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Chronic viral hepatitis: The histology report

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Abstract

In chronic viral hepatitis, the role of liver biopsy as a diagnostic test has seen a decline, paralleled by its increasing importance for prognostic purposes. Nowadays, the main indication for liver biopsy in chronic viral hepatitis is to assess the severity of the disease, in terms of both necro-inflammation (grade) and fibrosis (stage), which is important for prognosis and therapeutic management. Several scoring systems have been proposed for grading and staging chronic viral hepatitis and there is no a general consensus on the best system to be used in the daily practice. All scoring systems have their drawbacks and all may be affected by sampling and observer variability. Whatever the system used, a histological score is a reductive approach since damage in chronic viral hepatitis is a complex biological process. Thus, scoring systems are not intended to replace the detailed, descriptive, pathology report. In fact, lesions other than those scored for grading and staging may have clinical relevance and should be assessed and reported. This paper aims to provide a systematic approach to the interpretation of liver biopsies obtained in cases of chronic viral hepatitis, with the hope of helping general pathologists in their diagnostic practice.

Keywords: Chronic viral hepatitis; GIPAD report; Liver biopsy; Scoring systems

1. Introduction

The definition of chronic hepatitis applies to a protracted necroinflammatory liver disease, irrespective of its etiology. Indeed, chronic hepatitis can be the consequence of a variety of noxious stimuli, among which hepatitis viruses are the most common. A fundamental feature of this disorder is its tendency to evolve, giving it the potential to culminate in cirrhosis and, eventually, hepatocellular carcinoma.

As chronic hepatitis is a silent process in the vast majority of cases, a suspected case of chronic hepatitis has for many years represented the main reason for performing a liver biopsy.

Paul Herlich performed the first liver aspiration procedure more than 100 years ago [1]. In 1958, the technique was refined by Menghini, who introduced the "Menghini needle" and the so-called "one-second needle biopsy of the liver" [2], which became widespread thanks to a low mortality rate and relatively limited morbidity. The use of liver biopsy procedures peaked in the last two decades of the 20th century. Until serological tests for detecting hepatitis virus infections were developed, liver biopsy was performed mainly for diagnostic purposes, to distinguish chronic hepatitis from other acute and chronic disorders, and to offer prognostic insight. The prognosis of chronic hepatitis relied on a simple morphological classification [3,4], which distinguished *chronic active hepatitis* (characterized by the presence of

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interface hepatitis, or what was formerly called piecemeal necrosis) from *chronic persistent hepatitis* or *chronic lobular hepatitis* (with no interface hepatitis). Only chronic *active* hepatitis was considered at risk of developing into cirrhosis. With the identification of hepatitis C virus (HCV) and advances in our understanding of the mechanisms of fibrosis onset and progression, this classification was subsequently challenged for two main reasons [5–7]. First, it became clear that the progression of fibrosis is influenced not only by interface hepatitis, but also by the overall severity of the necroinflammatory picture, so chronic *active* and chronic *persistent* hepatitis should no longer be considered as distinct entities, but – more appropriately – as different stages of the same disease, one possibly evolving into the other. Second, the specific etiology was recognized as a major factor influencing the rate of cirrhotic development and response to treatment. A new diagnostic approach was thus devised, integrating etiology with morphological findings to establish prognosis and treatment indications [8].

The development of effective anti-viral treatments led to the need to assess the histological severity of chronic viral hepatitis more objectively (and possibly in a more reproducible manner) in clinical trials, prompting the use of numerical grading and staging systems [9]. Several clinical-pathological studies have demonstrated the practical value of grading and staging in the management of patients with chronic viral hepatitis, providing evidence that both grade and stage affect disease progression and treatment efficacy.

Although grading and staging are the primary reasons for performing a liver biopsy nowadays, they clearly tell only a part of the story when it comes to the pathological assessment of chronic viral hepatitis because they fail to take into account the whole spectrum of morphological changes that might influence outcome and/or treatment.

The aim of this paper is to take a systematic approach to the pathological assessment of liver biopsies obtained in cases of chronic viral hepatitis, focusing particularly on analyzing the different scoring systems available for grading and staging liver damage, and on the practical value of adjunctive information.

2. Epidemiology

2.1. Hepatitis B virus infection

The prevalence of hepatitis B virus (HBV) infection varies widely, ranging from 0.1% to 20% in different parts of the world [10]. The Far East and part of the Middle East, sub-Saharan Africa and the Amazon basin are regions with a "high" prevalence (i.e. hepatitis B surface antigen [HBsAg] positivity rates >8%). The viral infection is highly endemic in these areas and it is often acquired perinatally or early in childhood. Japan, the Indian subcontinent, parts of central Asia and the Middle East, Eastern and Southern Europe, and parts of South America, are all areas with an "intermediate" prevalence (2% to 7% HBsAg positivity) of chronic HBV infection. Regions with a "low" prevalence (<2% HBsAg positivity) include the United States, Northern Europe, Australia, and the southern part of South America [11]. Italy is a low-prevalence area (less than 1%) with few regional variations and this is due to both the routine vaccination of the newborn and to improved socio-economic conditions. In Italy, as in other developed countries, the reservoir of infection is expected to be maintained by immigration and adoption [12].

HBV is present in the blood, saliva, semen, vaginal secretions, menstrual blood and, to a lesser extent, perspiration, breast milk, tears and urine of infected individuals. The virus can survive outside the body and is easily transmitted through contact with infected body fluids. Sexual activity (particularly heterosexual) and injected drug use account for the majority of cases of HBV transmission in low-prevalence areas.

2.2. Hepatitis C virus infection

Hepatitis C virus is an RNA virus first identified in 1989, belonging to the Flaviviridae family of viruses, and it spreads primarily through direct contact with the blood or body fluids of infected individuals. The sharing of needles amongst intravenous drug users, inadequately sterilized instruments used in medical procedures, tattooing and body piercing are generally considered the main risk factors for acquiring HCV infection.

It is estimated that 3% of the world population is infected with HCV [13]. Most populations in the Americas, Europe, and South-East Asia have HCV prevalence rates below 2.5%. In the Western Pacific regions and parts of South America, its prevalence is higher (2.5–4.9%), while in populations in the Middle East and Africa it ranges from 1% to 12%. In Italy, the global prevalence of infection is about 3%, but there is a great regional variability, the south having the highest prevalence (12–16%). The prevalence of HCV infection is also age-related, children and adolescents exhibiting very low rates (0.4%) [14].

HCV is highly heterogeneous. Eleven HCV genotypes with several distinct subtypes have been identified around the world. Different strains do not differ significantly in their virulence or pathogenicity, but different genotypes vary in their sensitivity to interferon/ribavirin combination therapy. This heterogeneity also hinders the development of vaccines, since vaccine antigens from multiple serotypes will probably be needed for global protection. In the absence of a specific vaccination, HCV infection thus remains a major global health problem and, although efficient therapies are now available, HCV-related end-stage liver disease is still the most frequent indication for liver transplantation in adult patients.

3. Clinical and laboratory aspects

The most frequent clinical circumstances arousing a suspicion of chronic viral hepatitis involve an unexplained rise in ALT levels, but the incidental discovery of high liver enzymes

is also very frequent. The patient's medical history usually orients the physician's interpretation of these biochemical changes and, when viral disease is suspected, the next step in the clinical workup of viral hepatitis involves an evaluation using serological or molecular biological methods.

Once chronic hepatitis is suspected, serological and molecular assays can confirm whether or not a virus-related chronic hepatitis exists. When the diagnosis is confirmed and the etiology determined, then the severity of the disease needs to be established and suitable therapy arranged.

