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Rethinking the Role of Liver Biopsy in the Era of Personalized Medicine

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53120>

1. Introduction

Indications and methods of liver biopsy have changed over the past few years [1]. However, an histological diagnosis may be needed for optimal management of a patient [2, 3].

Although modern biochemical, immunological, and radiographic techniques have facilitated the diagnosis and management of liver diseases they have not made liver biopsy obsolete. Clinicians rely on information derived from the liver biopsy to inform patients and to make their therapeutic options [4].

There are, however, many controversies surrounding liver biopsy resulting potential limitations, such as sampling errors and interobserver variations [5], which can lead to misclassification therefore, P. Bedossa et al. consider that when it comes to liver biopsy the term “best” standard is more appropriate than “gold” standard [6].

It is essential, when analysing the indications, contraindications, complications and other aspects of the liver biopsy, to consider present hepatology and personalized medicine.

Practiced since the late 19th century, liver biopsy remains the criterion standard in the evaluation of the etiology and extent of disease of the liver. Paul Ehrlich performed a percutaneous liver biopsy in Germany in 1883. [7]. Since then, this method has been improved with the introduction of different needle types for cutting and aspiration. But, until the 1950s, when Menghini developed an aspiration technique which led to a wider use of the procedure and broadened its applications, it was not common. While in the early 1960 and 1970s the liver biopsy was used for making a diagnosis in cases of suspected medical liver disease, today it is more often performed to assess the prognosis or evaluate therapeutic strategies [1].

With regards to the technique used to carry out the liver biopsy there has also been a major change, it used to be performed blindly by clinicians, specialists in gastroenterology or hepatology at the patient's bed whereas at present, percutaneous biopsies are performed primarily by radiologists.

Currently, a liver biopsy can be obtained either transvenously or transcutaneously, or by combining imaging modalities such as ultrasound, computed tomography, and laparoscopy. The choice of one technique over another is based on availability, personal preference, and the clinical situation.

Liver biopsy techniques: Percutaneous, transjugular or laparoscopic

- Percutaneous liver biopsy can be transthoracic, with an intercostal liver access or subcostal, when the patient has an enlarged liver extending below the costal margin. Clinicians have now discarded blind liver biopsies in favour of ultrasound-guided biopsies.
- Transjugular or transvenous liver biopsy was first described in 1964. It is a technique used in order to avoid percutaneous liver biopsy in patients who are at a higher risk of bleeding. However, it has its limitations and is considered an inferior biopsy due to the fragmentation of the obtained specimen, which may reduce the accuracy of the diagnosis. It is performed in a vascular catheterisation laboratory by a radiologist with special training in interventional radiology. Videofluoroscopy equipment and cardiac monitoring are mandatory due to the risk of cardiac arrhythmia as the catheter passes through the right atrium. With this method, hepatic venography, wedged hepatic venous pressure, caval pressure and atrial pressure measurements can also be obtained during the procedure. The most frequent indications for the transjugular route are: severe coagulopathy, ascites, obesity, suspected vascular tumour or peliosis hepatis.
- Laparoscopic liver biopsy. This technique is well established and its use varies between centers. It is indicated in centers where access to transvenous liver biopsy is not available, and in patients with focal liver lesions and coagulopathy for whom obtaining histology is essential for their management.

The decision to use a particular technique is based on the risk profile of the patient. If he or she has advanced liver failure with coagulopathy and ascites, liver biopsy is unnecessary, but the diagnosis of the underlying disease is crucial in specific circumstances in order to determine a therapy, for example in cases of liver transplant. Before a liver biopsy it is necessary to carry out an ultrasound to quantify vascular permeability and because it may rule out anatomical abnormalities and can identify mass lesions that are clinically silent. When cirrhosis is suspected on clinical grounds, or by non-invasive methods liver biopsy is usually avoided.

2. Contraindications to percutaneous liver biopsy

Absolute contraindications: the main contraindication to percutaneous liver biopsy is significant coagulopathy, others are: uncooperative patient, history of unexplained bleed-

ing, prothrombin time 3–5 seconds more than control, platelet count less than 50,000/mm³, the use of a non-steroidal anti-inflammatory drugs, (unless discontinued 7 to 10 days previously), blood for transfusion unavailable, suspected hemangioma, another vascular tumor or echinococcal cysts in the liver, and the inability to identify an appropriate site for biopsy.

Relative contraindications: Morbid obesity, ascites, hemophilia, infection in the right pleural cavity or below the right hemidiaphragm.

Accepted indications: Given the new developments that have proved the efficacy of liver biopsy, its role in the management of patients with chronic liver diseases has much evolved in recent years and will continue to evolve as new non invasive technologies are developed.

• Diagnosis
1. Many parenchimal liver diseases
2. Abnormal liver tests
3. Fever of unknown origin
4. Focal or diffuse abnormalities on imaging studies
• Prognosis-Staging of known parenchimal disease
• Management –Developing treatment plans based on histologic analysis
Contraindications for percutaneous liver biopsy
• Absolute: uncooperative patient, severe coagulopathy, infection of the hepatic bed, extrahepatic biliary obstruction.
• Relative: ascites, morbid obesity, possible vascular lesions, amyloidosis, hydatid disease.

Table 1. Indications and contraindications for liver biopsy

Its importance in diagnosis, staging and prognosis largely depends on the indication and the clinical question relying on an answer from the histological result.

2.1. Is liver biopsy always necessary?

The utility of routine liver biopsy has been the subject of debate in recent years. Due to liver biopsy being associated with a small but definite risk, a biopsy should only be performed when the findings contribute to a better management of the patient. It is argued that for the purposes of management, liver biopsy is neither needed in cases with advanced fibrosis nor those diagnosed with cirrhosis by other methods, nor in patients with mild disease, for whom a therapeutic decision is not urgent. Until recently, liver biopsy played a key role in the evaluation of chronic liver disease, but now in the presence of better diagnostic tests on disease etiologies and treatments its role has to be re-evaluated. Recognition and confirmation of the pattern of injury (chronic hepatitic, chronic cholestatic, steatohepatitic, etc.) is the pathologist's priority when evaluating the liver biopsy.

Moreover, liver biopsy provides information on the severity and distribution of lesions (codified in the staging and grading of chronic liver disease), the presence of confounding patterns of injury (such as steatohepatitis coexisting with chronic viral hepatitis), and the presence of additional findings such as steatosis or iron accumulation that may have prognostic or therapeutic relevance.

2.2. Who should be biopsied?

As a rule patients with standard clinical and radiological features are not biopsied. However, in the presence of non concordant or atypical results, a biopsy may be recommended. The decision whether to perform a liver biopsy in some patients is clear, however in cases with a suspected concomitant diagnosis or when results from other methods are non conclusive confirmation is needed [8,9].

Type of Injury	Causes
Fatty change	Ethanol, fatty liver disease, obesity, diabetes, drugs
Councilman bodies	Viral hepatitis, drugs, toxins, ischemia (acidophilic bodies)
Mallory bodies * (hyaline) (see pages: 13, 15, 17 and 18)	Ethanol, obesity, diabetes, drugs, Wilson disease, biliary tract disease, hepatocellular carcinoma
Hydropic change (ballooning degeneration)	Viral hepatitis, drugs, cholestasis, fatty liver disease
Cholestasis	Duct obstruction or injury, drugs, viral hepatitis
Interlobular duct injury	Primary biliary cirrhosis, primary sclerosing cholangitis, hepatitis C
Piecemeal necrosis	Viral hepatitis, primary biliary cirrhosis, drugs, Wilson disease
Increased iron stores	Hemochromatosis, transfusions, hemolysis
Granulomas** (see pages: 19, 20 and 21)	Sarcoid, infections (tuberculosis, fungi), drugs

Table 2. Patterns of liver cell injury found in liver biopsies and clinical differential diagnosis

3. The generally accepted indications of liver biopsy are the following

- Diagnosis for a better scoring of grading and staging of chronic viral hepatitis C or hepatitis B, alcoholic liver disease, non-alcoholic steato-hepatitis, or autoimmune hepatitis.
- In patients with raised ferritine for the diagnosis of hemochromatosis
- If there are suspected disorders of copper metabolism for the diagnosis of Wilson’s disease, with quantitative estimation of copper in liver tissue.
- Evaluation of possible autoimmune hepatitis

- In cholestatic liver diseases: primary biliary cirrhosis, primary sclerosing cholangitis and overlap syndromes.
- Evaluation of abnormal results of biochemical tests of the liver in association with a serologic workup that is negative or inconclusive
- Evaluation of the efficacy or the adverse effects of treatment regimens (e.g., methotrexate therapy for psoriasis).
- Alcohol related disease. Non-alcoholic fatty liver disease (NAFLD) or Non-alcoholic steatohepatitis (NASH).
- Diagnosis of a liver mass, in selected cases, when image tests are inconclusive.
- Evaluation of fever of unknown origin, with an eventual culture of liver tissue.
- Evaluation of the status of the liver post transplantation or of the donor liver pre transplantation.

4. Methods: How to handle a liver biopsy

Liver samples should be fixed in 10% neutral buffered formalin because this enables all routine histochemical and immunohistochemical staining to be carried out. 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.

As for stains, a good collagen stain to assess fibrosis is mandatory. Perls' stain for iron is recommended and the Periodic Acid-Schiff (PAS) stain with and without diastase digestion is useful for assessing hepatocyte cytoplasmic glycogen content.

