. It must have at least 6 portal spaces, for adequate scoring of the inflammatory activity and the stage of fibrosis. Liver needle biopsies are subject to sampling errors, with an estimated accuracy of diagnosing cirrhosis depending on the size of the biopsy and the number of cores obtained. In a study done to evaluate the effect of size in the accuracy of diagnosing cirrhosis, only 14 of 20 cases with cirrhosis were detected when the length of the biopsy decreased from 25 to 20 mm. When the sample was 10 mm long, only 11 biopsies were correctly diagnosed. In another study done immediately before autopsy, cirrhosis was missed by a single pass in 20% of the cases, and three passes were needed for 100% accuracy in the diagnosis of cirrhosis (7).

While fine gauge liver needle biopsies performed by interventional radiologists are adequate for the diagnosis of cancer, they are inadequate for semi-quantitative scoring of the biopsy, because the few portal spaces present are very often incomplete. Cutting (Tru-cut, Bard) needles make staging more reliable than suction needles, (Menghini, Klatskin), which tend to produce fragmented biopsies.

**II. Grade.** Grading the necro-inflammatory activity refers to assessing the degree of inflammation in the periportal and lobular areas. *Interface hepatitis* is the current terminology for piecemeal necrosis or periportal hepatitis. Piecemeal necrosis is no longer used to describe this histologic pattern of cell death, because it is considered to be due to apoptosis rather than necrosis. An example of interface hepatitis is illustrated in Figure 1. Observe the inflammatory cells surrounding hepatocytes at the periphery of the limiting plate. It is important not to misinterpret the portal inflammation, which is always present, with interface hepatitis, since it is the later that is significant in determining the progression of the disease. *Lobular necrosis* or degeneration refers to apoptosis, occurring singly or in clusters, observed in the liver parenchyma, away from the portal areas. Figure 2 illustrates an example of lobular necrosis. *Bridging*



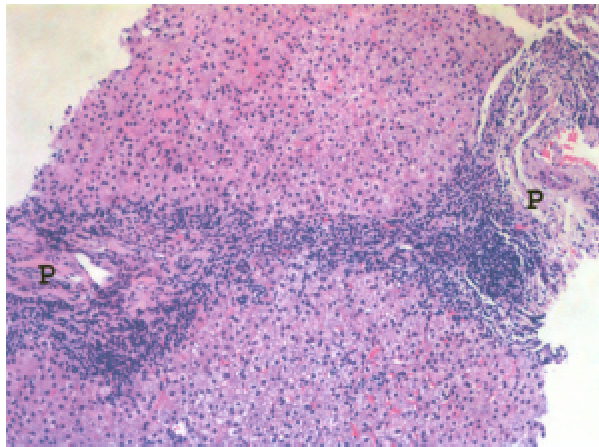
**Figure 1.** H&E. 40X. Portal space with moderate lymphoplasmacytic infiltrate. Observe the inflammatory cells surrounding hepatocytes at the periphery of the limiting plate (Arrows).



**Figure 2.** H&E 40X. Lymphoplasmacytic aggregate within the liver parenchyma away from portal or central areas.

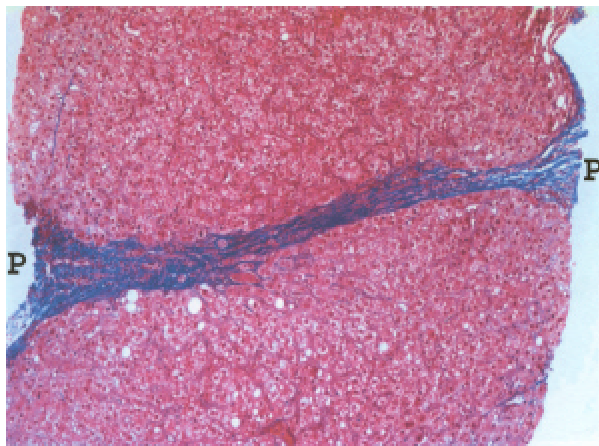
*necrosis* refers to the necro-inflammatory process linking portal tracts (Zone 1) to other portal tracts, or to hepatic veins (Zone3). Most observers restrict bridging necrosis to the linkage of portal tracts to hepatic veins. An example of porto-portal bridging necrosis is illustrated in Figure 3. Confluent necrosis comprises bridging necrosis, multilobular or multiacinar necrosis and massive hepatic necrosis.

