Chapter 5

Risks and Benefits of Liver Biopsy in Focal Liver Disease

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1. Introduction

Even with the recent evolution of imaging techniques, and with the ever-increasing role of serum markers, direct analysis of tissue samples maintains its role in modern medicine. This is especially true for the diagnosis and assessment of the prognosis and evolution of a series of viral, tumoral and inflammatory liver diseases. Thus, liver biopsy and histological assessment of the liver parenchyma can still be called by many the "gold standard" in diagnosis and staging of associated disease. However, liver biopsy in itself implies a series of risks and inherent discomfort for the patient. With the increasing availability of other non-invasive methods routinely used in diagnosis and staging of liver-related diseases, many debate the necessity and ethical implications of tissue sampling.

In the following pages, we will try and synthetize the historical evolution of liver biopsy, describe the techniques used over the years and present its current recommendations and their alternatives, with focus on the so-called "virtual liver biopsy" techniques currently employed.

2. Historical landmarks and recent developments in liver biopsy

The first written documented report of a successful liver biopsy was made by Paul Ehrlich in the book "On diabetes" published in 1884. He published an account of the procedure performed in 1880 in Berlin, along with graphical illustrations of the instruments and the liver samples collected. This came detailed account was based on previous theoretical advantages of this technique discussed by the French physician AGM Vernois in 1844, who in turn based his assumption on successful procedures performed for punctur-

ing purulent echinococcus, as early as 1825 (Récamier) and 1833 (Stanley). Cytology was reported as a diagnosis method for liver disease by L. Lucatello (in Rome) in 1895, while F. Schupfer performed liver and spleen biopsies with a thicker needle twelve years later, in 1907. This new approach provided cylindrical-shaped tissue samples which could be histologically prepared and analyzed [1].

Other scarce accounts of successful procedures followed in the next couple of decades (Olivet, 1926; Huard 1935; Silverman, 1938; Baron, 1939; Kofler, 1940; Dible, 1943), using different aspiration techniques performed with different modified biopsy needles [1].

A new stage in modern liver biopsy techniques was reached when, in 1957 and repeated in the following year, Menghini performed and reported on the first "one-second needle biopsy" performed with a special small caliber needle with no trocar and a sharp bevel. This was the first time needle liver biopsy was introduced worldwide as a praised diagnostic technique capable of providing enough histological material for an accurate interpretation of the pathological changes present in the parenchyma [1].

Following this radical advancement, liver biopsy became more spread and the technique evolved once modern imagistic methods allowed for better and safer puncturing of the liver parenchyma. Thus, the technique entered the image-guided age of investigation performed under computed tomography (CT) or ultrasound (US) real-time screening. Reports from Denmark, China, the United Kingdom, France or the United States of America populated the 1960–1980 literature, once the technique became widespread and fully acknowledged by the academic community. Its utility in diagnosing liver diseases and later on in staging hepatitis or malignancies was undisputed for entire decades of the 20th century [1].

Recent advancements, based on the advent of new imagistic high-accuracy techniques based on both US and CT/RM approaches, highly diminished the role played by this invasive investigation. The term "virtual biopsy" became more and more present in recent literature, once both doctors and patients alike became more confident and were introduced to these high-yield methods, such as Transient or Acoustic Radiation Force Elastography. Moreover, advanced serum markers (such as, for example, the Fibrotest-Actitest battery of tests) allow for an accurate non-invasive staging in hepatitis. The introduction of arterial uptake contrast-enhanced US and CT/RM techniques substantially decreased the role of biopsy in diagnosing liver biopsy [2–4].

However, histology remains one of the most accurate methods for evaluating liver parenchymal changes, and is always used in malignancies when the diagnosis is uncertain or when other non-invasive methods fail to provide an accurate staging for hepatitis. Along with these non-invasive techniques came a revolution in in-situ biopsy methods. Such is probe-based confocal laser endoscopy (pCLE), which uses miniaturized probes connected to a laser source through fiber optics, small enough to fit inside a biopsy needle, thus providing rapid live assessment of liver architecture [5].

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3. Modern liver biopsy techniques and sampling adequacy

3.1. Percutaneous biopsy

All modern percutaneous liver biopsy techniques have rapidity as a common denominator. Either cutting or suction needles can be used for transthoracic or subcostal biopsy, either after palpation or imaging assessment of the puncturing zone, or, preferably, under continuous image guidance. The transthoracic approach is the preferred method used, under real-time US or (more rarely) CT guidance and after a thorough imaging investigation of the liver and puncture route. All percutaneous methods imply two phases, one extra-hepatic corresponding to the needle puncturing the skin and reaching the needle, and a hepatic stage in which the needle passes the liver capsule, collects the parenchyma material, and is swiftly extracted. It is considered a relatively safe procedure, complication rates varying between studies, from 0.75% up to 13.6% [6].

Trucut needles and their modified versions driven by spring-loaded biopsy guns are increasingly used and are the instruments of choice in many centers worldwide, especially in Europe [7]. Needle diameters vary between 1.20 mm to 1.60 mm, smaller calibers being used when a high risk of complications is suspected.

Suction needles are less expensive and their operation allows for rapid intra-hepatic handling, thus being easier to use and possibly imply less bleeding-related complications. The most widespread types are the Menghini, Jamshidi and Klatskin needles, which remained virtually unchanged since their introduction in the second half of the last century. The maximum required time for a complete syringe suction of the cytological material and the consecutive needle retraction is 0.5 seconds. The intrahepatic phase is reduced to as low as 0.1 seconds when the needle is operated by an expert practitioner [8].