Special stains for special circumstances are ordered if indicated by the clinical situation. For instance, the Ziehl-Nielsen is ordered for mycobacteria, and Grocott's silver methanamine stain is used when granulomas are observed or when fungi infection is suspected. The Congo Red stain is requested when amyloid is suspected to be present. Rhodamine stain, Victoria blue or orcein stain is used to detect copper deposition when there is clinical suspicion of Wilson's disease. Immunohistochemistry is used to confirm the presence of Hepatitis B surface antigen and Hepatitis B core antigen [10].

Cultures could be indicated in selected cases such as Mycobacterias [11].

5. Histologic diagnosis and clinical correlation

The pathological report that used to be too descriptive now has to include etiology, aspects related to prognosis and possible therapy [12,13].

In order to promote the clinico-pathological diagnostic correlation with the intention of improving communication and clarifying individual cases, regular meetings between clinicians

and pathologists are necessary. It is not only important to hold formal conferences but also to increase daily exchanges. To facilitate the communication between pathologists, radiologists, surgeons and clinicians it is desirable, when feasible, for the same teams to work together.

6. Writing the histology report

In order to produce a clinically relevant liver histology report pathologists should follow the internationally accepted guidelines.

1. The adequacy of the biopsy should be assessed by measuring the length of the specimen and counting the number of portal tracts. The data should be written up in the final report to make clinicians aware of any potential sampling error in the grading and staging. To reduce sampling error the amount of tissue required is usually 1 to 4 cm long and needs to include at least four portal tracts.
2. The type and severity of necroinflammation and fibrosis should be described in words. By only using numbers to report the presence or not of bridging necrosis for example, some clinically useful information might be omitted. A validated scoring system should be used for grade of activity and stage of fibrosis.
3. As well as being described, the existence of adjunct data should be scored subjectively, such as steatosis graded on a scale of 0-3 and siderosis graded on a scale of 0-4.
4. Other diagnostic criteria may be useful in differential diagnoses:
 - Differentiating viral hepatitis from other chronic disorders, such as cell dysplasia and thus separately reporting the presence of cell changes.
 - Using immunostaining when appropriate, for example Hepatitis B Virus antigens [14].
 - Searching for any concomitant diseases.
5. Chronic viral hepatitis, primary biliary cirrhosis and autoimmune hepatitis have typical histological lesions and it is advisable to consider the characteristics of: portal tract inflammation, interface hepatitis, lobular necrosis and bile duct damage, separately [15].
6. Concomitant histological features in liver specimens of hepatitis C cases: auto-immunity, co-infections, steatosis, hemosiderosis, malignancy –related changes, hepatitis due to drugs and/or vascular problems. Furthermore, biopsy frequently detects associated lesions such as steatosis or steatohepatitis providing information related to management and prognosis of patients with chronic hepatitis C.
7. Finally the conclusions should be written in order to make the histological diagnosis, stating whether the pathological findings are consistent with chronic hepatitis or not, whether a specific viral etiology may be suspected or whether there are changes related to concomitant diseases, specifying which.

	Chronic viral hepatitis	Primary Biliary Cirrhosis (PBC)	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)	Duct destruction present	May be present

Table 3. Specific features in liver biopsy differential diagnosis and pathological findings

7. Use of liver biopsy in clinical practice

Here we will discuss some of the most prominent findings of liver biopsy in the following clinical settings:

- a. Viral Hepatitis : Hepatitis B, Hepatitis C.
- b. Metabolic diseases: Hemochromatosis, Porphyria, Wilson disease, Alpha 1- anti-trypsin disease.
- c. Autoimmune and cholestatic diseases: Auto-Immune Hepatitis, Primary Biliary Cirrhosis (PBC), Primary Sclerosing Cholangitis (PSC), Overlap Syndrome.
- d. Alcoholic liver disease
- e. Non-alcoholic Fatty Liver Diseases and Non-Alcoholic Steato-Hepatitis (NAFLD and NASH). Liver steatosis.
- f. Drug-Induced Liver Injury (DILI).
- g. Infections and pyrexia of unknown origin.
- h. Cirrhosis. Fibrosis progression.
- i. Hepatocellular Carcinoma (HCC) and other benign or malignant focal lesions. The role of the fine needle aspiration biopsy (FNAB) and other imaging diagnostic tools.
- j. New evolving fields for liver biopsy: Liver transplantation and living donors. Bone marrow transplantation. Morbid obesity

7.1. Chronic viral hepatitis C and B

In the past the majority of liver biopsies were performed in chronic hepatitis C patients. However, recently this has changed, due to a better understanding of the etiology, pathogenesis, the natural history of the disease and available therapies.

As the ability to treat hepatitis C effectively improves, the value of information gained from a liver biopsy decreases. The most effective therapy currently available, a combination of pegylated interferon α and ribavirin, can induce sustained viral clearance, implying a definitive cure and improved long term prognosis. This occurs, after anti-viral treatment in up to 80% of patients infected with genotypes 2 and 3. In patients with genotype 1 receiving recently approved telaprevir and boceprevir, triple therapy constituents, an average of 70-75% can achieve sustained viral clearance. Due to the high percentage of positive response in persons with genotypes 2 and 3, the need for a liver biopsy in such cases has been questioned.

The terminology used to assess the appearance of liver biopsies with chronic viral hepatitis has also evolved.

The first classification of chronic hepatitis based on histological criteria was published in 1968. At that time, only three diseases causing chronic hepatitis could be diagnosed, hepatitis B, non A-non B (hepatitis C, since 1989) and autoimmune hepatitis. This classification which also had prognostic implications only had two categories, namely "chronic persistent hepatitis" and "chronic active hepatitis". Three years later, the term chronic lobular hepatitis was added to represent findings similar to those observed in acute hepatitis.

During the 1990s, there were great changes in the understanding of chronic viral hepatitis by pathologists and hepatologists. The new concepts recognized that the traditional categorization of pathologic changes (chronic persistent hepatitis, chronic lobular hepatitis, and chronic active hepatitis) was inadequate for assessing histological changes during clinical trials. Pathologic processes were separated rather than considered as part of a continuum of pathologic changes that occur in chronic hepatitis C. Pathologists introduced the idea of staging for fibrosis and grading for the inflammatory component to the pathological evaluation of chronic hepatitis C.

7.1.1. Grading and staging of chronic hepatitis C Scoring Systems (Table 4) [16-20]

Grading is the assessment of the activity of a disease, which may increase and decrease as a disease flares and subsides, or may remain static throughout the disease.

Grade and stage evaluation is a standard part of the pathologic assessment of liver biopsies in chronic hepatitis. Pathological staging has focused on the assessment of fibrosis as the best surrogate marker of the disease process. Staging divides the fibrotic continuum into discrete categories and all of the existing staging systems have cirrhosis as their highest stage. Several systems exist for grading and staging of chronic hepatitis and all have been used effectively to assess changes in pathology following therapeutic intervention. These systems include the methods of Scheuer, Desmet, Batts and Ludwig, and the METAVIR system used to score individual features of inflammation and fibrosis semi-quantitatively in clinical studies [14-20].

Steatosis and Steatohepatitis in chronic hepatitis C Steatosis in hepatitis C is mainly macrovesicular and a common finding in genotype 3 [21] it is also related to a high body mass index and older age. More recently, steatosis has been recognized as a feature worthy of study, from an etiologic standpoint and especially in terms of its clinical significance. Estimation of the degree of steatosis has been hampered by the lack of standard definitions and

breakpoints between grades. Although the intrinsic mechanism and involved factors for accelerated fibrosis are unclear, steatosis has been associated with increased inflammation, hepatocellular apoptosis and the presence of perisinusoidal fibrosis [22].

Utility of biopsy in hepatitis C. Nowadays, the majority of Hepatitis C patients can be managed without having to undergo a liver biopsy since liver biopsy rarely identifies unsuspected etiology and hepatitis C diagnosis relies on blood antibody and HCV RNA determinations. However, a biopsy allows to identify patients most in need of therapy or to find clinically unsuspected cirrhosis, which when found it is necessary to screen for varices and hepatocellular carcinoma.

Moreover clinical and laboratory surrogates for biopsy may be useful in identifying cirrhosis and biopsy is not necessary if clinical, image and analytical data concur. Post-treatment biopsy is not needed, nevertheless a new liver biopsy, could be performed if new treatments or clinical trials arrive in order to stratify patients by prognosis.

Fibrosis stage	Knodel et al. 1981 [16]	Scheuer, 1991 [17]	METAVIR, 1994 [18]	Batts and Ludwig, 1995 [19]	Ishak et al. 1995 [20]
0	No Fibrosis	No Fibrosis	No Fibrosis	No Fibrosis	No Fibrosis
1	Fibrous portal expansion	Enlarged fibrotic portal tracts		Portal fibrosis	Fibrous expansion of some portal areas, with or without short fibrous septa
2		Periportal or portal-portal septa but intact architecture	Enlargement of portal tracts	Periportal fibrosis	Fibrous expansion of most portal areas, with or without short septa
3	Bridging Fibrosis (portal-portal or portal-central linkage)	Fibrosis with architectural distortion but no obvious cirrhosis		Septal fibrosis	Fibrous expansion of most portal areas with occasional portal to portal bridging
4	Cirrhosis	Probable or definite cirrhosis		Cirrhosis	Fibrous expansion of portal areas with marked bridging (portal to portal as well as portal to central)
5					Marked bridging with occasional nodules (incomplete cirrhosis)
6					Cirrhosis probable or definite

Table 4. Comparison of commonly used scoring systems for fibrosis staging in chronic Hepatitis C

7.1.2. *Natural history*

The degree of inflammation, fibrosis stage, and steatosis seen on liver biopsy are key histological predictors of progression to cirrhosis. (Table 5) (see page 28).