3.1. Hepatitis B virus-related chronic hepatitis

3.1.1. Diagnosis

Patients with chronic hepatitis B (CHB) are diagnosed from the persistence of hepatitis B surface antigen (HBsAg) in serum for more than 6 months, so serological rather than molecular assays are needed to establish whether the patient is an active or inactive carrier, or to distinguish between acute and chronic HBV-related liver disease [15].

There are two main forms of HBsAg-positive hepatitis [16,17] i.e. the HBeAg-positive form associated with wild-type infection, and the HBeAg-negative form associated with core promoter and/or pre-core mutant viruses. In the former, active carrier status is defined by HBV DNA levels $\geq 1.8 \times 10^4$ IU/ml; viraemia levels above this threshold are generally associated with liver disease. In patients with HBeAb, HBV DNA or ALT levels tend to fluctuate in time, so repeatedly measuring HBV DNA levels helps to distinguish between active and inactive carrier status (the latter being characterized by HBV DNA levels below 1.8×10^4, by ALT levels that are normal or up to twice as high, and by the absence of liver disease). Because of these fluctuating levels, HBV DNA and ALT need to be monitored for at least 12 months to rule out active infection in HBeAg-negative patients with HBeAb. The absence of liver damage, as evaluated directly on liver biopsy or assumed in the absence of antiHBc IgM, is nonetheless needed to diagnose an inactive HBV infection (Table 1).

HCVAb, HDVAb and HIVAb should be sought not only for the purposes of differential diagnosis, but also to exclude any co-infections.

Screening for hepatocellular carcinoma by abdominal US is a further step in the diagnostic work-up of HBV chronic hepatitis, because liver cancer can develop in cases of HBV infection with or without liver cirrhosis.

3.1.2. Treatment options

Antiviral treatment is indicated in patients with chronic hepatitis B in the active replication phase [16–19]. High levels of HBV DNA and serum ALT are characteristic of active HBV infection, which is usually associated with variable degrees of liver fibrosis. Treatment decisions are currently based on HBV DNA, but it may be necessary to assess the grade and stage of liver damage by histology.

There are several goals of treatment for patients with chronic HBV infection, some more easily achieved than others [17]. The short-term goals of antiviral therapy are to convert patients from the high replication phase (demonstrated by HBeAg) to the low replication phase characterized by the appearance of HBeAb. This endpoint is associated with lower or normal ALT levels and less hepatic inflammation. HBeAg/HBeAb seroconversion, with the loss of serum HBV DNA is an intermediate objective, while the ultimate aim of treatment is HBsAg/HBsAb seroconversion. The long-term goals are to delay or prevent histological progression to cirrhosis and hepatocellular carcinoma, and to improve survival. Patients with compensated cirrhosis consequently warrant treatment when their HBV DNA levels are >200 IU/ml, whatever their ALT levels, with a view to stopping or slowing the progression of their liver disease and preventing viral reactivation.

Treatment options for chronic hepatitis B include Peg-interferon and antiviral drugs such as nucleoside or nucleotide inhibitors [17]. Interferon therapy is of finite duration, whereas a long-term therapy should be planned when using nucleoside analogs.

The usual regimen for Peg-interferon is a weekly dose for 12 months. Nucleoside treatment should be continued for 6 months after seroconversion in HBeAg-positive patients, or after HBV DNA levels have become undetectable in HBeAg-negative patients.

After interferon treatment, HBeAg seroconversion occurs in 25–40% of patients, and loss of HbsAg in 5–10%.

Table 1
Definitions of chronic infection and carrier conditions in HBV-infected patients

	Chronic infection	Active carrier	Inactive carrier
HBsAg	+ (6 mos)	+ (>6 mos)	+ (>6 mos)
HBeAg	+/–	–	–
antiHBe	+/–	+	+
antiHBs	–	–	–
antiHBc	+	+	+
HBV DNA serum	>2000 if HBeAg–	>20,000	<2000
HBV DNA serum	>20,000 if HBeAg+		
HBV DNA tissue	+	+	+
Liver enzymes	↑ (persistent or intermittent)	normal	normal
Liver biopsy	Inflammatory activity present	Present (90%)	Absent (>50%)

3.2. Hepatitis C virus-related chronic hepatitis

3.2.1. Diagnosis

When HCV infection is clinically suspected, its diagnosis is based on the presence of both HCVAb and HCV RNA. Prior infections need to be distinguished from currently active infections, however, since up to 40% of patients infected with HCV undergo spontaneous HCV RNA clearance. A molecular assay with a sensitivity of ≤50 IU/ml is therefore needed to exclude ongoing viral replication [15,20].

HCV infection is associated with six different viral genotypes, but no association has been demonstrated as yet between severity of liver damage and genotype [21]. Randomized controlled trials have shown that quantitative measures of HCV RNA levels do not correlate with the severity of histological damage, so they cannot be used as markers of severity or as surrogate markers of progressive liver damage. As a direct consequence of these findings, in the debate raised by recent studies that support or question the role of liver biopsy for patients with chronic HCV infection, it should be emphasized that molecular assays are no substitute for a histological assessment for prognostic purposes.

3.2.2. Treatment options

All HCVAb-positive patients with detectable HCV RNA are potential candidates for treatment [22]. In chronic hepatitis C infection, treatment outcome has improved with the advent of Peg-interferon and ribavirin combination therapy [23]. Two different types of Peg-interferon – alpha 2a and alpha 2b – are available and recommended in combination treatment with ribavirin. With these treatment regimens, overall sustained virological response (SVR) rates are 55–60%, ranging from 40% in patients with genotype 1 infection to 75–80% in those with genotypes 2 and 3. Given such different rates of response, HCV genotyping is required before starting any treatment. With the marked improvement in SVR rates, patients' preferences regarding therapy, irrespective of any biopsy findings, and cost–benefit analyses may influence decisions concerning whether or not to go ahead with antiviral therapy. Liver biopsy is therefore no longer required for patients infected with genotypes 2 and 3, who respond well to antiviral therapy, or for patients with persistently normal ALT levels, who typically have mild disease. On the strength of these findings, it has become standard practice to use liver biopsy only in selected cases, rather than routinely, for managing patients with chronic hepatitis C [23].

Among the baseline factors predicting the success of antiviral therapy, genotype, viral load and histology are the most important: all these factors can be used to predict a relatively better or worse response to treatment (Table 2). It has also recently been demonstrated that response while on the treatment, and particularly an undetectable HCV RNA by week 4, is the best predictor of SVR after combination treatment.

3.2.3. Treatment monitoring

It is important to monitor virological response during the treatment not only to predict a favorable outcome (undetectable HCV RNA by week 4, regardless of genotype, predicts a high SVR rate), but also to decide when to discontinue the treatment because there is little chance of any SVR being achieved (HCV RNA still positive at week 12, or a <2 $\log_{10}$ drop in HCV RNA levels by comparison with the baseline values). Very low levels of circulating HCV RNA at the end of treatment are potentially useful for identifying patients likely to relapse after its discontinuation.

At the end of treatment lasting 24 weeks, finding undetectable HCV RNA levels with assays affording a sensitivity ≤50 IU/ml ensures that the treatment can be considered curative.