Image guidance has become mandatory in centers where the gastroenterologist can perform his or her own US exam. Real-time surveillance of the procedure greatly decreases the risk of complications (such as bleeding) and minimizes post-procedural complaints such as pain or hypotension. Hepatologists in the United States usually prefer to have a radiologist performing the procedure under CT or US guidance [8].

3.2. Transjugular (transvenous) biopsy

The transjugular route is preferred when the risk for complications is high and therefore a percutaneous approach is not considered safe enough for the patient. Patients with clinical ascites, known hemostatic defect, cirrhotic liver with clinical signs of organ deficiency (smaller size and increased palpatory stiffness) or morbid obesity are usually prime candidates for this approach. Another situation when the transvenous approach is preferred is when additional pressure measurements in the hepatic vein are required [8].

The resources needed for this procedure are higher than percutaneous approaches; however, complication rates are lower (2.5% up to 6.5%) according to some authors [9], with mortality rates of approximately 0.09% in high-risk patient groups [10]. The expertise of the

performing physician also plays a crucial role in the success rate of this procedure, and should be considered along with the higher resource costs when choosing this access route for a lower-risk patient [1].

Another very important aspect is the lower quality of the tissue specimens collected through the transjugular approach. The tissue cylinders are thinner and more fragmented than those obtained through percutaneous biopsy, and usually represent only 1-2 cm of the liver parenchyma, containing fewer portal fields [11].

3.3. Surgical or laparoscopic biopsy: Novel approaches for liver biopsy

This approach is preferred in patients with peritoneal involvement when an abdominal cancer is present, with associated ascites or peritoneal disease with ascites of suspected hepatic origin. Also, focal hepatic lesions can be targeted for biopsy through the laparoscopic channel.

Biopsy can thus be performed with either normal needle systems, or by wedge resection. However, the later approach may overestimate the level of fibrosis, as the resection is performed too close to the fibrotic capsule that envelops the liver. The procedure is always conducted under general anesthesia and requires controlled pneumoperitoneum by infusion of nitrous oxide, always performed by trained physicians, allowing for a good control of bleeding and a minimum set of complications due to the large working area created. In direct comparison with percutaneous biopsy, the laparoscopic approach provides a higher level of accuracy as it allows the evaluation of the surrounding peritoneum [12]. The main complications are related to the general anesthesia used for the procedure, the local abdominal and intra-peritoneal traumas associated, as well as the risk of bleeding, which is also present in the other types of biopsy.

Advancements to surgical techniques led to the development of the natural orifice transluminal endoscopic surgery (NOTES), a new surgically-derived endoscopic technique that uses a transgastric or transanal route to facilitate the access to the abdominal cavity. One recent study presented a liver biopsy performed through a transgastric flexible endoscopic device which permitted the inspection of the liver and surrounding intraperitoneal space. The technique can be applied to morbidly obese patients or to patients at high risk of complications [13]. This approach remains however limited at the present time to a few highly selected patients, and is performed only by trained surgeons and gastroenterologists, at moderate to high costs and in selected centers.

Recent studies also focused on evaluating the liver capsule in cirrhotic patients through pCLE inserted through a laparoscopic channel, this being a promising field in the advancement of minimally invasive biopsy techniques [14]. Another study describes the use of pCLE in a routine minilaparoscopy setting, performed under conscious sedation. The authors could describe subsurface serial images in real time, allowing for an in vivo analysis of the liver parenchyma [5]. This approach may lead the way to targeted biopsy through live assessment of the liver parenchyma, as well as immediate morphological and dynamic evaluation of intrahepatic structures.

3.4. Adequacy of liver biopsy samples

Analysis of the biopsy material under ultraviolet fluorescent light may be required in order to identify porphyria. Liver tissue obtained through biopsy is then quickly transferred into a buffer solution, usually 4% or 10% neutral formalin, to avoid the alterations it may sustain due to hepatic enzymes autolysis. It can then be subjected to various preparation techniques, in accordance to what diagnostic tests will follow with that specific sample (frozen section, RNA detection etc.) [1].

An adequate biopsy fragment is between 1 and 4 cm long, weighting between 10 to 50 mg, with a minimal diameter of 1 mm. Fragmented samples from Menghini needles are acceptable, as their added size is somewhere in the vicinity of 2 cm (usually range from 1 to 2.5 cm in length). In order to properly represent the parenchymal architecture, at least 10–11 portal tracts should be completely present, six being a minimally acceptable number. Specimens of inadequate lengths usually lead to understaging of fibrosis and underestimate the grade of inflammation. Cirrhotic parenchyma usually comes fragmented through biopsy, thus leading to approximately 20% sampling errors [15, 16].

As it is appreciated that a liver biopsy specimen represents 1/50 000 of the total organ mass, discussions regarding how representative it can be for diffuse lesions always existed in the literature [8, 17]. It is however appreciated that most diffuse (steatosis or inflammation etc.) or focal lesions (both malignant and benign), as well as structural lesions such as fibrosis can be visualized with a fairly high degree of accuracy, if the minimum amount of liver parenchyma and the required number of portal spaces are present. It was however demonstrated that the size of the sample is directly correlated to an underestimation of inflammatory changes [18], this paradigm being extended to fibrotic changes and has a direct effect on the subsequent grading and staging [1, 19, 20].