Based on retrospective data, it has been shown that most patients with moderate inflammation on initial liver biopsy developed cirrhosis after 20 years, and nearly all patients with severe inflammation or bridging fibrosis developed cirrhosis in 10 years. Patients with mild inflammation and/or minimal fibrosis have a low risk of progression to cirrhosis. Hepatic steatosis is also an emerging risk factor for fibrosis progression in hepatitis C [22]. Clinical information may help to refine prognosis, but cannot substitute the valuable information obtained from a liver biopsy. Poynard's group showed three clinical factors which are independently associated with faster progression of fibrosis: male, aged over 40 at the time of infection, and having a daily alcohol consumption of 50 grams or more [23]. Other factors predicting progression to cirrhosis include immunosuppression and co-infection with hepatitis B or HIV [24].

Utility of biopsy in hepatitis B Liver biopsy is not mandatory but may show moderate or severe inflammation which is why before starting antivirals, usually for a long period, our protocol is to perform a liver biopsy and to individualize the therapeutic decision [25]. It has been proved that long-term therapy may improve histology but the role of serial liver biopsies has yet to be established outside of clinical trials. Fibroscan has yet to be validated for patients with chronic hepatitis B but research on this is ongoing [26].

It is important to identify cirrhosis to indicate anti-hepatitis B therapy and hepatocellular carcinoma screening is also recommended for all hepatitis B surface antigen-positive (HBsAg+) patients, cirrhotic or not. New guidelines on anti-HBV treatment say that it is advisable to treat patients with elevated DNA-HBV and minimally elevated or fluctuating alanine aminotransferase (ALT), [27, 28].

Features typical of chronic viral hepatitis inflammation, like fibrosis, is considered to be one of the key characteristics of chronic viral hepatitis. It is a chronic necroinflammatory process in which hepatocytes are preferentially injured compared with bile ducts. The grade of inflammation is a stratification of the overall necroinflammatory changes into mild, moderate, and marked categories. Unlike the fibrosis systems, which are based on distinctive architectural changes that can be highlighted with special stains, assessment of inflammation is more subjective and hence shows more interobserver variations. Usually varying degrees of portal and periportal inflammation (with lymphocytes, plasma cells, and macrophages), lobulillar hepatitis, and fibrosis are to be individually considered and scored.

Interface hepatitis occurs when the inflammatory infiltrate crosses the limiting plate; it is usually associated with local hepatocyte damage, piecemeal necrosis, and inflammation.

Lobular inflammation is accompanied by some hepatocellular necrosis (acidophilic or Councilman bodies). Chronic hepatitis leads to progressive fibrosis and, without treatment, to cirrhosis. The fibrosis begins in portal areas, extends to periportal areas, bridging also other portal tracts and central veins. Histopathological findings in the liver

biopsy that help to predict etiology chronic hepatitis B may show some of the changes described previously, as well as a ground-glass change to the cell cytoplasm. This change reflects accumulation of hepatitis B surface antigen within the endoplasmic reticulum of the hepatocytes [29].

Chronic hepatitis C may be associated with prominent lymphoid aggregates within portal tracts, sometimes including germinal centers and, occasionally, bile duct damage, although not to the degree seen in line primary biliary disorders. In addition, biopsies may show focal, nonzonal macrovesicular steatosis [30].

Patterns of liver cell injury found in liver biopsy and differential diagnosis. Chronic viral hepatitis have no unique histopathologic features, it is therefore necessary to consider various causes. In addition to viral infection, chronic hepatitis may be autoimmune or drug related. Histological features of chronic cholestatic disease, including PBC, primary sclerosing cholangitis (PSC), autoimmune cholangitis, as well as metabolic diseases including Wilson disease and α_1 -antitrypsin deficiency, may overlap with some of the findings with “so called” chronic hepatitis.

7.2. Metabolic liver diseases

Many rare diseases originate in the liver, either affecting the liver directly or causing extra-hepatic disease [31]. For example, liver histology is usually normal in primary hyperoxaluria while the kidneys and other organs may be irreparably damaged; however, cure is only possible with a liver transplant. In other inherited disorders, the liver disease may remain asymptomatic until precipitous acute liver failure develops; the classic example is Wilson disease. Here we present the diseases most frequently observed in adult patients.

7.2.1. Hematochromatosis: The role of liver biopsy in the diagnosis of hepatic iron overload in the era of genetic testing [32]

Hemochromatosis is an autosomal recessive disorder that leads to massive deposits of iron in many organs, including liver, pancreas, heart, joints, and skin. The gene responsible for hereditary hemochromatosis, HFE, is located on chromosome 6. The two most common mutations are C282Y (present in up to 80% of cases) and H63D. The defining characteristic of this disease is the failure to prevent unneeded iron from entering the circulatory pool as a result of genetic changes compromising the synthesis or activity of hepcidin, the iron hormone. Hemochromatosis results from the interaction between genetic and acquired factors. Depending on the underlying mutation, the coinheritance of modifier genes, the presence of nongenetic hepcidin inhibitors, and other host-related factors, clinical manifestation may vary from simple biochemical abnormalities to severe multiorgan disease [33]. The indication of a liver biopsy in the era of genetic testing is being questioned. But, in our opinion, liver biopsy continues to play an important role in the diagnosis, prognosis and management of patients with elevated serum ferritin and abnormal liver function test results in general hepatology practice. Genetic tests for HFE mutations (C282Y, H63D) and liver biopsies are complementary in the workup of these patients.

Liver biopsy allows a quantitative iron concentration study and the identification of the grade of hepatic iron overload, localization pattern and associated liver pathology for diagnosis and management of patients [34].

Liver biopsies may be relatively normal or show bridging fibrosis or even micronodular cirrhosis. Untreated, hemochromatosis leads to the development of micronodular cirrhosis. Prior to the availability of genetic testing, the diagnosis of hemochromatosis was always determined with liver biopsy and quantitation of tissue iron. With the availability of genetic testing for the C282Y and/or H63D mutations, liver biopsy is more often reserved for evaluation of clinical status or complications (i.e. degree of fibrosis, development of hepatocellular carcinoma) rather than for primary diagnosis [35]. A biopsy can also help determine if other disease processes are present, such as hepatitis C or fatty liver disease [36].

We suggest that patients with suspected hemochromatosis undergo genetic testing for the C282Y and H63D mutations, especially if they have a family history of hemochromatosis, in order to establish the genotype of the patient and permit genetic counseling. A liver biopsy may not be necessary in young C282Y homozygotes or in heterozygotes without evidence of liver disease.

Disorders that have to be considered in the clinical differential diagnosis of hemochromatosis

The list of disorders associated with increased hepatic iron is long. The majority of patients with hepatic iron accumulation from any cause do not have hepatic iron concentration (HIC) that is above the upper limit of normal (approximately 1100 mg/μg dry liver weight). Furthermore the pattern of distribution of the iron in the liver may be of some help in establishing the diagnosis [37]:

- predominantly hepatocellular distribution of iron leads to a diagnosis of genetic hemochromatosis, alcoholic liver disease and/or porphyria cutanea tarda.
- predominant presence of iron in Kupffer cells, may be the result of multiple transfusions and/or hemolytic anemias.
- a mixed distribution of iron may be a sign of megaloblastic anemia or anemia secondary to chronic infection.

7.2.2. *Porphyria Cutanea Tarda (PCT)*

It is the most common form of porphyria across the world. PCT is usually an acquired liver disease caused by exogenous factors, such as excess alcohol intake, iron overload, chronic hepatitis C and oestrogen therapy.

The pathogenesis of PCT is varied; it may be hereditary or acquired, leading to hepatic iron loading and to an increase of oxidative stress. Iron loading is usually only mild or moderate in degree. However, in patients with excessive alcohol intake and/or chronic hepatitis C infection, hepcidin production by hepatocytes decreases. This decrease is responsible for increased iron absorption from the gut. The important role that PCT often plays in the hepatitis C virus setting has recently been emphasized [38].

7.2.3. *The role of liver biopsy in determining the diagnosis of Wilson disease*

Wilson disease is an autosomal recessive disorder of copper metabolism, characterized by excessive accumulation of copper in the liver and other organs. Genetic evaluation is difficult because most patients are compound heterozygotes. For patients with Wilson disease the norm is to perform a liver biopsy with a quantitative copper testing of the liver; levels are typically greater than 250 mg/g dry weight liver (normal level, 38 mg/g) [39]. When the diagnosis of Wilson disease is considered prior to liver biopsy other tests are undertaken. - Serum ceruloplasmin (less than 20 mg/dL in patients with Wilson disease; normal levels, 23 to 50 mg/dL). - 24-hour urinary copper (greater than 100 mg/dL; normal, less than 30 mg/dL). - Kayser-Fleischer ring has to be studied by ophthalmologic testing. The liver biopsy in this disease can present differently, depending on the patient's age. In children and young adolescents, the most common finding may be fatty change. In older adolescents and young adults, a liver biopsy may show chronic hepatitis with piecemeal necrosis. Adults tend to show cirrhosis, and Mallory bodies*. In adolescents or adults, confluent necrosis may lead to a severe hepatic failure that may require an urgent liver transplant [40].