Table 2
Predictors of SVR in chronic hepatitis C infection

	Likelihood of SVR
Host-related predictors	
Age	High if <45 yrs
Body mass index	High if >30
Race/genetics	Better in Asians than in Caucasians, Hispanics, or African Americans, in declining order
Cirrhosis/bridging fibrosis	High if absent
ALT levels	Higher if >3 times beyond the upper normal limit
Insulin resistance	Better if absent
Virus-related predictors	
HCV genotype	High if genotype 2
HCV RNA viral load	High if HCV RNA <400,000 IU/ml
Quasispecies	Low if more complex
Treatment-related predictors	
Peg-IFN type and dosage	Better if full dosage
Ribavirin dosage	Better if weight-based
Adherence to treatment	Better if >80% of dosage of both drugs are taken for 80% of planned duration

4. Basic pathology of chronic viral hepatitis

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 [24,25].

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 [25,26] (Diagnostic strength: level 3). The severity of portal inflammation may vary from one patient to another and from one portal tract to another. Portal inflammation may be associated with *interface hepatitis periportal hepatitis* (periportal hepatitis; called piecemeal necrosis in the older literature), characterized by lymphocyte infiltrate at the boundary between the portal tract and the adjacent parenchyma associated with hepatocyte damage (mainly apoptosis) and dropout. A variable degree of *lobular changes (lobular hepatitis)*, including focal and confluent necrosis, apoptosis and inflammation, completes the picture of necroinflammatory changes in chronic hepatitis.

Liver cell dysplasia may be observed, more often in HBV cases and in late stages.

As for fibrosis, the process usually starts in the portal tracts (which become enlarged) and proceeds with the formation of fibrous septa that may ultimately lead to the onset of cirrhosis.

"Ground-glass" hepatocytes are a hallmark of hepatitis B infection [27]. They are liver cells with an eosinophilic, granular, glassy cytoplasm on light microscopy. This appearance corresponds to a proliferation of smooth endoplasmic reticulum containing HBV surface antigens. Ground-glass hepatocytes may be seen in other conditions too, including Lafora's disease, cyanamide therapy for alcohol abuse, post-transplant complications, Still's disease, and metabolic disorders [28]. Immunohistochemistry should therefore be performed to confirm the presence of HBV surface antigens.

The triad of lymphocyte nodular inflammation in portal tracts, steatosis and bile duct damage is considered highly characteristic of chronic HCV hepatitis [29] Of course, this association is not pathognomonic and should always be interpreted in its clinical context. Mild iron deposition can be detected by means of specific stains and may have clinical implications (see below).

Pathologists should differentiate chronic viral hepatitis from other diseases presenting similar pictures [25]. The most common diseases entering into the differential diagnosis with viral hepatitis include autoimmune hepatitis and primary biliary cirrhosis (Table 3), but toxic damage and metabolic disorders such as Wilson's disease and alpha 1 antitrypsin deficiency should also be considered. A detailed discussion of the differential diagnostic criteria is beyond the scope of this article and readers can refer to the relevant textbooks. Close clinical-pathological correlations enable the etiological diagnosis to be established in most cases.

5. Liver biopsy in chronic viral hepatitis

With the refinement of serological and virological tests, liver biopsy is no longer needed to establish an etiological diagnosis, the only exception being liver-transplanted patients, in whom the main reason for taking liver biopsies is still to understand the cause(s) of abnormal liver enzyme levels.

When it comes to chronic viral hepatitis patients, pathologists are now required:

1. To assess the extent of necroinflammation and fibrosis, because this information has important prognostic and therapeutic implications;
2. To assess the presence of any adjunctive lesions, recognized as an important factor in disease progression and/or response to treatment and therefore potentially influencing treatment decisions;
3. To detect (or rule out) comorbid conditions, such as alcoholic and non-alcoholic steatohepatitis, hemochromatosis or other disorders that may be relevant to immediate patient management and long-term outcome assessment. When two or more concomitant causes of liver disease are recognized, the pathologist should specify which is the more important.

6. Assessing necroinflammation and fibrosis: grading and staging systems

The grading and staging systems, borrowed from oncological practice, take into account the whole spectrum of morphological lesions affecting progression to cirrhosis. *Grading* reflects the severity of necroinflammation, while *staging* quantifies the extent of fibrosis and indicates the point to which the disease has progressed along its putative path towards the cirrhotic endpoint.

Several systems have been developed for the grading and staging of chronic viral hepatitis [9]. The simplest method is to use descriptive terms (i.e. mild, moderate and severe) to report the overall severity of necroinflammation and fibrosis, but such a method is naturally highly subjective.

The more complex methods are all based on the same principles:

– the *grade* represents the sum of numerical scores attributed to each histological necroinflammatory lesion in a given picture of chronic viral hepatitis. Higher numbers correspond to more severe lesions. The assessment is semi-quantitative and the numbers represent not arithmetical measurements, but categories, and consequently require appropriate statistical analysis;
– the *stage* is obtained by assessing the extent and location of fibrosis and changes in liver tissue architecture. All systems use a single numerical scale, where 0 (zero) represents the absence of fibrosis and the highest number indicates cirrhosis. Different systems use different criteria (see below) to score intermediate stages. Here again, the numbers reflect not measurements, but mainly qualitative concepts, e.g. "portal fibrosis" or "septal fibrosis".

The first scoring system – The Histological Activity Index (HAI) – was designed by Knodell et al. [30] to assess the

Table 3
Major criteria to differentiate viral hepatitis from other chronic disorders

	Chronic viral hepatitis	Primary biliary cirrhosis	Autoimmune hepatitis
Portal tract inflammation	Mononuclear cells	Mononuclear cells; eosinophils	Mononuclear cells; plasma cells
Interface hepatitis	Common	Common; ductular reaction (i.e. biliary piecemeal necrosis)	Present
Lobular necrosis	Variable degree; usually focal	Variable; usually mild and focal	Severe, may be confluent
Bile duct damage	Common in hepatitis C (usually mild)	Present; duct destruction	May be present

efficacy of interferon in trials on patients with chronic viral hepatitis by providing histological information in a format suitable for statistical analysis. The HAI is now rarely used in its original version because of two main limitations, i.e. it combines necroinflammation (i.e. the cause) with fibrosis (i.e. the consequence), which do not necessarily coincide; and the method is based on scales with non-sequential scores. For a detailed analysis of the pros and cons of the Knodell scoring system, see [9,31–33].

The currently most widely-used scoring systems are analyzed below.

6.1. The Scheuer system (1991)

This was the first system to score necroinflammation and fibrosis separately [34] (Table 4).

Activity is graded by summing the scores for portal inflammation/piecemeal necrosis (i.e. interface hepatitis) and lobular lesions on a scale from 0 (absent) to 4. Taking the traditional view that the risk of progression is related not to the severity of portal tract inflammation, but only to interface hepatitis, the extent of portal inflammation is not assessed separately. Scheuer's original paper did not mention the criteria used to define the severity of piecemeal necrosis and lobular changes, which may differ from one portal tract/lobular area to the next. We recommend considering the worst situation rather than the mean severity.