Another issue highly debated in literature is the inter-observer variability; even with the wide usage of quantification scores for both inflammation and fibrosis such as the Knodell [21] scoring system and the revised Ishak version [22] or the METAVIR score [23]. All interpretations are subjected to the experience and training of the pathologist, which is an independent variable in itself, separated from the inherent sampling and procedural errors. A second opinion is always recommended, and two pathologists are usually present in most large referral centers. Collaboration between the pathologist and the clinician performing the liver biopsy is also preferred, as some studies indicated [24–26].

The most important quantification parameters refer to its geometry and relationship between the principal compartments – portal tracts and the elements of the arterial vascular system; the configuration adopted by hepatocyte plates; the sinusoids and the perisinusoidal compartment; the amount of connective tissue, fat and the number of ducts present, as well as other normal cellular infiltrates of lymphoid origin [8]. Regenerative nodular hyperplasia or macronodular cirrhosis can be sometimes classified as normal parenchyma, and the inherent variations of normal inflammatory cellular infiltrate can be misleading for an inexperienced pathologist when observing low grade inflammatory lesions [8, 27].

4. Risks, complications and post-procedural complaints of liver biopsies

The main risks for a patient subjected to liver biopsy were already briefly discussed in the previous paragraphs. Their frequency and predisposition in certain patient groups are determinant factors for choosing one biopsy technique in favor of another. The risk of bleeding cannot be excluded with any instrument, and liver biopsy is not recommended in most cases of suspected primary liver cancers because of a needle track seeding of tumor cells. These however do not exclude liver biopsy as a last resort diagnostic tool, when imagistic or serum tests proved constantly inconclusive or do not converge to an outcome.

The most commonly occurring complication of percutaneous liver biopsy is pain, present in up to 84% of procedures and ranging from mild discomfort to severe pain [28]. It is usually located in the right upper quadrant and it is referred to the right shoulder, with various intensities and time of installment. Moderate to severe pain is present in fewer than 5% of all patients, and may be the sign of a more severe complication such as bleeding or the puncturing of the gallbladder [16, 29]. Mechanisms that lead to pain after the biopsy maneuver are not fully understood, however it is likely to be caused by bile or blood extravasation with subsequent capsule swelling (the only liver component with sensitive nervous terminations) [30]. Another cause of upper abdominal pain is the traction of the falciform ligament after the puncture. Cervical pain, as well as pain in the right shoulder, may also be caused by the irritation of the phrenic nerve. Subcapsular hematoma may lead to respiratory pain and irritation of the pleura or peritoneum may lead to vagal stimulation and consecutive vagal shock, manifested through bradycardia, severe hypotension, weak pulse and intense pain in the upper abdomen [1]. In some cases of extreme pain, hospitalization and further imaging tests are required to determine the correct course of action for these patients.

However, the most important complication of liver biopsy is bleeding. The most severe bleedings occur intraperitoneally, when they determine a drop in vital signs and can be visualized through imaging [16, 31]. Urgent hospitalization and blood transfusion, even followed by surgery or radiological intervention may be required. Nevertheless, these cases are scarce, with 1 in 2 500 up to 10 000 biopsies incidence, while less severe cases which do not require blood transfusions or surgical maneuvers are more frequent, approximately 1 in 500 biopsies [16]. Serious bleeding-related complications usually occur within 2 hours of the procedure, and over 90% of all bleedings become evident within 24 hours of the procedure. Clinical symptoms are revelatory, as patients experience hypotension and shock. Age and the underlying conditions also are predictive factors, as older patients and liver masses are more frequently associated with post-puncture bleeding. A correlation between the needle type and the risk for bleeding was also cited in literature, as cutting needle seem to pose an increased risk compared to their suction counterparts [15]. Other factors are related to operator experience, the diameter of the needles and their diameter [16].

A correlation between conventional coagulation tests and the risk of bleeding has not been sufficiently demonstrated until now; therefore no certain recommendations in this regard are currently in place [16]. The option to insert coagulation agents on the needle tract is considered, especially in the US, with no definite data on its ability to prevent possible bleed-

ings. As already mentioned, the transvenous approach is preferred in certain categories of patients as it is considered safer, even though several pooled analyses showed similar risks with standard percutaneous methods [10,16].

The singular major complication of liver biopsy, caused in turn by consecutive severe bleeding is patient death. No consistent data regarding post-procedural mortality exists in the literature, the most commonly quoted rate being less or equal to 1 in 10 000 biopsies [16], and seems to be greater after biopsies of malignant liver masses compared to diffuse parenchymal disease [6].

Other complications of liver biopsies include the perforation of other viscous organs, bile peritonitis (major complication which can result in death), infections (especially in post-transplant patients due to immunosuppressive medication), hemobilia, pneumothorax (instantly recognized on radiographs, essentially to diagnose quickly due to high risk of death) or hemothorax. Correct usage of imaging methods both when choosing the biopsy site and for surveillance of the procedure minimizes many of these risks, especially those related to puncturing adjacent structures [16]. The risk of needle track seeding when puncturing liver malignancies exists in 1 to 3% of all cases [32], as will be detailed below.