7.2.4. *Alfa₁-antitrypsin (A₁-AT) deficiency on liver biopsy*

A₁-AT is the major circulating inhibitor of serine proteases (Pi). Its primary target is the potent elastase found in polymorphonuclear cells (PMNs). It is a glycoprotein synthesized in the liver. Many of the Pi variants are associated with fairly normal serum concentrations and function and thus are of little clinical significance. However, a few, result in low circulating levels of α_1 -AT (i.e., PiZZ) and are of pathologic significance. Liver biopsies from affected patients demonstrate classic PAS-positive, diastase-resistant globules within periportal hepatocytes. Portal fibrosis and chronic hepatitis may also be present. Liver cell dysplasia may be seen, and patients older than 50, especially men, are at risk of developing hepatocellular carcinoma. The presence of PAS-positive, diastase-resistant globules is not always diagnostic for A₁-AT deficiency because various inflammatory conditions may be associated with overproduction of the enzyme, as is the case in cardiac congestion or hypoxia. For this reason clinical correlation is required [41].

7.3. Autoimmune Hepatitis (AIH)

Autoimmune hepatitis (AIH) is an inflammatory condition of the liver that can affect patients of all ages, sexes, and races [42].

Timely diagnosis and immunosuppressive therapy may control disease activity in almost all affected patients and various case series have reported near normal or normal life expectancy in patients diagnosed and treated adequately [43]. Untreated AIH, however, has 5-year mortality above 50%.

It was first described as a form of chronic hepatitis in young women, showing jaundice, elevated gammaglobulins and amenorrhea, which eventually leads to cirrhosis. There is not a single test to diagnose AIH but a set of diagnostic criteria has been suggested in order to classify patients as having probable or definite AIH depending on a score.

Clinical appearance ranges from an absence of symptoms to a severe fulminant presentation. It is usually clinically associated with other autoimmune diseases such as rheumatoid arthritis, ulcerative colitis, autoimmune thyroiditis, or diabetes mellitus. A family history has been reported [44].

A liver biopsy should be obtained at first diagnosis before therapy for grading, staging and confirmation of the diagnosis. Histological appearance is not characteristic, although typical features such as periportal hepatitis with lymphocytic infiltrates, plasma cells and piecemeal necrosis, with more advanced disease bridging necrosis, are frequent. Variable degrees of portal fibrosis are present [45]. In non treated patients or in non-responsive to corticosteroid therapy cirrhosis eventually occurs.

Differential diagnosis which has been revisited recently by the International Autoimmune Hepatitis Group comprises: chronic hepatitis not caused by other etiologies (viral, drug-induced), acute hepatitis alone or acute hepatitis superimposed on underlying chronic liver disease and autoimmune diseases with associated duct damage and duct loss [47].

Early diagnosis may be difficult because the clinical picture is heterogeneous and the liver histology sometimes shows atypical features.

A simple and accurate diagnostic scoring system for AIH has been established but not totally validated yet. In 1993, the International Autoimmune Hepatitis Group (IAIHG) proposed specific diagnostic criteria, which were revised in 1999. These criteria were made by expert consensus and introduced to allow comparison of studies from different centers [46]. Some of the items were of questionable value which is why in 2008 the IAIHG published a new simplified scoring system for wider applicability in routine clinical practice, based on the data of patients with well-established diagnoses and validated in another group of patients [47]. The new score includes autoantibodies, immunoglobulin G, histology, and exclusion of viral hepatitis, allowing a reliable diagnosis of AIH applying simple scores.

7.3.1. *Primary Biliary Cirrhosis (PBC)*

PBC is a chronic progressive cholestatic liver disease that occurs in middle-aged patients, usually women, and is often associated with other autoimmune diseases. Patients may present with jaundice and pruritus in advanced cases. Laboratory testing reveals serum anti-mitochondrial antibody (AMA) as well as increased alkaline phosphatase, bilirubin, and γ -glutamyl transpeptidase [48]. The histological staging of PBC considers the degree of bile duct damage and fibrosis [49].

- **Stage 1** early disease is characterized by damage to septal and larger interlobular bile ducts, reflected by biliary epithelial damage with infiltration of the duct by lymphocytes, plasma cells, eosinophils, and rare polymorphs. The inflammatory infiltrate confined within the portal tract, may include granulomas and lymphoid follicles (florid duct lesion).
- **Stage 2** the inflammatory process extends beyond the portal tract, and changes of interface hepatitis (piecemeal necrosis) may be seen. Bile ducts begin to disappear and proliferation of

bile ductules (cholangioles) may also be present along the edges of the portal tracts. These changes are associated with features of chronic cholestasis, including feathery degeneration within the cytoplasm of hepatocytes, accumulation of bile pigment, periportal accumulation of copper (not generalized as in Wilson disease), and, occasionally, Mallory bodies*.

- **Stage 3** is associated with increasing fibrosis and bridging between portal areas, with decreased amounts of inflammation.
- **Stage 4** represents biliary cirrhosis, usually micronodular. In the past the diagnosis was done in very advanced disease, biliary cirrhosis, hence its name.

7.3.2. *Primary Sclerosing Cholangitis (PSC)*

Primary sclerosing cholangitis (PSC) is a disease with a variable clinical course, with obliteration of the biliary tree that leads to biliary cirrhosis and its complications such as portal hypertension and liver failure. The term “primary” is used to distinguish this condition from the bile duct strictures that are secondary to bile duct injury, cholelithiasis or ischaemia [50].

Patients may present with increased alkaline phosphatase and positive perinuclear anti-neutrophil cytoplasmic antibodies (pANCA). In this disease, liver biopsy does not have a crucial role in the diagnosis. Ultrasound is used for the initial investigation and may show bile duct dilatation and liver and splenic changes; however, it is unspecific for PSC. [51,52]. The classic lesion of PSC in the histological study is onion-skin or concentric periductular fibrosis, with damage to the ductal epithelium, but it is rarely seen on percutaneous biopsy. The most common findings on a biopsy in early-stage disease are non-specific [46], fibrosis with inflammation of portal tracts and paucity of normal bile ducts. In addition, in patients with extrahepatic obstruction, proliferation and dilatation of interlobular ducts and an increased number of periportal PMNs can be observed. Endoscopic retrograde cholangiopancreatography (ERCP) is the next choice test for diagnosis, but it is invasive, for this reason its role is under debate [53]. Transhepatic cholangiography can be used if ERCP is unsuccessful, but again is invasive. Non-invasive alternatives to ERCP are: magnetic resonance cholangiopancreatography (MRCP), which is increasingly used and is useful for excluding other disease and evaluating the biliary system [54]. Transient elastography (FibroScan®) has potential as a non-invasive method for detection of cirrhosis in patients with more advanced liver disease [55].

PSC shares many clinical biochemical and pathologic features with primary biliary cirrhosis, although PSC, can affect both intrahepatic and extrahepatic ducts. PSC is strongly associated with inflammatory bowel disease, particularly ulcerative colitis. Due to its major morbidity and mortality the diagnosis has to be confirmed. At the time of diagnosis, PSC typically involves both intra and extrahepatic bile ducts in the majority of cases. The most dismal sequel of PSC is the development of cholangiocarcinoma (CC) in 14% of patients (which may not be demonstrable radiographically with the usual diagnostic methods) [56].

A wide spectrum of disease severity exists, ranging from patients who present with advanced liver disease requiring liver transplantation within a short time to those who remain

asymptomatic for decades. The natural course of PSC is determined by interindividual variability, the rate of progression and the development of CC, which can occur at any time.

The differential diagnosis has to be established among : autoimmune hepatitis, overlap syndromes, infectious hepatitis, other bile duct diseases presenting as acute or chronic cholangitis, and biliary strictures, cholangiocarcinoma, gallstones, hepatomegaly and primary biliary cirrhosis.

Liver biopsy in PSC is only needed to diagnose small-duct PSC or to exclude other diseases that may be associated with PSC or with similar features and confounding aspects. Liver biopsy also may be useful for staging the disease. However, serial liver biopsy in monitoring the disease is not indicated [57].

Recently some authors have developed the Mayo clinic risk score, a multivariate statistical survival model, on the basis of the long-term course of the disease in 486 PSC patients seen at three centers in United States. In this score, the need for liver biopsy has been eliminated. This scoring system has its advantages; it is non-invasive and was found to be well correlated to actual survival. It also performs better than the Child-Pugh classification for cirrhosis, which does not predict survival with PSC [58].

7.3.3. Autoimmune Hepatitis (AIH) with overlap variants

Overlap syndromes of AIH are not uncommon but are not well defined. Histology, clinical and serological indicators imply more than one liver disease at the same time.

The diagnosis of an overlap syndrome relies on the biochemical profile, either cholestatic or hepatic in addition to the auto-antibodies pattern and elevated gamma globulins. The histopathology can show portal inflammation with or without involvement of bile ducts [59].

In adult patients with an overlap of PBC and AIH, which is the the most common, antinuclear as well as antimitochondrial antibodies are present. Chronic hepatitis C may trigger autoimmune activation, with concomitant positive autoimmune antibodies. AIH may be associated with Ig G4 autoimmune cholangitis (IAC). In contrast to PSC, IAC-IgG4, has no associated intestinal bowel disease and pancreatitis [60].

The value of a biopsy in liver diseases such as PSC or suspected metastatic disease, which is characterized by a zonal affection of the liver has to be dealt with individually and completed with other imaging techniques.

Liver biopsy is advisable if diagnostic tests show abnormal liver function results which may be indicative of many etiologies e.g. nonalcoholic steatohepatitis with strongly elevated antinuclear antibodies and abnormal iron studies, or co-infection with HIV and hepatitis C in a patient with abnormal liver function tests taking hepatotoxic drugs etc.