**III. Stage of fibrosis.** Several special stains can be useful in underscoring the level of fibrosis present in a biopsy, with Masson's trichrome being the most popular. Type I collagen is stained blue, highlighting the portal areas, hepatic vein tributaries and Glisson's capsule. Another commonly used stain is reticulin, a silver stain, which is helpful in visualizing the type III collagen that lines the sinusoids. *Fibrosis* is characterized by the

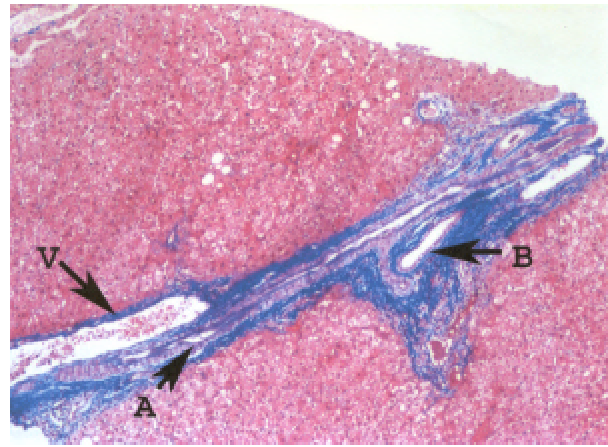


**Figure 3.** H&E 10X. Linkage of two portal spaces by necroinflammatory process. P: Portal space.

increased deposition of collagen fibers, in the scarring events that may lead to cirrhosis in patients with chronic viral hepatitis C. *Portal fibrosis* is the increased deposition of collagen type I around the portal tract. This fibrosis is a sine qua non of chronic hepatitis in untreated patients. *Fibrous septae* refers to a wall of fibrous tissue that divides the liver parenchyma. Fibrous septae may extend between portal spaces or between portal spaces and central veins. The walls of fibrous tissue that bridge portal and central spaces represent a more advanced stage in the route towards cirrhosis. Portal based fibrosis characterizes chronic viral hepatitis, chronic cholestatic diseases, autoimmune diseases and hemochromatosis, while central based fibrosis characterizes alcoholic liver disease, non alcoholic steatohepatitis (NASH) and chronic venous obstruction. Bridging fibrosis is shown in Figure 4. It should not be confused with tangential sections of fibrotic portal tracts, as shown in Figure 5. The traversing bile

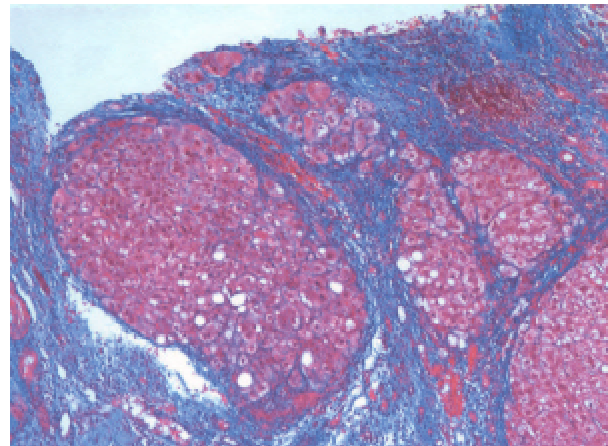


**Figure 4.** Trichrome stain. 10x. Fibrosis connecting two portal spaces. P: Portal space.



**Figure 5.** Trichrome stain. 10x. Tangential section of hepatic artery and bile duct. A: Artery. V: Vein. B: Bile duct.

ducts and vessels help to make the distinction. The endpoint of fibrosis is cirrhosis, where normal liver architecture is substituted by structurally abnormal nodules. Cirrhotic nodules are shown in Figure 6. The cirrhosis of chronic viral hepatitis is characterized by nodules of different sizes, while chronic cholestatic diseases lead to regular nodules with central venules.