5. Current recommendations regarding conditions that require liver biopsy

The indications for liver biopsy were greatly reduced since the recent introduction of accurate non-invasive tests which can evaluate liver parenchyma with minimal or no patient trauma. The concept of liver biopsy may evolve even further, if in vivo direct histological methods such as pCLE will provide important additional data. It is most likely that the recommendations for liver biopsy will suffer further changes in following years. A series of these advancements will be discussed separately within this chapter. Below, we will describe some of the main indications for liver biopsy, either for diagnostic purposes or for evaluating and staging liver disease.

5.1. Grading and staging of chronic viral hepatitis

The recent outburst of viral hepatitis cases (especially as a result of the increasing number of newly diagnosed virus C infections) represents a major health burden worldwide. With almost four million people being infected in the United States alone, and between 130 and 170 million worldwide, chronic hepatitis C virus (HCV) infections and more than double those figures for hepatitis B virus (HBV) infections, this ensemble of viral diseases currently represent the main cause of liver-related morbidity [33, 34].

Nowadays, the role of liver histology in the positive diagnosis of chronic viral hepatitis has greatly diminished. However, it still plays a central role when assessing both activity and progression of the disease [8, 35]. Sampling issues arise when evaluating liver parenchyma affected by chronic hepatitis, as the quality of the obtained specimens can greatly influence

the semi-quantitative scores developed in the last four decades to quantify disease progression. There are a number of changes present within the liver and their heterogeneity makes the "10-complete portal spaces" paradigm essential when evaluating disease severity. All scoring systems are bound to yield significantly different results, primarily because of sample variability, but also as a result of the different levels of expertise from the pathologist involved in their evaluation. All modifications of the liver parenchyma – inflammation, necrosis or fibrosis – exhibit particularities and can be subjectively interpreted even in a scoring system [8].

The first approach to liver biopsy scoring for chronic hepatitis dates from the early 1980s when the histological activity index (HAI) was introduced by Knodell and Ishak [21]. This model did not clearly delimited between disease grades (that is, the importance of any inflammatory activity present) and stage, which refers to the degree of fibrosis and parenchymal remodeling. The later modification performed by Ishak resolves most of these issues and is currently used worldwide, partially replacing or at least complementing the earlier alternative Knodell classification. The preferred approach is a parallel evaluation using several scoring methods, such as the modified HAI, the Scheuer or the Ludwig systems and the Knodell classification, or the METAVIR algorithm devised in France [23].

5.2. Abnormal hepatic biochemical tests, alcoholic and non-alcoholic liver disease

Chronically elevated hepatic biochemical parameters are a common concern for many patients during routine screenings or general consults. Gastroenterologists facing abnormal aspartate aminotransferase/alanine aminotransferase, gamma-glutamyltransferase or alkaline phosphatase levels have to conduct a thorough anamnesis to determine the underlying condition. Many such patients either acknowledge high alcohol consumption or are diagnosed with non-alcoholic liver disease (NAFLD) associated with their lifestyle, while few remain undiagnosed until they begin to display signs of liver cirrhosis (cryptogenic cirrhosis or cirrhosis of unknown etiology). The latter two classes are usually diagnosed through liver biopsy, as no other condition can be found from either their background or non-invasive investigations and blood tests [8, 16].

The most common aspect revealed by liver biopsy in these patients is macrovesicularsteatosis, intracellular lipid accumulation exceeding 5% of the total cellular population. This macrosteatosis is generally coined as fatty liver disease (FLD) and can either be identified as either alcoholic liver disease (ALD), when regular alcohol consumption above established thresholds is established, or NAFLD when obesity, type 2 diabetes mellitus and/or hyperlipidemia are associated. Steatohepatitis, either of alcoholic origin (alcoholic steatohepatitis – ASH) or metabolic (non-alcoholic steatohepatitis – NASH) share histological similarities. NASH is recognized as a form of NAFLD with ballooning hepatocytes and necroinflammatory changes, as well as fibrosis and parenchymal remodeling. The NAFLD activity score (NAS) was developed in an attempt to objectively quantify the extension of this disease. This score sums the three pathologic features – steatosis, lobular inflammation and hepatocellular ballooning on a 0 to 8 scale, 5 being the cut-off point for a certain diagnose of NASH and 3–4 being labeled as borderline steatohepatitis [36, 37].

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Currently, even though liver biopsy is still regarded as the "gold standard" when diagnosing these conditions, no consensus has been reached. Liver biopsy remains therefore a controversial decision which ultimately has to be performed only when a clear diagnosis cannot be extracted from serum values, imagistic findings and clinical features [38].

5.3. Metabolic liver disease

Diseases that determine intrahepatic iron accumulation are the main indications for liver biopsy when a metabolic condition is suspected, besides NAFLD or ALD. Hereditary hemochromatosis, in its various forms identified today, is routinely diagnosed and staged through liver biopsy [8, 39]. The metabolic syndrome (syndrome X) represents the increased accumulation of iron within hepatocytes, in the context of NAFLD. These deposits are not distributed equally among various regions of the liver, therefore deeper biopsies are needed in order to collect more tissue for analysis [8, 40]. For this purpose, at least two scores are currently used – the Deugnier and the Brissot scores [41, 42]. The hepatic iron index is calculated through a mathematical formula which takes into account the hepatic iron concentration (evaluated by liver biopsy), its atomic weight as well as the age of the patients. An index above 1.9 is an indicator of hemochromatosis; however its sensitivity is low as it is dependent on the timing of the liver biopsy [8].