7.4. Alcohol: Fibrous progression related to alcohol injury

Many patients with ethanol injury show initial scarring around central veins with delicate fibrosis along the sinusoids [61]. Eventually, bridging fibrosis connects central veins

and portal tracts. When cirrhosis is fully developed, most of the native central veins have been obliterated. Alcoholic cirrhosis is micronodular and the scarring is relatively uniform throughout the liver. With complete alcohol abstinence, the nodules can regenerate to a larger size, but the central veins are decreased in number and the nodules may lack some portal tracts [62].

7.5. Non-Alcoholic Fatty Liver Disease, (NAFLD) and Non-Alcoholic Steatohepatitis, (NASH)

The histological appearance in these disorders may be very similar to the injury related to alcohol. In non-alcoholic steatohepatitis, the liver exhibits fat and perivenular sinusoidal collagen deposition and may be indistinguishable from alcoholic perivenular fibrosis on histological grounds alone. Clinical correlations are basic for its diagnosis [63].

Sometimes a biopsy shows a pattern which looks like alcoholic hepatitis, but the patient denies alcohol use. A differential diagnosis for alcoholic hepatitis has to be done, and non-alcoholic fatty liver disease, (NALDF) and non-alcoholic steatohepatitis, (NASH) should be considered [63].

For many decades, typical “alcoholic hepatitis” was often diagnosed with liver biopsy, and in some patients’ medical records were completed with somewhat judgmental comments about their persistent denial of alcohol intake. Now, there are other known causes for Mallory bodies (*) and steatosis found in liver biopsies which, in the past, were classified as alcohol related liver injury. In retrospect, we now know that many patients with “alcoholic hepatitis” were treated unfairly [64].

It is clear that similar patterns of injury can be seen in non-alcoholics, especially in the setting of diabetes and obesity, referred to as nonalcoholic steatohepatitis (NASH) or nonalcoholic fatty liver disease (NAFLD). This represents a significant form of chronic liver disease in both adults and children, with a spectrum ranging from indolent to end-stage liver disease. It may be an underlying cause of cryptogenic cirrhosis and has been reported to recur after a liver transplant. Other conditions associated with NASH include acute starvation, accelerated weight loss, intestinal bypass, disorders of lipid metabolism, and various drugs. Careful clinicopathologic correlation is required to determine the cause. Liver biopsy evaluation allows us to establish the degree of steatosis, inflammation, and fibrosis stage [65].

Liver steatosis

The diagnosis of liver steatosis has several implications in chronic liver diseases.

- Liver steatosis is associated with liver fibrosis progression and a decreased rate of sustained viral response in chronic hepatitis C.
- Donor liver macrovesicular steatosis is independently associated with graft failure at one year after liver transplantation.
- After major hepatic resection, liver steatosis induces an increased risk of post-operative complications and elevated risk of death.

- Finally, liver steatosis is the main lesion observed in non-alcoholic fatty liver disease (NAFLD) which, as a consequence of the worldwide burden of visceral obesity, is now an important cause of chronic liver disease in western countries.

At present, the histological examination of a liver biopsy continues to be the reference for evaluating liver steatosis despite its limitations. The procedure is invasive and impaired by sampling bias, which results in imperfect reproducibility and only allows for a semi-quantitative grading of steatosis [66]. The non-invasive diagnosis of liver steatosis is done by imaging techniques and blood tests, but diagnostic accuracy remains to be validated and their use in clinical practice has yet to be recommended. Ultrasonography is considered the imaging technique of choice for steatosis screening, but its sensitivity in detecting fatty liver is only 60–94% and is operator dependent. Other techniques, such as computed tomography, proton magnetic resonance spectroscopy and magnetic resonance imaging offer high accuracy for quantification of liver fat but have low availability, high cost and lack standardization [67].

The diagnosis of hepatic steatosis and steatohepatitis or non-alcoholic steatohepatitis (NASH) is not yet possible without liver biopsy. Therapeutic targets of drug development are in early stages. As regards the study of factors most likely associated with disease progression, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) has sponsored the NASH Clinical Research Network (CRN) who has developed a histological scoring system, which is used for clinical trials for NASH [68].

The histological lesions for the diagnosis of NASH are: zone 3 macrosteatosis, hepatocyte ballooning and mixed lobular inflammation. Other findings that are common include mild-moderate portal inflammation, acidophil bodies, glycogenated nuclei, lipogranulomas and perisinusoidal fibrosis. In addition, the following may be present: Mallory's hyaline (*) in hepatocytes, megamitochondria and mild siderosis.

(*) Mallory bodies or Mallory's hyaline are irregular, rope-like eosinophilic intracytoplasmic strings that represent aggregates of cytokeratin filaments. The cytokeratins form a filamentous support network within the hepatocytes. Cellular damage is due, for example, to ethanol producing hepatocyte ballooning degeneration, which can cause the keratins to misfold and aggregate. Mallory bodies may be found in alcoholic, nonalcoholic steatohepatitis, and Wilson disease, cholestatic conditions such as primary biliary cirrhosis (PBC) and with certain drugs, such as amiodarone. Although the fat and neutrophils can resolve relatively quickly after alcohol abstinence, hyaline can take up to 6 weeks to disappear [69].

The histological severity of NAFLD is determined by the Non-alcoholic fatty liver disease Activity Score (NAS) and the Fibrosis Score, developed and validated by the CRN [68]. This scoring system is very useful for assessing change in clinical trials but it is not meant to replace a full interpretation of histological findings by a pathologist [70].

Some investigators have observed that there is significant sampling variability and that the histological lesions of NASH are unevenly distributed throughout the liver parenchyma and can lead to substantial misdiagnosis and staging inaccuracies. For example, Ratziu et al. reported that on 51 patients with NAFLD who underwent paired biopsies, the

discordance rate for steatosis would have been missed in 24% of cases if only one biopsy had been done and a difference of one stage of fibrosis or more was seen in 41% of paired biopsies [71].

7.6. Liver injury caused by drugs

Drug and toxin induced liver injury is a common cause for abnormal liver tests in humans [72]. Liver injury related to drugs can be subdivided into intrinsic and idiosyncratic injury. Intrinsic injury is produced through direct or indirect mechanisms and idiosyncratic injury may be mediated by hypersensitivity or by metabolic toxic metabolites [73].

Drug induced liver cell injuries have different morphological patterns such as, hepatocellular injury, cholestatic injury, bile duct injury, vascular injury, portal fibrosis, neoplasia or miscellaneous (pigments and inclusions).

The list of implicated products is very long and in some cases mixed lesions can be found. Drug “signature” is a well-known concept which implies that the drugs responsible for the injury can be identified from the different lesions it causes to the liver. For example, diclofenac and minocycline produce a chronic hepatitis pattern, steatohepatitis can be induced by amiodarone and tamoxifen, vascular toxicity may be associated with azathioprine etc. [74].

Histological changes that suggest drug- or toxin-related liver injury are atypical therefore, in some cases, depending on the findings, it is worth the pathologist asking the clinician specific questions in order to do a differential diagnosis and to identify the drug [75]:

Is the patient’s blood analysis compatible with hepatitis? Has viral injury been excluded?

- What are the patient’s toxic exposures at work, home, or play?
- Has every drug been sought and disclosed?
- Granulomas (**) may also be part of the inflammatory reaction in drug injury [76].

If granulomas have been found, have other causes of granulomas been excluded? (see below) [77]

If significant fatty change is found is there any possibility that it could be related to toxic ethanol injury?

If an abundance of eosinophils is observed in a liver biopsy, a hypersensitivity reaction is suspected which may resemble viral hepatitis. Eosinophils may also be present nonspecifically in viral hepatitis, in connective tissue disorders, and in some neoplasms (usually in Hodgkin’s disease infiltrates). However, when eosinophils are a striking feature, it is advisable that the clinician search for a drug, a toxin, or even a nutritional supplement (“natural medicines”).

If numerous liver cell mitotic figures show up in the liver biopsy, this may suggest that a short episode of drug exposure is to blame.

7.6.1. Drug Induced Liver Injury (DILI) examples

The American Association of Rheumatology has provided guidelines for monitoring patients receiving Methotrexate therapy as there is a known relation between this treatment and hepatotoxicity [78].

A few years ago methotrexate was used for treating rheumatoid arthritis. Now patients with psoriasis are also treated with this drug, albeit at a lower dose. Many potentially hepatotoxic medications, used in such cases are worth investigating [78].

Amoxi-clavulanic acid is one of the examples of a broadly used antibiotic which has been implicated in liver toxicity. Typically the patient with this toxicity presents with jaundice. After excluding other causes, such as viral hepatitis, autoimmunity, or other etiologies, and in presence of a normal biliary tree, a liver biopsy is recommended, which may show a cholestatic hepatitis pattern. After discontinuation of the drug the evolution is usually favourable [79].

7.6.2. Granulomas in liver biopsies

Granuloma is defined as an aggregate of histiocytes and can only be diagnosed through histopathological examination.

Causes of granulomas in the liver: most systemic granulomatous diseases involve the liver to some extent; tuberculosis and sarcoidosis are the most common causes [80]. Other infectious agents include bacteria (brucellosis, nocardiosis, tularemia, Q fever [*Coxiella burnetii*], spirochetes), various fungi, protozoa, and viruses (cytomegalovirus, Epstein-Barr virus). Noninfectious causes in addition to sarcoidosis include PBC, drug reaction, extrahepatic inflammatory disease, such as chronic inflammatory bowel disease, rheumatoid arthritis), neoplasms (Hodgkin disease) and foreign substances (talc, mineral oil) .