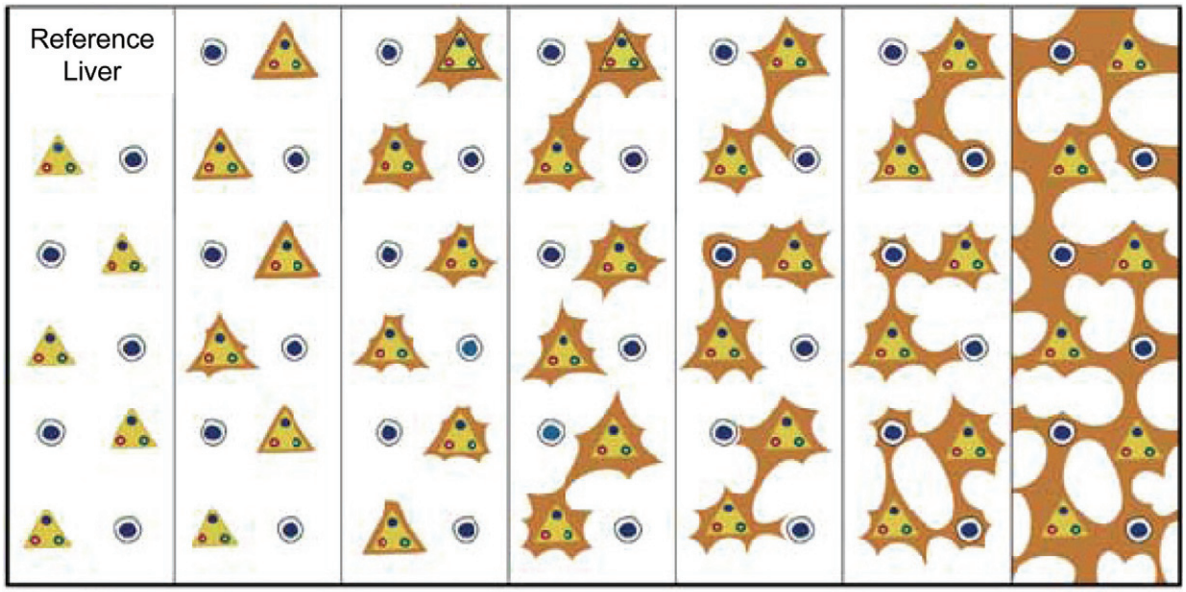
As for fibrosis, this is also scored on a scale from 0 to 4 (Table 4 and Fig. 1). Enlarged portal tracts (Stage 1) and periportal fibrosis (Stage 2) may be difficult to distinguish, but periportal fibrosis is characterized by irregular, stellate portal tract contours, which are smooth in the case of portal fibrosis; this distinction has no proven prognostic significance, however.

Table 4
The Scheuer system for grading and staging chronic hepatitis

Activity grade		
Portal/periportal activity	***Lobular activity***	
None	None	0
Portal inflammation alone	Inflammation but no necrosis	1
Mild piecemeal necrosis	Focal necrosis or acidophilic bodies	2
Moderate piecemeal necrosis	Severe focal cell damage	3
Severe piecemeal necrosis	Damage includes bridging necrosis	4
Fibrosis stage		
No fibrosis		0
Enlarged, fibrotic portal tracts		1
Periportal fibrosis or portal-portal septa, but intact architecture		2
Fibrosis with architectural distortion, but no obvious cirrhosis		3
Probable or definite cirrhosis		4

6.2. The Metavir system (1994)

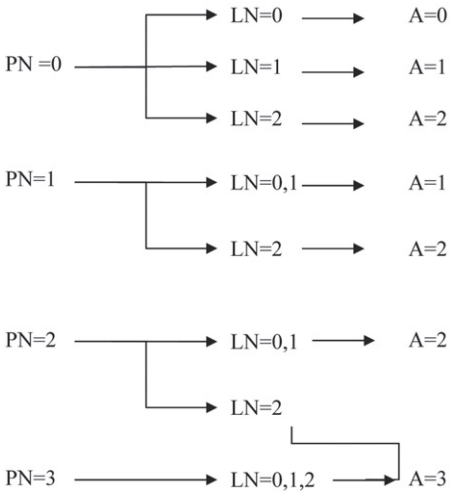
This system was specifically designed for chronic HCV hepatitis [35], but it is also used for hepatitis B. The activity grade is obtained by combining piecemeal and lobular



Lesions	Fibrosis of some portal tracts	Portal/periportal fibrosis (most portal tracts)	Fibrous septa: portal to portal	Fibrous septa: portal to portal & portal to central	Fibrous septa with architectural disturbance	Cirrhosis
Ishak score	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Scheuer score	Stage 1	Stage 2	Stage 2	Stage 3	Stage 3	Stage 4
Metavir score	Stage 1	Stage 1	Stage 2	Stage 3	Stage 4	Stage 4

Fig. 1. Hepatitis staging: analogue scale.

Table 5
The Metavir algorithm for grading chronic viral hepatitis



PN = piecemeal necrosis (i.e. interface hepatitis); LN = lobular necrosis; A = grade of activity.

necrosis in an algorithm, producing 3 grades of severity: A1 = mild, A2 = moderate, and A3 = severe (Table 5).

As with Scheuer's score, portal inflammation is not considered a separate lesion, so it does not enter the algorithm. The criteria for scoring piecemeal necrosis and lobular necrosis are as follows:

- *piecemeal necrosis*: 0 = absent, 1 = focal alteration of periportal plate in some portal tracts, 2 = diffuse alteration of periportal plate in some portal tracts, or focal lesions around all portal tracts, 3 = diffuse alteration of periportal plate in all portal tracts; what was actually meant by "focal" and "diffuse" alterations was not specified in the original paper;
- *lobular necrosis*: 0 = less than one necroinflammatory focus per lobule, 1 = at least one necroinflammatory focus per lobule, 2 = several necroinflammatory foci per lobule, or bridging necrosis. The cut-off between "at least one" and "several" was not mentioned, nor was it specified whether bridging necrosis includes portal-central bridges alone or portal-portal bridges too.

Like Scheuer's score, the Metavir system grades fibrosis on a scale of 0 to 4. Stage 1 represents portal fibrosis without septa. Stage 2 and 3 are assigned when rare or numerous septa are present, respectively. We are not told whether the "septa" include both incomplete and bridging septa. We recommend assigning a score of 2 only when there is bridging fibrosis; this is consistent with most clinico-pathological studies, which consider stage 2 (according to the Metavir scoring system) as "clinically significant" fibrosis. Stage 2 includes portal-to-portal and portal-to-central septa in the Metavir system (Fig. 1).

6.3. The Ishak et al. system (1995)

This method is also known as the *modified HAI*; it was generated to overcome the weaknesses of Knodell's original HAI and it is much more detailed than the previous systems [36] (Table 6).

Table 6
The Ishak et al. scoring system

	Score
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous, around <50% of tracts or septa)	3
Severe (continuous, around >50% of tracts or septa)	4
B. Confluent necrosis	
Absent	
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis + occasional portal-central (P-C) bridging	4
Zone 3 necrosis + multiple P-C bridging	5
Panacinar or multiacinar necrosis	6
C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation*	
Absent	
1 focus or less per ×10 objective	1
2–4 foci per ×10 objective	2
5–10 foci per ×10 objective	3
More than 10 foci per ×10 objective	4
D. Portal inflammation	
Absent	
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
Fibrosis	
No fibrosis	
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 portal areas with marked portal-to-portal (P-P) as well as portal-to-central (P-C) bridging	4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6

*Does not include diffuse sinusoidal infiltration by inflammatory cells.

For grading purposes, all elementary lesions (interface hepatitis, "focal" lobular changes, confluent necrosis and portal inflammation) are separately assessed, thereby emphasizing their different contributions to the progression of fibrosis. The system limits the designation of bridging necrosis to portal-central (P-C) bridging, which is thought to have a different (more severe) prognostic and pathogenic significance than portal-portal (P-P) bridging.

Ishak's scoring method introduces some "quantitative" concepts (few, some, most) that are not very adequately explained in the original paper. The meaning of "few" or "most" portal tracts (or zone 3 areas) obviously depends on the size of the specimen: 3 are "few" in a long sample with numerous portal tracts, but "most" if only 4 portal tracts are counted. We use "few" and "some" when less than half

of the portal tracts or central areas are involved, regardless of their number, while "most" is used when more than half of the portal tracts/central areas are involved. As for focal lobular changes, the exact number of foci per ×0 objective is recommended for each grade. Mononuclear cells in the sinusoids are not counted.