5.4. Focal liver lesions

Discovery of a focal liver lesions (FLL) can occur after imaging tests used routinely for either screening or diagnosis. The practitioner may encounter lesions of various sizes, number and location, some of them being associated with pre-existing conditions. This is especially the case of primary liver malignant tumors, either hepatocellular carcinoma (HCC) or cholangyocarcinoma (CC). Early discovery of a FLL is possible in up to 60% of all cases, especially in developed countries where surveillance programs are well established and health services are available to the majority of the population, irrespective of their location and economic status [43, 44].

Imaging alone is currently the main diagnostic procedure for HCC, as modern contrast-enhanced techniques, either by CT or MRI, are sufficient to highlight the hallmark pattern of tumor vascularization. Diagnostic criteria in the United States of America, Europe and Asia stipulate that imaging techniques are sufficient to diagnose the majority of HCC lesions, biopsy being reserved for the few situations where imaging is unclear, discordance between two methods exists, or tumor size does not allow a precise imaging diagnosis [43–45]. A defining criteria for evaluating FLLs is the presence of an underlying hepatic condition such as hepatitis or cirrhosis.

When HCC is suspected in cirrhotic patients, criteria for liver biopsy are set by the size of the tumor. In nodules between 1 and 2 centimeters, diagnosis should ideally be based on non-invasive criteria; however, confirmation through biopsy should be sought whenever possible. The evaluation should be performed ideally by a pathologist with extensive experience in evaluating liver biopsies. In case of inconclusive findings after the initial biopsy, a

second one should be performed if no other imaging criteria are present during the evaluation period. Nodules larger than 2 centimeters discovered through routine US should be ideally diagnosed through non-invasive procedures; however, when radiological findings are atypical, a liver biopsy should be obtained as confirmation [43–45]. A panel of immunohistochemical markers was proposed as diagnostic when evaluating liver biopsies for HCC. A combination of glypican 3, heat shock protein 70 and glutamine synthetase are recommended for the differential diagnosis between early HCC and high grade dysplastic nodules [46] (Di Tomaso et al, 2009). A final recommendation of the EASL-EORTC guidelines is that liver biopsy should be performed within controlled settings of scientific research, for identifying new markers for HCC and for tissue bio-banking[44].

The current tendency in diagnostic medicine is to avoid liver biopsy when evaluating HCC [44]. The main reasons against performing liver biopsy are the high rate of sampling errors which would diminish the sensitivity of the investigation; a higher rate of recurrence post-transplant in patients who underwent liver biopsy and finally the small but well-established risk of needle track seeding. In transplant referral centers, liver biopsy is performed more frequently, as there is an increased need for a correct final diagnosis; however, these procedures are subject to wide variation depending on country-specific regulations [43, 44]. Another argument for liver biopsy in HCC cases that benefit from chemotherapy would be the importance of histological grading. Response to local or systemic anti-angiogenic or anti-proliferative agents might be dictated by the microscopic configuration of the tumor and the amount of angiogenesis markers present on histological samples [16].

The second most important primary liver malignancy is CC. It can also develop in the presence of an underlying liver condition, such as chronic biliary tract diseases. Imaging diagnosis is sometimes difficult, as it may present similar contrast-enhancing patterns to those of HCC – the majority of CCs are solitary masses present in the hilum, while a minority can develop in other regions [43, 44]. Mixed forms of CC/HCC may also be present, their non-invasive diagnosis being even more difficult. All these forms of either atypical CCs or mixed presentations are usually subjected (with various degrees of variability, depending on setting and context) to liver biopsy. Surgical intervention, either by resection or liver transplant, are the approaches that yield the best survival chances for the patient. Therefore, liver biopsy may be indicated, as well as concomitant biopsy of lymph nodes in the upper abdominal area [16].

Metastases have the overall highest incidence amongst malignant liver lesions [47]. When a secondary malignant liver lesion is suspected and the physician cannot identify the primary point, liver biopsy is usually diagnostic, even when imaging fails to provide enough detail. If an underlying parenchymal disease is also suspected, biopsy should be performed outside the lesion site as well, for an extended and more precise diagnosis. A vast panel of markers may be employed in an immunohistochemistry study; however, the histologic architecture identified through normal techniques may be sufficient for an expert pathologist to determine the primary site of origin [1, 16].

Other rare primary liver parenchyma or bile duct malignant or benign neoplasms can ultimately be identified through histological analysis, after careful imaging-guided liver biop-

sy is performed. This diagnosis is often not possible on cross-sectional imaging studies as well as tumor serum markers, as their specificity for such lesions is inadequate. An expert hepatologist should closely collaborate with an experimented pathologist, as the diagnosis is difficult most of the times. These lesions may develop in the presence of an underlying liver condition, which would aid the clinical diagnosis or suspicion on the part of the clinician [1, 16].

The majority of lesions discovered through imaging techniques in patients without pre-existing liver conditions are benign in origin, mostly solitary or occasionally multiple. They exhibit particular vascular patterns in contrast-enhanced imaging techniques and are thus easily diagnosed without the use of invasive techniques. Such is the case of liver hemangiomas, mostly solitary benign tumors with characteristic contrast enhancement throughout all phases of an imaging investigation. Other lesions such as focal nodular hyperplasia are also usually solitary and may display distinct features such as "central scarring" or particular enhancement patterns (spiked wheel enhancement etc.). All these particularities have a morphological substrate: central hypoechoic areas which do not show vascular hyperenhancement usually correspond to areas of necrosis; intense signal enhancement zones are indicators of high microvessel density and neo-angiogenesis vessels; the US or CT peripheral rim translate in certain particularities of fibrous capsules [1, 16, 44].