7.6.3. Can negative stains for fungi and acid-fast bacilli exclude infection in patients with fever of unknown origin?

Definitely not. Cultures for these organisms are more sensitive than special histological stains. If infection is a possibility, a core of liver should be submitted with sterile precautions and without fixative to the microbiology laboratory. In addition, tissue in formalin should be sent to the pathology laboratory for microscopic sections. A tissue sample may also be sent for molecular analysis to determine whether an infectious agent is present, depending on the possibilities [82, 83].

7.6.4. Different types of granulomas useful in determining specific diagnosis

- Epithelioid granulomas are nodular aggregates of plump macrophages, often associated with multinucleated giant cells, lymphocytes, and plasma cells. They are typically seen in sarcoidosis. The presence of central caseating necrosis suggests tuberculosis.
- Fibrin-ring granulomas are formed by a fibrin band encircling a lipid droplet, with associated inflammation. They were first described with Q fever but may also be seen after in-

fection with cytomegalovirus or Epstein-Barr virus as well as with drug (allopurinol) toxicity and in association with systemic lupus erythematosus.

- Lipogranulomas are composed of lipid deposits and vacuolated macrophages. They are formed in the presence of exogenous or endogenous fat accumulation.
- Microgranulomas may be a nonspecific finding, they are usually subtle and composed of small, round clusters of plump Kupffer cells.

There are many causes of hepatic granulomas, including local irritants, infections, infestations and hypersensitivity to drugs. The constituents of these lesions, depending on the etiology and inflammatory cytokines produced include large epithelioid cells, multinucleated giant cells, varied numbers of mononuclear cells and eosinophils. The causes vary in frequency from one country to another. Although the etiology may be determined from the histological features, from special stains for micro-organisms, from culture of part of the biopsy specimen or polymerase chain reaction of the paraffin-embedded specimen, or from clinical and serological data, the cause of hepatic granulomas remains unknown in one third of cases. It is likely that approximately one third of granulomatous liver reactions are caused by drugs, including allopurinol, carbamazepine, procainamide, diphenylhydantoin, quinidine, isoniazid, and sulphanilamide.

7.7. The role of liver biopsy in infections and pyrexia of unknown origin

Although the usefulness of liver biopsy in the diagnosis of fever of unknown origin is still controversial, a review of the literature shows that liver biopsy can be effective in confirming histopathological diagnosis and microbiological analysis [83].

Based on the findings of a liver biopsy evaluating Fever of Unknown Origin (FUO), we can conclude that abnormal liver biopsy is helpful in determining the cause of the FUO. The most common cause of fever was of an infectious origin. Other causes were neoplastic disorders or inflammatory [87].

Liver biopsy was performed after routine studies were negative. Therefore results such as histoplasmosis and tuberculosis indicate that, despite advances in diagnostic technology, liver biopsy continues to be useful in the diagnosis of FUO. In endemic areas, histoplasmosis and tuberculosis should be considered in the differential diagnosis of FUO [85].

7.8. Cirrhosis

Cirrhosis is pathologically defined as a diffuse process in which the normal anatomical lobules are replaced by architecturally abnormal nodules separated by fibrous tissue. Therefore, focal scarring, even if significant and associated with nodules, is not cirrhosis because the process is not diffuse [86]. In the past the description of a liver as “cirrhotic” implied an ominous prognosis in a patient with liver disease. In chronic hepatitis, the most important goal is to delay or to stop the development of cirrhosis. Nowadays, treatments to prevent its progression are available. At present there are many known stages as opposed to before

when there only one was considered, G Garcia-Tsao in the article “In search of a pathological classification of cirrhosis.” [86].

7.8.1. *Fibrosis progression*

One of the most crucial developments is the reformulation of the concept of cirrhosis from a static to a dynamic process. This concept is likely to be even better defined in the future.

As fibrotic scars advance and extend the normal architecture changes and nodules are formed [88]. Moreover, the angiogenic process that naturally accompanies scar formation permits the creation of abnormal channels between central hepatic veins and portal vessels, resulting in the shunting of blood around the regenerating parenchyma. Normal vascular structures, along with sinusoidal channels, may be obliterated, leading to portal hypertension. Some authors describe cirrhosis as a vascular disease [89]. Clinical consequences of cirrhosis result from the decreased ability of the parenchyma to synthesize clotting factors and other substances combined with the complications related to portal hypertension [90].

Knowledge on the level of fibrotic progression between normal histology to cirrhosis has considerable prognostic weight. Patients with bridging fibrosis on biopsy are much closer to end-stage liver disease than those with minimal or no fibrosis. Fibrosis is not an autonomous feature, but rather a tissue progressive lesion resulting from other pathologic mechanisms such as inflammatory, degenerative or dystrophic processes [91].

The first transition in this process occurs between the normal, non-fibrotic state and the expansion of the portal area by fibrosis, to the extension of short, incomplete septations around the portal area, change that gives to the portal areas an irregular stellate shape.

In the next transition, development of bridges between vascular structures, portal-portal bridging fibrosis and portal-central bridging, occur. Gradually, more and more bridges are formed, accompanied by distortion of the architecture due to hepatocellular regeneration and contraction of fibrotic scars. When these changes diffusely involve the biopsy, it is classified as cirrhosis [92].

Progressive fibrosis leads to cirrhosis and it is now known that cirrhosis can be reversible. There was a lot of controversy surrounding this issue a few years ago [93]. For patients in a precirrhotic stage of fibrosis, liver biopsy remains the gold standard of assessment. Prior to 1995, there was no published system which subdivided advanced stages of cirrhosis. Only the Ishak modification of the Histologic Activity Index (HAI) subdivided cirrhosis into three categories [94].

Nowadays, since Garcia-Tsao et al. reported compensated and decompensated phases in the clinical evolution of liver cirrhosis, many prophylactic measures and controls have been implemented in order to improve survival and quality of life [87]. Cirrhosis is usually clinically evident. Once the pathologic stage of cirrhosis has been reached, clinical scales such as the Child-Pugh score have to be used because they represent the prognosis and the staging of

the liver disease better [95]. The present debate questioning the need for liver biopsy versus non invasive tests will be discussed below.

7.8.2. A needle biopsy specimen does not always permit the diagnosis of cirrhosis

Micronodular cirrhosis (nodules of 3 mm or less), which may develop as a result of ethanol injury, biliary tract disease, or hemochromatosis, is usually uniform throughout the liver, and nodules may be identified on a needle specimen. However, macronodular cirrhosis (nodules greater than 3 mm), due most commonly to chronic viral hepatitis, constitutes a less uniform pattern [96].

7.9. Hepatocellular Carcinoma (HCC) and other benign or malignant focal lesions: The role of Fine Needle Aspiration Biopsy (FNAB) [97]

Indications of liver biopsy with regards to diffused or local lesions

Liver biopsy is useful for diagnosis of a diffused disease and guided liver biopsy remains essential for the diagnosis of localized lesions.

7.9.1. Fine needle aspiration biopsy (FNAB)

This technique has a crucial role in the evaluation of focal liver lesions or localized lesions. Liver tumors appear as nodular or localized lesions which can be malignant or non-malignant and can be either primary from the liver or metastatic. If clinical, biochemical and radiologic findings are inconclusive, some phases of the diagnostic process may require a liver biopsy in order to establish the diagnosis and their staging and management [99].

Malignant lesions. Hepatocellular carcinoma (HCC), the most frequent malignant liver cancer, is usually discovered during screening programs in cirrhotic patients. Regarding treatment, the only curative option is surgery, both limited hepatectomy of the tumor or liver transplant in very select cases [100].

In liver lesions with typically recognized features of HCC, defined by using advanced radiological methods, liver biopsy has no place. However, a liver biopsy will be performed in patients with atypical liver tumors suggestive of a possible colangiocarcinoma. These cases require another form of therapy and the prognosis is worse [99].

Besides, when surgery is indicated in a patient with suspected liver cirrhosis, a liver biopsy has to be performed in the non-neoplastic liver. Pathological diagnosis may help to assess the functional capacity, specific prognosis and whether surgery could be performed.

Metastasis of the liver with an unknown primary tumor should be biopsied to obtain information of the primary tumor in order to determine therapy.

Concern has been expressed about the risk of spreading malignant cells via the needle tract, but this rarely occurs when using needles with a diameter of less than 1.3 mm, which also minimizes the risk of bleeding. The procedure is simple, safe and painless [101].

7.9.2. *Non-malignant lesions*

In cases of Hemangioma or Focal Nodular Hyperplasia (FNH), diagnosed and confirmed by radiology, biopsy is usually not necessary.

FNH and hepatic adenoma are benign tumors and are less frequently observed than HCC. Their diagnosis is done using imaging techniques (ultrasound or helicoidal scanner). However, differential diagnosis is necessary because, although FNH only requires radiological follow-up, in some cases, higher risk circumstances have been recognized and surgery is recommended [102].

7.9.3. *Most prevalent mass lesions [102, 103]*

- Benign: cysts, hemangioma, adenoma, liver abscess (amebic or pyogenic), focal nodular hyperplasia, fatty infiltration, rare primary liver neoplasms.
- Malignant: hepatocellular carcinoma, cholangiocarcinoma, metastatic, rare primary liver neoplasms, rare primary bile duct neoplasms.

7.10. **New evolving fields for liver biopsy: Liver transplantation, Bone marrow transplantation, Living donors and Morbid obesity**

7.10.1. *Liver transplantation*

With regards to liver transplantation, liver biopsy remains very useful in the management of transplanted patients. In this clinical situation, if a rejection is suspected and other complications have been ruled out, a guided biopsy will be performed. This procedure can be of great value in order to confirm the specific diagnosis and to indicate treatment [104].