With Ishak et al. scoring system, fibrosis is assessed in more detail and the scale ranges from 0 to 6, making it more accurate in comparing paired biopsies. The system clearly distinguishes incomplete (i.e. "short" = scores 1 and 2) from complete septa formation, and keeps P-P and P-C septa separate (Table 6 and Fig. 1).

6.4. Which system is best?

There is no general consensus as to which is the best scoring system and all those described here have been widely used, both in routine practice and for research. This means that pathologists can choose what they (and the clinicians with whom they work) prefer. At most centers, the simplest (and most reproducible) systems are preferred for routine use [37–39], while the more detailed are used only for special purposes, e.g. clinical trials.

In practice, what matters is that clinicians be familiar with the chosen system and that its name be clearly indicated in the pathology report, partly because otherwise the numbers are meaningless and also because patients might be followed up at different centers during the course of their disease.

6.5. Problems relating to grading and staging

6.5.1. Sampling error

Due to the possibly uneven distribution of the lesions, liver biopsy size may affect grading and staging, and the ideal sample size has been much debated in recent years. The risk with small samples is that the damage may be underestimated [40]. Grading and staging accuracy ultimately depends on the availability of a representative number of portal tracts, since they are the elective site of damage in chronic viral hepatitis. The number of portal tracts depends on the size of the biopsy, which in turn depends on the size of the needle. A study by Colloredo et al. [41] demonstrated that the risk of underestimating grade and stage in chronic viral hepatitis is low with liver biopsy samples containing at least 11 portal tracts, which can be achieved with specimens no less than 2 cm long obtained using a 16-gauge needle. A study by Bedossa et al., using virtual biopsies [42], indicated that a sample at least 2.5 cm long (1 mm wide) is needed to evaluate fibrosis accurately using a semiquantitative score. There are also data indicating that grading and staging accuracy is severely limited by the use of samples obtained with fine needles (≤21G) [43] and wedge biopsies pose further problems, since most of the liver tissue comes in this case from the subcapsular area, where fibrous septa spreading from the Glissonian may give rise to an overestimation of the fibrosis. Non-specific necroinflammatory lesions relating to the surgical procedure, which are commonly encountered in the subcapsular area, can also influence the grading of the inflammation [44]. In addition, it is important to emphasize the need for biopsies of comparable size when assessing disease progression or the effect of antiviral therapy [45]. Cutting-type needles may provide less fragmented biopsies and are purported to be better than suction-type needles for evaluating cirrhosis [46,47].

Transjugular liver biopsy (TJLB) has been proposed as a useful method to obtain "adequate" samples, since it allows for more than one pass without any significant risk of bleeding [48]. Nonetheless, while TJLB with 3 passes almost always produces optimal biopsies for diagnostic purposes, they are only adequate for staging and grading in 38% (≥25 mm) or 25% (≥11 CP) of cases [48].

Taking the above considerations into account, pathologists should recommend that clinicians (radiologists or hepatologists) performing liver biopsies obtain samples 2–3 cm long using a 16-gauge needle [40,50,51], avoiding any use of fine needles and limiting the use of TJLB to conditions in which this procedure is specifically warranted.

6.5.2. Observer reproducibility

Observer variation has been documented in the grading and staging of chronic hepatitis [52–55]. Available studies indicate that, whatever the system used, inter-observer agreement is better with systems that use simpler scales. Fibrosis scores and diagnoses of cirrhosis are much more reproducible (k statistic = 0.80–0.91 with the Metavir score) than necroinflammatory lesions. The French experience has shown that reproducibility is higher when assessments are conducted simultaneously by two observers [56] and it is influenced by the pathologists' level of experience (including their specialization and also how long and where they have worked) [57].

7. Adjunctive information

7.1. Steatosis

Steatosis is a common finding in liver biopsies from HCV-infected individuals [58] and several studies have correlated its presence and severity with the severity and progression of fibrosis [59] (Level of evidence: III).

A meta-analysis, conducted by Leandro et al. [60] by pooling data from 10 different centers confirmed the association between steatosis and fibrosis, irrespective of the center, and the association also held for large sub-groups of patients, including those with genotype 1 and a BMI below 25. Steatosis has also been associated with a lower rate of response to antiviral therapy [61–63] and this has prompted the recommendation of weight reduction programs prior to treatment in patients with steatosis. So assessing steatosis in liver biopsies is recommended because it may have practical consequences in hepatitis C. In the pathology report, the presence, type (macro- or micro-vacuolar), topography and severity of steatosis should be noted. Its topography may have diagnostic implications. In hepatitis C, steatosis is usually

mild, mainly macrovacuolar and without an elective topographic arrangement (diagnostic strength: level 3), so finding a more than mild steatosis located mainly in the pericentral zone 3 is a sign of potential concomitant non-alcoholic (or alcoholic) fatty liver diseases (N/AFLD) (see below). Steatosis is usually scored on a scale from 0 to 3, where: 0 = absent, 1 (mild) = ≤33%; 2 (moderate) = >33% to ≤ 66%; 3 (severe) = >66%.

Available data on the prevalence and significance of liver steatosis are less abundant and consistent for hepatitis B than for hepatitis C [64]. The prevalence of steatosis ranges from 22% to 59% in various studies, which are difficult to compare because different methods were used to detect steatosis. The current world trends of obesity and type 2 diabetes will probably mean increasing numbers of individuals with chronic hepatitis B and fatty livers. Metabolic factors have been associated with steatosis in HBV infection more strongly than viral determinants [65]. The effects of steatosis on response to antiviral therapy are not known. Finding steatosis in HBV-related hepatitis may help to explain abnormally high ALT levels in cases with very low viral replication rates.

In routine practice, identifying steatosis demands no special techniques and it is usually based on hematoxylin and eosin (H&E) staining. Inter- and intra-observer consistency is reportedly good or excellent, with k statistics ranging from 0.64 to 0.98 [66]. On the other hand, a recent study based on liver biopsy microphotographs [67] reported a poor inter-observer consistency (among experts) in the overall assessment of steatosis and in differentiating between macro- and micro-vacuolar steatosis. It is not easy to account for these discrepancies.

7.2. Iron deposition

Hepatic iron deposition (or hepatic siderosis) may be found in diseases of various etiology, including alcoholic and non-alcoholic fatty liver and viral infections. Iron overload is common in liver biopsies from HCV patients [68], and much more frequent than in cases of HBV. In CHC, generally mild-grade iron deposits may be seen in both hepatocytes and reticuloendothelial cells [68]. The exact mechanisms behind iron accumulation in the liver in CHC are not clear, but hepcidin, a recently-discovered circulating antimicrobial peptide produced in the liver, seems to have an important role [69].

Assessing hepatic iron overload in liver biopsies may be of practical use, since several experimental and clinical studies have suggested that iron is a cofactor in CHC, increasing the risk of fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [70–72]. Hepatic iron concentrations have also been inversely associated with response to antiviral therapy [73] and the absence of mesenchymal iron deposits in the baseline biopsy has been found to correlate with sustained viral response [74]. Some studies have demonstrated that phlebotomy may improve liver function test findings [75] and histology [76], increase the chances of a sustained HCV eradication after antiviral therapy [77–79], and limit the onset of HCC [80].

In the light of the above considerations, pathologists should assess iron deposition in liver biopsies from patients with CHC.