Overall, lesions may present as cystic, solid or vascular; all these particularities usually being identified through non-invasive procedures prior to liver biopsy. In the USA for instance, liver biopsy is performed by imagists as they can perform the pre-biopsy or real-time assessment of the procedure, while in Europe most gastroenterologists or hepatologists perform the procedure themselves, under US surveillance [43, 44]. A core biopsy is usually preferred to fine-needle aspiration, as histology is considered superior from a diagnostic perspective compared to cytology; another reason being that experts in evaluating histology are more numerous compared to cytologists. The risk of puncturing blood vessels, either major arteries in the normal parenchyma, or intra-tumoral vessels is considerably diminished by real-time imaging guidance, for instance US with color Doppler. The risk of track seeding exists, even if extremely low (one study estimates a risk of 0.13%, while in other studies no such incidents were reported) [48, 49]. A certain dependency on the technique and size of the needle was also proven [50]. Infectious lesions may be biopsied; even if echinococcal cysts were considered an absolute contraindication as puncturing can be associated with anaphylactic shock and death, it was proven that these lesions can be aspirated with 19 or 22-gauge needles, taking all preparations for possible anaphylaxis [51].

6. Novel techniques in liver biopsy; modern non-invasive alternatives

6.1. Probe-based confocal laser endomicroscopy

The latest development in histological evaluation of gastrointestinal structures is confocal laser endomicroscopy. It allows for the in vivo evaluation of dysplasia and malignancies of the gastrointestinal tract, or in order to obtain directed biopsies that would allow rapid and

more precise diagnoses [52, 53]. The first embodiments of this technique required dedicated endoscopes to be used for evaluating cavitary structures accessible from both ends of the digestive tracts.

Recent advancements however were able to miniaturize the technology so the imaging microprobe can be connected to 30,000 fiber-optic threads that enable point-to-point real-time detection at 12 frames/sec. The imaging device by itself measures less than 1.5 millimeters in diameter, thus allowing its use through 19G or tru-cut biopsy needles, or insertion by laparoscopy or NOTES [53]. This technology will allow in vivo, real-time imaging of liver histology, technically enhancing the capabilities of liver biopsy [54]. A few studies on animal models exist in the literature, detailing pCLE use for liver histological imaging [14, 55, 56]. The technique can be used for assessing the state of hepatocytes and the morphology of the liver tissue, or can be limited to the study of the exterior liver capsule, yielding interesting preliminary results in the setting of cirrhosis. Mennone et al reported interesting results regarding a fibrotic pattern and collagen deposits in animal models with cirrhosis induced by bile duct ligation [14]. The technology shows promise and may someday allow for safer histological assessment of patients with chronic liver disease irrespective of its advancement, either cirrhotic or having any extreme complications, such as HCC.

6.2. Non-invasive imaging and serum tests for the assessment of fibrosis

Transient elastography (TE, Fibroscan® developed by Echosens, Paris, France) and Acoustic radiation force impulse (ARFI) are two ultrasound-based methods for quantifying liver fibrosis without the need for histological assessment. Another approach is through serum markers of fibrosis quantification, processed in complex mathematical formulas which give a quantitative result for liver stiffness, such as the Fibrotest, Biopredictive and the aspartate transaminase to platelets ratio index (ARPI) approaches.

TE is a novel and rapid non-invasive examination which involves minimal patient discomfort over a relatively low time period (one examination may take up to 5-10 minutes depending on the skeletal and adipose conformations of the patient). The device consists of a hand-held vibrating unit with an ultrasound transducer probe mounted on its axis, which generates medium amplitude vibrations at a low frequency, thus inducing an elastic shear wave in the underlying tissue. The hand-held probe is connected to a modified tower US machine which registers the result and through the on-screen software interface presents the user with an elastogram as a function of depth in time. The patient lies on his/her side and the probe is placed against the skin on the median clavicle line, directed towards the anatomical location of the liver, at a 90 degrees angle with the skin surface. Its results are presented as kilo Pascals (kPa), units of applied force. A series of 10 measurements are mediated to present a final value of the liver stiffness, which is equivalent to an F-stage fibrosis measurement obtained through biopsy [2].

ARFI is another technology that uses short-duration, high-intensity acoustic pulses which in turn exert mechanical excitation upon the tissues, generating local displacement resulting in shear waves. Their velocity can be assessed in a selected cylindrical area of interest of 0.5 cm

(length) x 0.4 cm (diameter), up to 5.5 cm below skin level. Its results are expressed as velocities, in m/s [4].

Fibrotest-Actitest (Biopredictive, France) is a serologic marker-based algorithm which represents an alternative to invasive biopsy techniques. It received clinical validation in patients with chronic hepatitis B and C, ALD and NAFLD. Fibrotest consists of a panel of markers designed for appreciating liver fibrosis: Gamma-glutamyltranspeptidase (GGT), Total bilirubin Alpha-2-macroglobulin, Haptoglobin, and Apolipoprotein A1. Necroinflammatory activity is appreciated through the Actitest component, which adds Alanine transaminase (ALT) to the above mentioned serum markers [3, 57]. All these tests are performed in validated laboratories due to their complexity and variability of their different components and their results are inserted in a complex mathematical formula through a web-based interface, the end-result being correlated with other quantitative score systems such as METAVIR, Knodell or Ishak [58].