**Figure 6.** Trichrome stain. 40x. Three complete cirrhotic nodules of different sizes without central venules surrounded by fibrosis

**IV. Dysplasia.** The presence of dysplasia in a liver biopsy implies an increased risk for the development of hepatocarcinoma (8). Two types of liver cell dysplasia are recognized, with different implications in their relationship with cancer. Small cell dysplasia consists of intermediate and progenitor cells with increased nucleocytoplasmic ratio, small cytoplasm and nuclear crowding in a cirrhotic liver. Progenitor cells have the potential to differentiate towards hepatocytes or cholangiocytes. They express the same markers as hepatocarcinoma: alpha fetoprotein and

cytokeratins 7,19 and 14; but only a small minority will ever evolve into hepatocellular carcinoma. Large cell dysplasia consists of mature atypical hepatocytes with large nuclei, pleomorphism and nucleoli, but with normal nucleocytoplasmic ratio. Large cell dysplasia does not progress to cancer, but is associated to cirrhosis related hepatocarcinoma, probably because the same alterations lead to both dysplasia and cancer. Dysplastic foci measure less than 1mm, while dysplastic nodules are by definition larger than 1 mm. Dysplastic nodules are considered more advanced precursors of hepatocarcinoma than dysplastic foci.

**V. Steatosis.** Macrovesicular steatosis is a common finding in the liver biopsy of patients with chronic viral hepatitis C. While alcoholic patients have fat globules of equal size, occurring in central areas and uniformly along the lobule, Hepatitis C patients tend to have fat globules of different sizes, occurring focally and in periportal areas. Liver steatosis accelerates the development and progression of fibrosis in patients with chronic viral hepatitis C, as demonstrated by several clinical studies (9). Steatosis was statistically associated with the stage of fibrosis in patients with chronic viral hepatitis C in a study of a population of which one third had previous excessive alcohol consumption, but in another study steatosis was considered to be a cytopathic effect of HCV genotype 3 (3). HCV genotype 3 is an independent risk factor for steatosis, with a dramatic decrease in steatosis among patients with a sustained response to antiviral therapy (10). In genotype 1 patients, steatosis is associated to high body mass index and central adiposity, decreasing with weight loss. Thus, it is important to mention the degree of steatosis, when present, in the surgical pathology report. The amount of steatosis can be assessed as minimal when it is present in less than 5% of the biopsy; mild, when it affects 5 to 30% of hepatocytes; moderate, affecting 30 to 60% of hepatocytes and severe, when it affects over 60% of liver cells.

**VI. Other histologic findings.** Granulomas are seen more often in chronic viral hepatitis C than in viral hepatitis B and have been associated to interferon therapy. Hemochromatosis and hepatocellular carcinoma have also been identified.

### Scoring Systems

Since 1981, when Knodell published the Hepatitis Activity Index (HAI) for semi-quantitative scoring of the liver biopsy, three additional scoring systems have been published and used widely in the western world: Ishak's modification to Knodell's HAI (11), the Scheuer (12) and the METAVIR (2) systems. There is no standard scoring

system for chronic liver disease, thus different systems are being used. However, a scoring system is essential in the therapeutic trials for treatment of viral hepatitis and can be helpful in making therapeutic decisions in daily practice. Simpler scoring systems have the advantage of being more practical in routine daily practice, but may not be as useful in clinical studies of response to therapeutic regimens because of their insensitivity to subtle differences in the resolution or progression of the disease.

A simplified comparison of these available systems is included in Table 1. Ishak's modification of the Knodell

**Table 1**

Criterion	Knodell HAI	Ishak	Scheuer	METAVIR
Periportal hepatitis	0-10	0-4	0-4	0-2
Intralobular degeneration	0-4	0-4	0-4	0-2
Confluent necrosis		0-6		+/-
Portal inflammation	0-4	0-4		
Fibrosis	0-4	0-6	0-4	0-4
Highest activity score	22	18	8	3
Fibrosis stage		6	4	4