Iron deposits in liver tissues may be suspected from routine H&E-stained sections, but a thorough interpretation of their nature and grade of pigmentation demands a specific stain. Perls' stain is the most sensitive (and the most popular) method for accurately assessing hepatic siderosis. Iron pigment may be observed in both parenchymal and non-parenchymal cells, and in the connective tissue of portal tracts. For routine practice, the pattern of cell deposition, i.e. mainly or exclusively parenchymal, or mainly or exclusively non-parenchymal, or mixed should be reported along with the severity of iron deposition. A simple scoring system on a scale from 0 to 4 [81] is adequate for daily routine, while more sophisticated scoring systems (such as the one proposed by Deugnier et al. [82]) might be required for special investigations.

7.3. Liver cell dysplasia

The term large cell dysplasia (LCD) was coined by Anthony et al. [83] and describes a change characterized by nuclear and cellular enlargement (with a preserved nucleocytoplasmic ratio), nuclear pleomorphism and multinucleation of hepatocytes. This change was found in 65% of patients who had cirrhosis associated with HCC (mainly in HBsAg-positive cases), suggesting that it identified a group of patients at high risk of liver cancer. Subsequent studies demonstrated that large cell dysplasia occurs in 6–28% of cases in chronic hepatitis C and 13–32% in chronic hepatitis B. The incidence is higher in explanted cirrhotic livers, i.e. 71–85% in HCV-related cirrhosis and 100% in HBV-related cirrhosis [84].

Watanabe et al. [85] modified the original definition of LCD to include a "small cell" variant. In contrast with LCD, the small cell dysplasia (SCD) is characterized by a higher nuclear to cytoplasmic ratio in the hepatocytes, with cytoplasmic basophilia and without multinucleation or large nucleoli. On the basis of their morphological and morphometric studies, Watanabe et al. suggested that it is small cell dysplasia, rather than large cell dysplasia, that is the precancerous lesion in man. The incidence of SCD in cirrhotic livers ranges from less than 1% in biopsy specimens up to 50% in explanted livers [84].

For the time being, there is no final consensus on the preneoplastic nature of LCD. Existing data suggest that it may be a heterogeneous entity with two types, one tumor-related and the other innocent [84]. The biological nature of LCD seems to depend on the setting in which it occurs. In HBV infection, the characteristics of LCD are more consistent with dysplastic than with reactive hepatocytes. The pathogenetically noncommittal term "large cell change" (LCC) has consequently been recommended [84] as an alternative to "large cell dysplasia". From the clinical point of view, both prospective studies based on multivariate analyses and retrospective studies have shown LCC as the most important predictor of HCC, identifying a subset of patients at higher risk of developing cancer

[84,86–89]. This was confirmed more recently in a series of 181 patients with chronic hepatitis B who underwent needle liver biopsy [90]: patients with LCC had a significantly higher cumulative likelihood of developing HCC than those without LCC ($p = 0.016$). The presence of LCC coincided with an approximately 3-fold risk of developing HCC, with positive and negative predictive values of 15.9% and 94.9%, respectively. Although these data do not prove that LCC are direct precursors of HCC, they do support the clinical significance of these lesions as important tissue markers, which may help us to identify a high-risk subgroup of patients requiring more intensive screening for HCC.

As for small cell dysplasia (now termed "small cell changes" [SCC] by analogy with LCC), there are numerous data supporting its precancerous nature: SCC are characterized by the inactivation of cell cycle checkpoints, short telomeres, and accumulated DNA damage [91,92]. A significant correlation between the presence of SCC and HCC has been demonstrated [89], but large-scale prospective studies are lacking.

From the pathologist's perspective, since identifying SCC and LCC in liver biopsies from patients with chronic viral hepatitis may, with time, mean a greater risk of HCC, their presence should be recorded in pathology reports.

7.4. HBV antigens

HBsAg and HBcAg can be identified in liver tissue using simple immunostains. In the liver transplant setting, this may help to differentiate recurrent (or *de novo*) infection from rejection. HBsAg and HBcAg expression patterns correlate with the phase of infection. In chronic hepatitis, HBsAg may be seen in the cytoplasm and/or membrane and a diffuse membranous expression is usually associated with active viral replication [93]. HBcAg expression may be nuclear and/or cytoplasmic [94]. Inactive carrier status is usually characterized by the presence of HBsAg in clusters of hepatocytes and negative staining for HBcAg [93].

Assessing HBsAg/HBcAg patterns may have clinical relevance: pure cytoplasmic staining has been associated with the presence of mutations that block the translocation of HBcAg [94], while the absence of HBcAg may predict response to treatment, particularly in HBeAg-negative patients. Pathologists should therefore perform immunostaining procedures and report on the HBV antigen expression pattern in liver biopsies from cases of chronic hepatitis B.

8. Assessment of concomitant diseases

Patients with chronic hepatitis B or C may develop other liver diseases, which can affect their management, but which may or may not be suspected by clinicians. A recent study from Toronto demonstrated that, in about 1 in 5 (20.5%) of 1,842 consecutive patients with chronic type B or C hepatitis, liver biopsy revealed other liver diseases potentially affecting disease progression and/or patient management [95].

Considering the increasing prevalence of risk factors for metabolic syndromes in the general population, a major concern is the association of non-alcoholic fatty liver disease (NASH) with chronic viral hepatitis which is probably becoming increasingly common. In CHC the concomitant presence of NASH may accelerate progression of fibrosis [96,97]. However, the chances of recognizing histological signs of NASH in CHC pose some issues because some of the elementary lesions (steatosis and lobular inflammation) are common to both conditions. In adults, and particularly in those infected with non-3 HCV genotypes, moderate–severe steatosis should point to a diagnosis of concomitant NASH, especially when it is restricted to the acinar zone 3 or associated with hepatocyte ballooning and/or perivenular fibrosis [98]. Studies are needed, however, specifically focusing on the strength of histological criteria for clarifying the NASH/HCV overlap.

In conclusion, pathologists always should (and usually do) consider comorbid conditions when they examine liver biopsies. It is of paramount importance to correlate clinical and serological findings to obtain a specific diagnosis.

9. How to handle a liver biopsy

Liver samples should be fixed in 10% neutral buffered formalin because this enables all routine histochemical and immunohistochemical stains to be performed. A small portion of the sample could be snap-frozen for adjunctive molecular studies for diagnostic or research purposes, particularly when multiple etiologies are clinically suspected. This should be done with caution, however, to avoid being left with too small a piece for accurate grading and staging. As for stains, a good collagen stain to assess fibrosis is mandatory: which one is a matter of personal preference or experience. Perls' stain for iron is recommended, particularly in cases of hepatitis C; and the PAS stain after diastase digestion is useful for assessing hepatocyte cytoplasm content.

10. 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.

1. Assess the adequacy of the biopsy by measuring the length of the specimen and counting the number of portal tracts. Write these data in the final report to make clinicians aware of any potential sampling error in the grading and staging.
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.
3. Describe any presence and severity of adjunctive lesions:
   - steatosis (strongly recommended): graded on a scale from 0 to3
   - 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).

Conflict of interest

The authors have no conflict of interest to report.