The best results are provided by a combination of two or more non-invasive methods, one study in particular finding that Fibrotest and Fibroscan offers the best diagnosis performance compared to liber biopsy as a gold standard, at least for advanced fibrosis (F values beyond 2) or cirrhosis (F3 or F4) [2]. This conclusion was reached by another, more recent study performed by Boursier and his collaborators [59]. They diminish the number of patients who require liver biopsy, however, this procedure is not excluded in all cases. Some studies have shown a high variability between Fibroscan results, dependent of the body-mass index and population factors [60, 61]. A discordance between liver biopsy staging and the estimation provided by non-invasive methods has also been identified [34]. It was approximated that 30–40% of all patients investigated by a combination of non-invasive imagistic and marker-based methods still require liver biopsy, during either sequential or simultaneous protocols [60, 61].

7. Conclusion

Despite all its limitations and the advances in modern lesser invasive techniques, liver biopsy remains the gold standard for evaluating a wide array of liver diseases.

The main concern when turning to tissue sampling through biopsy is the risk/benefit ratio, the decision ultimately belonging to the clinician involved. The risks may at times be higher than the implied diagnostic outcome, in which case other methods are preferred for the diagnosis.

Currently, it is recommended that all interpretations should be based on proper tissue blocks, with the correct technique applied. It is preferred that more than one pathologist with extensive experience in liver pathology should formulate the final histological diagnosis. This is especially true for FLLs and liver malignancies, as benign features may at times overlap, making the diagnosis uncertain.

Modern imagistic techniques allow for precise non-invasive evaluation of liver fibrosis in the context of hepatitis; however, the correct methodology for interpreting these tests is yet to be established. Novel imagistic approaches may in time open new perspectives for liver biopsy, by providing in vivo, real time data on liver parenchymal features which would prove useful for accurate diagnosing of otherwise difficult to interpret pathologies.

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References

[1] Kuntz E, Kuntz H–D. Liver biopsy and laparoscopy. In: Hepatology: Textbook and Atlas. 3rd Edition (2008); pp 149–176. Springer, USA. 3540768386.

[2] Castéra L, Vergniol J, Foucher J et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C.Gastroenterology. 2005;128:343-50.

[3] Halfon P, Munteanu M, Poynard T. FibroTest-ActiTest as a non-invasive marker of liver fibrosis". GastroenterolClin Biol. 2008; 32: 22–39

[4] Crespo G, Fernández-Varo G, Mariño Z, et al. ARFI, FibroScan®, ELF, and their combinations in the assessment of liver fibrosis: A prospective study. J Hepatol. 2012;57:281-7.

[5] Goetz M, Kiesslich R, Dienes HP et al. In vivo confocal laser endomicroscopy of the human liver: a novel method for assessing liver microarchitecture in real time. Endoscopy. 2008;40:554-62.

[6] Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. Liver Int 2008, 28:705-12.

[7] Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344:495-500.

[8] Zimmermann A. Biopsy and laparoscopy. In: Textbook of hepatology: From Basic Science to Clinical Practice, 3rd Edition. Rodes J (Ed.). (2007); pp 489–99. Wiley-Blackwell. Massachusetts, USA. 978-1-4051-2741-7.

[9] Mammen T, Keshava SN, Eapen CE, Raghuram L, Moses V, Gopi K, Babu NS, Ramachandran J, Kurien G. Transjugular liver biopsy: a retrospective analysis of 601 cases. J VascIntervRadiol 2008, 19:351-8.

[10] Kalambokis G, Manousou P, Vibhakorn S, et al. Transjugular liver biopsy - Indications, adequacy, quality of specimens, and complications - A systematic review. J Hepatol 2007;47:284-294.

[11] Cholongitas E, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK. A systematic review of the quality of liver biopsy specimens. Am J ClinPathol 2006, 125:710-21.

[12] Denzer U, Arnoldy A, Kanzler S, et al. Prospective randomized comparison of minilaparoscopy and percutaneous liver biopsy: diagnosis of cirrhosis and complications. J ClinGastroenterol 2007;41:103-110.

[13] Steele K, Schweitzer MA, Lyn-Sue J, Kantsevoy SV. Flexible transgastricperitoneoscopy and liver biopsy: a feasibility study in human beings (with videos). GastrointestEndosc 2008;68:61-66.

[14] Mennone A, Nathanson MH. Needle-based confocal laser endomicroscopy to assess liver histology in vivo. GastrointestEndosc. 2011;73:338-44.

[15] Goessling W, Friedman FS (2006). Evaluation of the Liver Patient. In: The clinician's guide to liver disease, K. Rajender Reddy (Ed.),. SLACK Inc. USA: 1–31.

[16] Rockey DC, Caldwell SH, Goodman ZD et al. Liver biopsy. Hepatology. 2009;49:1017-44.

[17] Guido M, Rugge M. Liver biopsy sampling in chronic viral hepatitis. Semin Liver Dis. 2004;24: 89–97.

[18] 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, 239–244.

[19] Regev A, Berho M, Jeffers LJ et al. Sampling error and intra-observer variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol. 2002; 97, 2614–2618.

[20] Siddique I, El-Naga HA, Madda JP et al. Sampling variability on percutaneous liver biopsy in patients with chronic hepatitis C virus infection. Scand J Gastroenterol. 2003; 38, 427–432.

[21] Knodell RG, 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;1:431-435.

[22] Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.