In the first few weeks and months after transplantation, the major causes of abnormal liver tests include preservation injury, acute rejection, opportunistic infections (e.g., cytomegalovirus, hepatitis), vascular compromise, and/or biliary stricture. Of these, acute allograft rejection is the most common and results from direct alloantigenic stimulation of recipient T cells by donor dendritic cells (antigen-presenting cells). The effector T cells can then preferentially injure biliary epithelial cells of both interlobular and septal bile ducts as well as endothelial cells of intrahepatic arteries and veins.

The main histological features of acute rejection Acute rejection is characterized by an infiltration of mixed, predominantly mononuclear cells within portal tracts. The inflammatory infiltrates include lymphocytes, macrophages, plasma cells, polymorphonuclear neutrophils and eosinophils. The inflammatory cells typically infiltrate the bile duct epithelium and are associated with bile duct damage. Subendothelial inflammation (endothelialitis), which may involve both portal and central veins, is also a typical feature. The most common grading system is the Banff schema, a consensus document proposed by an international panel of pathologists and liver transplant physicians [105]. Criteria helping to distinguish recurrent hepatitis C after transplantation from allograft rejection; Hepatitis C (HCV) recurs in virtually all patients transplanted for that disease.

Distinguishing recurrent hepatitis from acute allograft rejection, which can overlap, is difficult. There are usually three main phases to recurrent HCV:

- Graft reinfection (from 0 to 3 months post-transplant). HCV-related inflammation is rarely seen at this time. Liver biopsies may show mild lobular disarray, few necrotic hepatocytes (acidophil bodies), and fatty change.
- Established graft infection (from 3 to 6 months), acute hepatitis including ballooning degeneration of hepatocytes, acidophil bodies, and Kupffer cell prominence can be observed. Varying degrees of portal tract inflammation may also be present.
- Progressive liver damage (after 6 months), features related to chronic HCV infection such as, mononuclear portal infiltrates and interface hepatitis are observed. Bile duct damage, although mild, may occur, and granulomas may be detected. Up to half of patients will have histological evidence after 1 year.

The role of liver biopsy in the evaluation of abnormal liver tests after the first year post transplantation Common causes after the first year include acute rejection, opportunistic infection, recurrent viral hepatitis, chronic rejection, steatohepatitis, or recurrent diseases. Chronic rejection occurs as a consequence of repeated episodes of acute rejection that are unresponsive to immunosuppression. The main histological abnormalities are loss of small bile ducts (ductopenic rejection) and/or obliterative vasculopathy (affecting large and medium-sized arteries). Unlike acute allograft rejection, the degree of bile duct damage is typically out of proportion to the degree of inflammation.

Complications of liver transplantation are not limited to acute and chronic rejection and recurrence of original disease, but include surgical complications, most commonly hepatic artery occlusion, infections, and development of de novo malignancies. In the early post transplantation period preservation injury, damage to the graft during harvesting and implantation, may lead to significant graft dysfunction. In post-perfusion biopsies, heavy neutrophilic infiltrate and hepatocyte necrosis may be predictive of initial poor graft function.

Ischemic complications, such as hepatic artery thrombosis, are one of the most serious complications and may lead to early graft loss or biliary stricture. In these patients liver biopsy is usually not performed.

Infectious complications that generally occur after transplantation, cytomegalovirus (CMV) for example, remains common and is frequently associated with parenchymal microabscesses which are found in the liver biopsy of CMV patients.

7.10.2. Bone marrow transplantation

A liver biopsy is effective in the evaluation of a bone marrow transplant recipient with elevated liver tests [106]. Known complications of bone marrow transplantation include veno-occlusive disease (VOD) and graft-versus-host disease (GVHD). A biopsy is necessary to diagnose VOD. It develops within 1 to 4 weeks after transplantation and is characterized by occlusion of central veins, sinusoidal fibrosis, and pericentral hepatocyte necrosis. Acute GVHD develops within 6 weeks after transplantation and affects the skin, gastrointestinal

tract, and liver. It is characterized by degenerative bile duct lesions with some degree of mononuclear inflammation. Cholestasis may be present. Chronic GVHD is a multiorgan process that develops 80 to 400 days after transplantation and is often preceded by acute GVHD. The changes in the liver are similar to those in acute disease, but the ducts show more prominent changes and are likely to be reduced in number or destroyed. A prominent periportal mononuclear infiltrate, or even piecemeal necrosis, may be seen.

7.10.3. *Liver transplant living donor*

Liver biopsies detect silent donor disease in potential living liver donors, especially patients suffering subclinical non-alcoholic fatty liver disease (NAFLD). The contribution of liver biopsy or even the need to perform this, when a potential donor is being evaluated is a controversial issue [107]. In the University of Pittsburgh Medical Center a retrospective study of the histopathologic examination and diagnoses of 284 patients, who were evaluated as living donors from 2001 to 2005 was carried out. Hepatic histology was correlated with liver injury tests and with demographic characteristics in an otherwise normal healthy population. A minority (n=119; 42%) of biopsies from this population of 143 males/141 females (average age=36.8years; mean BMI=26.6) were completely normal. The remainder showed steatosis (n=107; 37%), steatohepatitis (n=44; 15%), or unexplained low-grade/early stage chronic hepatitis, primary biliary cirrhosis, or nodular regenerative hyperplasia (n=16; 6%). Biopsy findings disqualified 29/56 donors, negative factors were: obesity, age and liver iron content, contributing to NAFLD pathogenesis. The conclusion was that liver biopsy provides valuable information about otherwise undetectable liver disease in potential liver donors.

7.10.4. *Morbid obesity*

About 90 per cent of morbidly obese patients show histological abnormalities of the liver. Morbid obesity may lead to severe disease showing steatosis, ballooning degeneration, lobular inflammation and fibrosis in the study of liver biopsy. These features are similar to the lesions observed in alcoholic hepatitis and may end in cirrhosis and liver failure. Many factors such as, alcohol, drugs, diabetes, viruses, can contribute to progressive liver damage. The development of severe fatty liver disease may be asymptomatic showing a poor correlation with liver function tests. It has been reported that after bypass surgery, weight loss is accompanied by improvement in fatty change and the liver function tests are normal.

Histopathologic findings in the liver of 160 patients who were undergoing laparoscopic gastric bypass or gastric banding for morbid obesity, were recorded, also clinical data (gender, age, BMI and associated diseases) and laboratory evaluation were obtained [108]. The diagnosis obtained were : 63 non-nonalcoholic steatohepatitis (non-NASH), 54 NASH, 26 chronic hepatitis B (CHB), 15 alcoholic steatohepatitis and NASH, and 2 chronic hepatitis C (CHC). The coexistence of clinical and histological features of steatohepatitis with another chronic liver disease may reflect the biological significance of the chronic inflammatory condition in the obese population, which requires further investigation.

8. Non-invasive tests for liver disease and assessment of fibrosis and cirrhosis

Limitations of biopsy have led clinical investigators to study alternative methods to investigate liver disease especially for the assessment of liver fibrosis. Since fibrosis is of sufficient importance in chronic liver disease and because it progresses to cirrhosis it is frequently used as the outcome and prognostic variable in clinical studies. Hepatic fibrosis is currently viewed as a dynamic process that may regress after successful treatment of chronic liver diseases. Serum markers, such as non-invasive markers, offer an attractive alternative. They are objective, allowing a dynamic calibration of fibrosis, can be performed repeatedly, are more cost effective and many of them are performed as a routine analysis [109].

Indirect markers of liver fibrosis: among them, the AST to Platelet Ratio Index (APRI), combines aspartate aminotransferase (AST) with platelet count. It was used in several studies conducted in cohorts of patients with hepatitis C and showed a rather good diagnostic performance and reproducibility, [110] particularly for cirrhosis. Forns and colleagues reported a fibrosis index (Forns' index) based on platelet count, γ -glutamyl transferase (GGT), and cholesterol levels [111]. It is rather good for excluding significant fibrosis, but only average for diagnosing significant fibrosis. However, one important limit of both APRI and Forns' indexes are that they leave almost half of the patients unclassified.

Another widely investigated combination set of noninvasive markers of liver fibrosis is the Fibrotest; a combination of five blood tests based on a mathematical formula: GGT, bilirubin, haptoglobin, apolipoprotein A1, α_2 macroglobulin adjusted for gender and age. According to the investigators, it could exclude cases with significant fibrosis (METAVIR > F2), having 100% of negative predictive value, and more than 90% positive predictive value, using liver biopsy as a reference [112].

8.1. Elastography (FibroScan)

Another noninvasive method for the assessment of liver fibrosis is elastography (FibroScan) [113].

FibroScan device (EchoSens, Paris, France) uses a mild-amplitude, low frequency vibration transmitted through the liver. It induces an elastic shear wave that is detected by pulse-echo ultrasonography as the wave propagates through the organ. The velocity of the wave correlates with tissue stiffness which correlates well with the degree of fibrosis.

This device is in widespread use in many parts of the world, but is not yet approved in the United States.

Most of the studies have been conducted on patients with chronic hepatitis C but a few studies have also covered fibrosis and cirrhosis due to other etiologies.

This technique, however, has its limitations: it uses expensive equipment, and has decreased accuracy in obese patients and in patients with ascites. Elastography results are not valid in presence of hepatic steatosis, cholestasis, necroinflammation, or portal hypertension. The patient’s age and levels of aminotransferases need to be taken into account when interpreting results of liver stiffness [114].