References

[1] von Frerichs FT. Uber den Diabetes. Berlin: Hirschwald; 1884.
[2] Menghini G. One-second needle biopsy of the liver. Gastroenterology 1958; 35:190–195.
[3] De Groote J, Desmet VJ, Gedigk P, et al. A classification of chronic hepatitis. Lancet 1968;2:626–8.
[4] International group. Acute and chronic hepatitis revisited. Lancet 1977; 29:914–9.
[5] Schmid M, Flury R, Buhler H, et al. Chronic viral hepatitis B and C: an argument against the conventional classification of chronic hepatitis. Virchows Arch 1994; 425:221–6.
[6] Ludwig J. The nomenclature of chronic active hepatitis: an obituary. Gastroenterology 1993; 105:274–8.
[7] Gerber MA. Chronic hepatitis C: the beginning of the end of a time honored nomenclature? Hepatology 1992; 15:733–4.
[8] Desmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–20.
[9] Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. Hepatology. 2000;31:241–6.
[10] Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507–39.
[11] Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97–107.
[12] Zanetti AR, Romano L, Zappa A, et al. Changing patterns of hepatitis B infection in Italy and NAT testing for improving the safety of bloody supply. J Clin Virol 2006;36(Suppl 1):51–5.
[13] Te HS, Jensen DM. Epidemiology of hepatitis B and C viruses: a global overview. Clin Liver Dis. 2010;14:1–21.
[14] Bialek SR, Terrault NA. The changing epidemiology and natural history of hepatitis C virus infection. Clin Liver Dis 2006;10:697–715.
[15] Mangia A, Antonucci F, Brunetto M, et al. Italian Association for the Study of the Liver (AISF). The use of molecular assays in the management of viral hepatitis. Dig Liver Dis 2008;40:395–404.
[16] Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48:335–52.
[17] Holomán J, Glasa J. EASL clinical practice guidelines. J Hepatol 2009;51(4):821–2.
[18] Loomba R, Ling TJ: Treatment of chronic hepatitis B. Antivir Ther 2007;12(Suppl 3):33–41.
[19] Keefe EB, Marcellin P: New and emerging treatment of chronic hepatitis B. Clin Gastroenterol Hepatol 2007;5:285–94.
[20] Strader DB, Wright T, Thomas DL, et al.; American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. Hepatology 2004;39:1147–71.
[21] Pawlotsky JM: Hepatitis C virus genetic variability: Pathogenic and clinical implications. Clin Liver Dis 2003;7:45–66.
[22] EASL International Consensus Conference on hepatitis C. Paris, 26-27 February 1999. Consensus statement. J Hepatol 1999;31(Suppl 1):3–8.
[23] Practice guidelines for the treatment of hepatitis C: recommendations from an AISF/SIMIT/SIMAST Expert Opinion Meeting. Italian Association for the Study of the Liver; Italian Society of Infectious, Tropical Diseases; Italian Society for the Study of Sexually Transmitted Diseases. Dig Liver Dis 2010;42:81–91.
[24] Theise ND, Bodenheimer Jr HC, Ferrell LD. Acute and chronic viral hepatitis. In: Burt AD, Portmann BC, Ferrell LD, editors. MacSween's Pathology of the Liver. 5th ed. Philadelphia: Churchill Livingstone; 2007, pp. 399–441.
[25] Ishak KG: Pathologic features of chronic hepatitis. A review and update. Am J Clin Pathol 2000;113:40–55.
[26] Scheuer PJ, Lefkowitch JH. Liver Biopsy Interpretation. 7th ed. Philadelphia: Saunders; 2006.
[27] Hadziyannis S, Gerber MA, Vissoulis C, et al: Cytoplasmic hepatitis B antigen in "ground-glass" hepatocytes of carriers. Arch Pathol 1973;96:327–30.
[28] Lefkowitch JH, Lobritto SJ, Brown RS Jr, et al. Ground-glass, polyglucosan-like hepatocellular inclusions: A "new" diagnostic entity. Gastroenterology 2006;131(3):713–8.
[29] Scheuer PJ, Ashrafzadeh P, Sherlock S, et al. The pathology of hepatitis C. Hepatology 1992;15:567–71.
[30] Knodell KG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–5.
[31] Scheuer PJ. Scoring of liver biopsies: are we doing it right? Eur J Gastroenterol Hepatol 1996;8:1141–3.
[32] Hübscher SG. Histological grading and staging in chronic hepatitis: clinical applications and problems. J Hepatol 1998;29:1015–22.
[33] Desmet VJ. Knodell RG, Ishak KG, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis [Hepatology 1981;1:431–5]. J Hepatol 2003;38:382–6.
[34] Scheuer PJ. Classification of chronic viral hepatitis: a need for a reassessment. J Hepatol 1991; 13:372–4.
[35] METAVIR Cooperative Study Group. An algorithm for the grading of activity in chronic hepatitis. Hepatology 1996;24:289–93.
[36] Ishak K, Baptista A, Bianchi L et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–9.
[37] Hytiroglou P, Thung SN, Gerber MA. Histological classification and quantification of the severity of chronic hepatitis: Keep it simple!. Sem Liv Dis 1995;15:414–21.
[38] Thiese N. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. Mod Pathol 2007;20:S3–S14.
[39] Goodman Z. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol 2007;47:598–607.
[40] Guido M, Rugge M. Liver biopsy sampling in chronic viral hepatitis. Semin Liver Dis 2004;24:89–97.
[41] Colloredo G, Guido M, Sonzogni A, et al. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. J Hepatol 2003;39:239–44.
[42] Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449–57.
[43] Brunetti E, Silini E, Pistorio A, et al. Coarse vs. fine needle aspiration biopsy for the assessment of diffuse liver disease from hepatitis C virus-related chronic hepatitis. J Hepatol 2004;40:501–6.
[44] Geller SA, Pitman MB. Morphological diagnostic procedures (liver biopsy). In: Mc Sween RNM, Burt AD, Portmann BC, et al., eds. Pathology of the liver. London: Churchill Livingston; 2002, pp. 943–60.