HAI= Hepatitis activity index

score includes the following changes: a) a continuous scoring progression by including the numerical 2 category in all the criteria, b) separating the degree of inflammation from the level of fibrosis. This feature is extrapolated from the field of oncology, where grading refers to the inflammatory activity and staging to the level of fibrosis, as proposed by Scheuer in 1991, c) including three additional numbers for the description of the progression from fibrosis to cirrhosis: 2: portal expansion of most portal areas with or without portal septae; 4:porto-central bridging and 5: incomplete cirrhosis, d) confluent necrosis is assessed separately from the category of piecemeal necrosis, with detailed description of each level. Both the METAVIR and the Scheuer systems exclude the criterion of portal inflammation when calculating the inflammatory activity index because portal inflammation is inherent to the diagnosis of chronic hepatitis and has little value in predicting response in both the HAI and modified HAI scoring systems. Interface hepatitis (Piecemeal necrosis) alone has shown a greater predictive value (5). The METAVIR system keeps the concept of separating the grade of activity from the stage of fibrosis and assessing bridging necrosis separately from piecemeal necrosis, introduced by the Scheuer system. The differences between the Scheuer and the METAVIR semi-quantitative scoring systems reside in a) METAVIR being simpler in the assessment of inflammatory activity, with a maximum score of 3, b) METAVIR does not distinguish between

porto-portal versus porto-central fibrosis. The latter has a more ominous prognosis and, when using the Scheuer classification, a fibrosis score of 3 or 4 is a good predictor of non response to Interferon therapy (5), and c) in the METAVIR histology score, piecemeal necrosis (interface hepatitis) has precedence over lobular necrosis when grading inflammatory activity. Thus, if interface hepatitis is mild, the maximum activity index will be 2, even if severe lobular necrosis is present.

A recent study compared Ishak's modified HAI with the METAVIR system in the evaluation of liver biopsies from patients who had chronic viral hepatitis B or C. A good concordance in scoring inflammatory activity and an excellent concordance in assessing the fibrotic change was reported (13). The Ishak system was found to be more user friendly, reproducible and comprehensive. The Knodell score has been used more extensively and has served to compare different trials; however, the scores of necro-inflammatory activity should not be added to the fibrosis score when estimating the activity index, because fibrosis is considered the consequence, rather than the cause of the inflammatory reaction.

### **The Surgical Report**

The surgical report of liver biopsies of patients with chronic viral hepatitis C should include all the elements affecting the patient's prognosis, as well as the limitations of the specimen. Thus, we always start with a microscopic description of the biopsy, mentioning the type of biopsy (needle or wedge) and the amount of portal spaces. Then we proceed to describe the degree (mild, moderate or severe) and the pattern of inflammation, whether it is interface and/or lobular and the presence of bridging necrosis. The stage of fibrosis is also described, including whether this finding was confirmed by special stains, such as trichrome and/or reticulum stains. The degree of steatosis is assessed and graded, when present. The presence of dysplasia and other unexpected findings, such as granulomas are also mentioned. A final diagnosis wraps the report, including the etiology of the hepatitis when it is serologically known, the degree of inflammatory activity and the stage of fibrosis, complemented by one of the semiquantitative scoring systems. A report that incorporates all these elements offers invaluable information to the clinician and enhances the quality of the medical care of the patient.

### **Conclusion**

We have reviewed the clinical significance of the liver biopsy in patients with hepatitis C, followed by a detailed

description of the major histologic findings. The available and most often used systems were described and compared, and the elements of the surgical report have been synthesized.

### **Resumen**

La prevalencia de hepatitis C es de 3%, según estimada por la Organización Mundial de Salud. El uso de agentes terapéuticos en hepatitis C ha hecho de la biopsia de hígado un espécimen rutinario en patología quirúrgica. El clínico necesita saber el grado y tipo de actividad necroinflamatoria, así como la presencia y grado de fibrosis antes de tomar una decisión terapéutica. La biopsia es esencial para determinar la naturaleza y extensión del daño hepático, el grado de inflamación, el tipo de reacción inflamatoria, la distribución y extensión de fibrosis y la presencia de otros hallazgos que afecten el pronóstico del paciente. Un sistema de puntuación es esencial para medir los efectos terapéuticos en los estudios de tratamiento de hepatitis viral y puede ayudar en la decisión terapéutica. El objetivo de este artículo es resumir la terminología, los hallazgos histológicos, los sistemas de puntuación disponibles más usados y el reporte quirúrgico de hepatitis viral C crónica.

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