	IN FAVOUR of LIVER BIOPSY	AGAINST OF LIVER BIOPSY
Chronic HCV hepatitis	In selected indications for grading and staging	Not necessary for diagnosis. Possible use of non-invasive methods in follow-up controls
Chronic HBV hepatitis	Grading and staging advisable before starting treatment	Not necessary for diagnosis. Possible use of non-invasive methods in follow-up controls
Non-alcoholic steatohepatitis (NASH)	NASH is a always an histopathological diagnosis	Assessment of fibrosis possible with non-invasive methods
Alcoholic steatohepatitis (ASH)	ASH is a histopathological diagnosis. But in alcoholic acute hepatitis liver biopsy usually is not performed	Assessment of fibrosis possible with non-invasive methods,(in abstinent patients)
Autoimmune Hepatitis	For diagnosis and staging liver biopsy is needed	Non validated methods yet for non-invasive assessment of fibrosis
Primary Biliary Cirrhosis (PBC)	Not needed in typical mild cases without biliary duct damage	Possible non-invasive assessment of fibrosis
Hemochromatosis	In general, liver biopsy performed for diagnosis and staging, and iron content in the liver	Non validated methods for non-invasive assessment of fibrosis
Wilson disease	For diagnosis and staging and copper content in the liver	Non other options

Table 5. Indication for liver biopsy in different chronic liver diseases in the era of non-invasive methods

In spite of that, elastography is complementary as the combination of noninvasive markers and elastography improves the overall accuracy. In one of the metanalysis, for significant fibrosis, the area under the ROC for Fibrotest and FibroScan were 0.81 (95% CI 0.78-84) and 0.83 (0.03-1.00), respectively [115].

Fibrotest, and elastography (Fibroscan) as first line estimates of fibrosis in patients with chronic hepatitis C are recommended and liver biopsy will probably be indicated only as a second line diagnosis and reserved for cases of discordance or non-interpretability [112].

Some authors conclude that elastography appears reliable to detect significant fibrosis and cirrhosis in patients with chronic hepatitis C, besides it may turn out to be a valuable diagnostic procedure and follow-up of patients with chronic liver diseases of different causes [115].

8.2. Liver biopsy: Consensus among pathologists?

It is crucial that biopsy interpretation be done by experienced liver pathologists. Pathologists have tried to define the features (including length and number of complete portal tracts) of an adequate liver biopsy sample able to correctly assess the classification of liver fibrosis. Some authors have recommended big samples of 1 to 4 cm in length containing at least 11 complete portal tracts, which could be more reliable for adequate grading and staging [116, 117]

Feature	Effect on response to therapy	Effect on natural history
Fibrosis	Reduces response	Presence implies progression
Inflammation	No effect	Related to increased amount of current fibrosis and increased rate of progression
Steatosis	Reduces response	Related to increased amount of current fibrosis, unclear effect on progression
Iron accumulation	Unclear effect	Related to increased amount of current fibrosis, unclear effect on prospective rate
(adapt from Semin Liver Dis 2005)		

Table 6. Relevance of histological features of chronic hepatitis C to disease progression and therapeutic response

Many intraobserver and interobserver variations have been estimated in the assessment of features, classification, and scoring of liver biopsy assessment. One study reported differences in the evaluation of liver biopsies in chronic viral hepatitis C among 10 pathologists specializing in liver diseases. These pathologists independently reviewed 30 liver biopsy specimens of viral hepatitis C and completed a histological form for each of the specimens. Five pairs of pathologists were then randomly designated and they independently reviewed the biopsy specimens and filled out a new coding form. The interobserver variation was calculated for each item among the 10 individuals and then

among the five pairs. Five features showed an almost perfect or a substantial degree of concordance among the 10 observers (cirrhosis, fibrosis, fibrosis grading by Knodell index, steatosis and portal lymphoid aggregates). The 17 other indicators showed a weaker concordance. Five items had a higher concordance when viewed by a pair of pathologists than when studied by only one pathologist (steatosis, periportal necrosis grading by Knodell index, lobular necrosis grading by Knodell index, centrilobular fibrosis, and ductular proliferation). This study reveals that certain features of major importance in assessing disease activity show significant observer variation. The acceptable degree of concordance was related mainly to the fibrosis score, whereas other numerical items displayed substantial observer variations. Simultaneous observation by two pathologists increased the reproducibility of numerical scoring and of certain viral hepatitis C lesions. A classification of chronic hepatitis C based on dissociated semiquantitative assessment of necroinflammatory lesions and fibrosis offers more reproducibility than the use of a global numerical index [107].

As a single percutaneous liver biopsy yields only a minute percentage ($1/50\,000$ or 0.002%) of the total hepatic tissue, paired biopsies have been evaluated in several published studies, especially for assessing steatosis and NASH. For quantification, better references are required, such as imaging techniques or morphometry, which determines the area of steatosis on liver biopsy.

In fact, as liver steatosis is not homogeneous, classical optical examination of a liver biopsy by a pathologist for measuring liver steatosis by the determination of the percentage of hepatocytes containing lipid vesicles is highly subjective, and steatosis grading corresponds only to a semiquantitative scale [68].

The role of the liver biopsy in disease management is evolving nowadays and has to be reconsidered given the modern pathologic assessment of liver biopsy. Pathologists have made progress in the interpretation of liver biopsies and in processing the information in a concise and scientific way available to clinicians. After evaluating the disease state and interpreting the tests results, the clinician in charge of the patient should consider the individual patient when making recommendations with regards to treatment.

Role of the liver biopsy in personalized medicine

The liver biopsy specimen aims to obtain a valuable material for the assessment of fibrosis and cirrhosis. Despite limitations related to sampling and interpretation, histological examination remains the best standard for staging and diagnosing chronic liver diseases. Its indications are decreasing because new therapeutical options for chronic viral hepatitis have improved [118]. Moreover, new non-invasive tests have been developed and their use may increase in the future, especially for long term management [119] (Table 7).

All invasive procedures involve risks, consequently the benefits of obtaining liver for histology should always be weighed against the possible morbidity of the procedure. The decision to indicate a liver biopsy has to be taken depending on the center's facilities and the availability of experienced liver pathologists to interpret the biopsy.

Ethics related to liver biopsy mainly include issues on the indications, information on potential risks and benefits and validity of available alternative options. Patients should be adequately informed and participate in the decisions for liver biopsy and treatment [120].

	Liver biopsy	Transient elastography
Advantages	- Direct measure of liver fibrosis - Well established staging system - Assessment of architectural disturbances related to liver fibrosis - Evaluation of associated lesions (inflammation, steatosis, iron, alcohol)	- Non-invasive - Easy to repeat - No risk to patient - Performed in the outpatient clinic - Results available immediately - Reproducible - Highly performance for detecting cirrhosis
Disadvantages	- Invasive and painful - Difficult to repeat - Potentially severe complications - Contraindicated if ascites, coagulopathy etc. - Sampling and interobserver variability	- Unable to discriminate between intermediate stages of fibrosis - False positive in case of acute hepatitis, extrahepatic cholestasis and congestive heart failure

(adapt L Castera & M Pinzani, Gut 2010)

Table 7. Use of liver biopsy in clinical practice. Respective advantages and disadvantages of liver biopsy and transient elastography for assessing fibrosis in chronic liver disease

12. Conclusions

What will be the real impact of Liver Biopsy now and in the near future in the era of personalized medicine?

1. The practice of liver biopsy will remain as an important component in the evaluation of liver disease. However, the value of liver biopsy should be contemplated as a complementary tool in modern medicine because of the presence of new non-invasive diagnostic measures, better prognostication methods and more advances in imaging techniques.
2. Non invasive tests such as Fibroscan, or similar, adding serum markers will be increasingly used to identify the amount of fibrosis, and will spare, in most patients, the performance of a liver biopsy.
3. Liver biopsy provides information that is used in conjunction with other data to inform and to guide therapy. The team that joins pathologists, clinicians, radiologists and other specialists meets in order to make clinico-pathological correlations. New classifications incorporating clinical data in the histological dictamen will be implemented.

4. Therapies, etiology, pathogenesis, cellular and molecular mechanisms, changes in tissue architecture and invasive (HVPG) and noninvasive diagnostic approaches, should be added to the liver biopsy information.
5. In the liver transplant field, liver biopsy has allowed many scientific advances and in most of these patients liver biopsy will continue to be mandatory for their management.
6. Patients seeking a second opinion or who are referred to a tertiary care center, will require a deep review of previous obtained specimens in order to confirm and to plan their management.
7. Since chronic viral hepatitis is a prevalent disease in the general population, the number of liver biopsies will be limited in the next years because it is costly and aggressive so validated non-invasive methods will be favoured.
8. Nevertheless, some questions surrounding non-invasive markers will remain. Non-invasive markers have been validated against the biopsy, and the overall accuracy of biopsy is only 80%, it is probably statistically impossible for a marker to perform any better than a biopsy.
9. Increasing research on hepatic fibrosis, diagnoses and therapy, is ongoing so valuable results of predicting changes in fibrosis content over time have to be followed by histological liver assessment. Moreover, considering the possible regression of cirrhosis, now clinicians and pathologists have become more interested in studying histological features of tissue repair and fibrosis regression in the liver.
10. The number of liver biopsy will be sparing in common patients but it will play a crucial role in research; for example studying rare diseases, stem cells and genetic disorders. Moreover, its role is evolving in many research fields such as obesity, bone marrow transplant, and oncology.

Acknowledgements

I would like to thank Aisling Dowd, for her help while preparing the manuscript

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