[45] Guido M, Rugge M. Liver fibrosis: natural history may be affected by the biopsy sample. Gut 2004;53:1878.
[46] Colombo M, Del Ninno E, de Franchis R, et al. Ultrasound-assisted percutaneous liver biopsy: superiority of the Tru-Cut over the Menghini needle for diagnosis of cirrhosis. Gastroenterology 1988;95:487–9.
[47] Sherman KE, Goodman ZD, Sullivan ST, et al. Liver biopsy in cirrhotic patients. Am J Gastroenterol 2007;102:789–93.
[48] Cholongitas E, Quaglia A, Samonakis D, et al. Transjugular liver biopsy: how good is it for accurate histological interpretation? Gut 2006;55:1789–94.
[49] Hunt N, Fleming K. Reproducibility of liver biopsy grading and staging. Liver 1999;19:169–70.
[50] Guido M, Colloredo G, Fassan M, et al. Clinical practice and ideal liver biopsy sampling standards: Not just a matter of centimetres. J Hepatol 2006; 44:823–4.
[51] Rockey DC, Caldwell SH, Goodman ZD, et al. Liver biopsy. Hepatology 2009; 43:1017–44.
[52] Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614–816.
[53] Goldin RD, Goldin GJ, Burt AD, et al. Intra-observer and inter-observer variation in the histopathological assessment of chronic viral hepatitis. J Hepatol 1996; 25:649–54.
[54] Westin J, Lagging LM, Weistal R, et al. Interobserver study of liver histopathology using the Ishak score in patients with chronic hepatitis C virus infection. Liver 1999;19:183–7.
[55] Gronbek K, Christensen PB, Hamilton-Dutoit S, et al. Interobserver variation in interpretation of serial liver biopsies from patients with chronic hepatitis. C. J Viral Hep 2002; 9:443–9.
[56] METAVIR cooperative group. Inter- and intra-observer variation in the assessment of liver biopsy of chronic hepatitis C. Hepatology 1994;20;1:15–20.
[57] Rousselet MC, Michalak S, Dupré F, et al. Sources of variability in histological scoring of chronic viral hepatitis. Hepatology 2005;41:257–64.
[58] Negro F. Mechanisms and significance of liver steatosis in hepatitis C virus infection. World J Gastroenterol 2006;12:6756–65.
[59] Castéra L, Hézode C, Roudot-Thoraval F, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut 2003;52:288–92.
[60] Leandro G, Mangia A, Hui J, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology 2006;130:1636–42.
[61] Adinolfi L, Gambradella M, Andreana A, et al. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV gGenotype and visceral obesity. Hepatology 2001;33:1358–64.
[62] Negro F, Clement S. Impact of obesity, steatosis and insulin resistance on progression and response to therapy of hepatitis C. J Vir Hepat 2009;16:681–8.
[63] Westin J, Lagging M, Dhillon AP, et al. Impact of hepatic steatosis on viral kinetics and treatment outcome during antiviral treatment of chronic HCV infection. J Viral Hepat 2007;14:29–35.
[64] Thomopoulos KC, Arvaniti V, Athanasios C, et al. Prevalence of liver steatosis in patients with chronic hepatitis B: a study of associated factors and of relationship with fibrosis. Eur J Gastroenterol Hepatol 2006;18:233–7.
[65] Peng D, Han Y, Ding H, et al. Hepatic steatosis in chronic hepatitis B is associated with metabolic factors more than viral factors. J Gastroenterl Hepatol 2008;23:1082–8.
[66] Merriman RB, Ferrell LD, Patti MG, et al. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. Hepatology 2006;44:874–80.
[67] El-Badry AM, Breitenstein S, Jochum W, et al. Assessment of hepatic steatosis by expert pathologists: the end of a gold standard. Ann Surg 2009;250:691–7.
[68] Corengia C, Galimberti S, Bovo G, et al. Iron accumulation in chronic hepatitis C. Am J Clin Pathol 2005;124:846–53.
[69] Girelli D, Pasino M, Goodnough JB, et al. Reduced serum hepcidin levels in patients with chronic hepatitis C. J Hepatol 2009;51:845–52.
[70] Chapoutot C, Esslimani M, Joomaye Z, et al. Liver iron excess in patients with hepatocellular carcinoma developed on viral C cirrhosis. Gut 2000;46:711–4.
[71] Pietrangelo A. Metals, oxidative stress, and hepatic fibrogenesis. Semin Liver Dis 1996;16:13–30.
[72] Angelucci E, Muretto P, Nicolucci A, et al. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 2002;100:17–21.
[73] Olynyk JK, Reddy KR, Di Bisceglie AM, et al. Hepatic iron concentration as a predictor of response to interferon-alfa therapy in chronic hepatitis-C. Gastroenterology 1995;108:1104–9.
[74] Ferrara F, Ventura P, Vegetti A, et al. Serum ferritin as a predictor of treatment outcome in patients with chronic hepatitis C. Am J Gastroenterol 2009;104:605–16.
[75] Di Bisceglie AM, Bonkovsky HL, Chopra S, et al. Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who have previously not responded to interferon: a multicenter, prospective, randomized, controlled trial. Hepatology 2000;32:135–8.
[76] Kageyama F, Kobayashi Y, Kawasaki T, et al. Successful interferon therapy reverses enhanced hepatic iron accumulation and lipid peroxidation in chronic hepatitis C. Am J Gastroenterol 2000;95:1041–50.
[77] Fontana RJ, Israel J, LeClair P, et al. Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, control trial. Hepatology 2000;31:730–6.
[78] Piperno A, Sampietro M, D'Alba et al. Iron stores, response to alpha-interferon therapy, and effects of iron depletion in chronic hepatitis C. Liver 1996;16:248–54.
[79] Fargion S, Fracanzani AL, Rossini A, et al. Iron reduction and sustained response to interferon-a therapy in patients with chronic hepatitis C: results of an Italian multicenter randomized study. Am J Gastroenterol 2002;97:1204–10.
[80] Kato J, Kobune M, Nakamura T, et al. Normalization of elevated hepatic 8-hydroxy-20-deoxyguanosine level in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 2001;61:8697–702.
[81] Searle J, Leggett BA, Crawford DHG, et al. Iron storage diseases. In Mc Sween RNM, Burt AD, Portmann BC, Ishak KI, Scheuer PJ, Anthony PP eds. Pathology of the liver. London: Churchill Livingston, 2002:943–60.
[82] Deugnier YM, Loreal O, Turlin B, et al. Liver pathology in genetic hemochromatosis: a review of 135 homozygous cases and their bioclinical correlations. Gastroenterology 1992;102:2050–9.
[83] Anthony PP, Vogel CL, Barker LF. Liver cell dysplasia: a premalignant condition. J Clin Pathol 1973;26:217–23.
[84] Park NY, Roncalli M. Large liver cell dysplasia: a controversial entity. J Hepatol 2006;45:734–43.
[85] Watanabe S, Okita K, Harada T, et al. Morphologic studies of liver cell dysplasia. Cancer 1983;51:2197–205.
[86] Borzio M, Bruno S, Roncalli M, et al. Liver cell dysplasia is a major risk factor for hepatocellular carcinoma in cirrhosis: a prospective study. Gastroenterology 1995;108:812–7.
[87] Le Bail B, Bernard PH, Carles J, et al. Prevalence of liver cell dysplasia and association with HCC in a series of 100 cirrhotic liver explants. J Hepatology 1997;27:835–42.
[88] Ganne-Carrie N, Chastang C, Chapel F, et al. Predictive score for the development of hepatocellular carcinoma and additional value of liver large cell dysplasia in Western patients with cirrhosis. Hepatology 1996;23:1112–8.
[89] Libbrecht L, Craninx M, Nevens F, et al. Predictive value of liver cell dysplasia for development of hepatocellular carcinoma in patients with non-cirrhotic and cirrhotic chronic viral hepatitis. Histopathology 2001;39:66–73.
[90] Koo JS, Kim H, Park BK, et al. Predictive value of liver cell dysplasia for development of hepatocellular carcinoma in patients with chronic hepatitis B. J Clin Gastroenterol 2008;42:738–43.
[91] Marchio A, Terris B, Meddeb M, et al. Chromosomal abnormalities

in liver cell dysplasia detected by comparative genomic hybridisation. Mol Pathol 2001;54:270–4.
[92] Hytiroglou P, Park YN, Krinsky G, et al. Hepatic precancerous lesions and small hepatocellular carcinoma. Gastroenterol Clin North Am 2007;36:867–87.
[93] Naoumov NV, Portmann BC, Tedder RS, et al. Detection of hepatitis B virus antigens in liver tissue. A relation to viral replication and histology in chronic hepatitis B infection. Gastroenterology 1990;99:1248–53.
[94] Park YN, Han KH, Kim KS, et al. Cytoplasmic expression of hepatitis B core antigen in chronic hepatitis B virus infection: role of precore stop mutants. Liver 1999;19:199–205.
[95] Nair V, Fischer SE, Adeyi OA. Non-viral-related pathologic findings in liver needle biopsy specimens from patients with chronic viral hepatitis. Am J Clin Pathol 2010; 133:127–32.
[96] Clouston AD, Powell EE. Interaction of non-alcoholic fatty liver disease with other liver diseases. Best Pract Res Clin Gastroenterol 2002;16:767–81.
[97] Ong JP, Younossi ZM, Speer C, et al. Chronic hepatitis C and superimposed nonalcoholic fatty liver disease. Liver 2001;21:266–71.
[98] Bedossa P, Moucari R, Chelbi E, et al. Evidence for a role of nonalcoholic steatohepatitis in hepatitis C: a prospective study. Hepatology 2007;46:380–7.