[23] METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. Hepatology. 1994; 20, 15–20.

[24] Bejarano PA, Koehler A, Sherman KE. Second opinion pathology in liver biopsy interpretation. Am J Gastroenterol 2001;96:3158-3164.

[25] Tomaszewski JE, Bear HD, Connally JA, et al. Consensus conference on second opinions in diagnostic anatomic pathology. Who, what, and when. Am J ClinPathol 2000;114:329-335.

[26] Hahm GK, Niemann TH, Lucas JG, Frankel WL. The value of second opinion in gastrointestinal and liver pathology. Arch Pathol Lab Med 2001;125:736-739.

[27] Kay EW, O'Dowd J, Thomas R et al. Mild abnormalities in liver histology associated with chronic hepatitis: distinction from normal liver histology. J ClinPathol. 1997; 50, 929–931.

[28] Eisenberg E, Konopniki M, Veitsman E, Kramskay R, Gaitini D, Baruch Y. Prevalence and characteristics of pain induced by percutaneous liver biopsy. AnesthAnalg 2003;96:1392-1396.

[29] Janes CH, Lindor KD. Outcome of patients hospitalized for complications after outpatient liver biopsy. Ann Intern Med 1993;118:96-98.

[30] Caldwell SH. Controlling pain in liver biopsy, or "we will probably need to repeat the biopsy in a year or two to assess the response". Am J Gastroenterol 2001;96:1327-1329.

[31] Huang JF, Hsieh MY, Dai CY, Hou NJ, Lee LP, Lin ZY, et al. The incidence and risks of liver biopsy in non-cirrhotic patients: An evaluation of 3806 biopsies. Gut 2007;56:736-737.

[32] Yu SC, Lo DY, Ip CB, et al. Does percutaneous liver biopsy of hepatocellular carcinoma cause hematogenous dissemination? An in vivo study with quantitative assay of circulating tumor DNA using methylation-specific real-time polymerase chain reaction.AJR Am J Roentgenol. 2004;183(2):383-5.

[33] Lavanchy D. The global burden of hepatitis C. Liver Int. 2009;29 Suppl 1:74-81

[34] Kim SU, Kim JK, Park YN, Han K-H. Discordance between Liver Biopsy and FibroScan® in Assessing Liver Fibrosis in Chronic Hepatitis B: Risk Factors and Influence of Necroinflammation. PLoS ONE. 2012; 7(2): e32233.

[35] Gebo KA, Herlong HF, Torbenson MS et al. Role of liver biopsy in management of chronic hepatitis C – a systematic review. Hepatology, 2002; 36:S161–S172.

[36] Zafrani ES. Non-alcoholic fatty liver disease: an emerging pathological spectrum. Virchows Arch, 2004;444:3–12.

[37] Mendler MH, Kanel G, Govindarajan S. Proposal for a histological scoring and grading system for non-alcoholic fatty liver disease. Liver International. 2005; 25:294–304.

[38] Streba LAM, Cârstea D, Mitruţ P, Vere CC, Dragomir N, Streba CT. Nonalcoholic fatty liver disease and metabolic syndrome: a concise review. Rom J MorpholEmbryol 2008;49:13-20.

[39] Franchini M, Veneri D. Recent advances in hereditary hemochromatosis. Ann Hematol. 2005;84:347–352.

[40] Moirand R, Mendler MH, Guillygomarc'hA et al. Nonalcoholic steatohepatitis with iron: part of insulin resistance-associated hepatic iron overload? J Hepatol. 2000;33:1024–26.

[41] Deugnier YM, Turlin B, Powell LW et al. Differentiation between heterozygotes and homozygotes in genetic hemochromatosis by means of a histological hepatic iron index: a study of 192 cases. Hepatology. 1993;17:30–34.

[42] Brissot P, Bourel M, Herry D et al. Assessment of liver iron content in 271 patients: areevaluation of direct and indirect methods. Gastroenterology. 1981; 80:557–565.

[43] Bruix J, Sherman M. Management of Hepatocellular Carcinoma: An Update. Hepatology. 2011 53(3):1020-2.

[44] EASL-EORTC Clinical Practice Guidelines: Management of hepatocellular carcinoma. Journal of Hepatology 2012;56: 908-943.

[45] Omata M, Lesmana LA, Tateishi R, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int. 2010; 4:439-474.

[46] Di Tommaso L, Destro A, Seok JY, et al. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. J Hepatol. 2009;50:746-54.

[47] Kasper HU, Drebber U, Dries V, Dienes HP. [Liver metastases: incidence and histogenesis].Z Gastroenterol. 2005;43(10):1149-57.

[48] Tung WC, Huang YJ, Leung SW, Kuo FY, Tung HD, Wang JH, et al. Incidence of needle tract seeding and responses of soft tissue metastasis by hepatocellular carcinoma postradiotherapy. Liver Int 2007;27:192-200.

[49] Bialecki ES, Ezenekwe AM, Brunt EM, Collins BT, Ponder TB, Bieneman BK, et al. Comparison of liver biopsy and noninvasive methods for diagnosis of hepatocellular carcinoma. ClinGastroenterolHepatol 2006; 4:361-368.

[50] Maturen KE, Nghiem HV, Marrero JA, Hussain HK, Higgins EG, Fox GA, et al. Lack of tumor seeding of hepatocellular carcinoma after percutaneous needle biopsy using coaxial cutting needle technique. AJR Am J Roentgenol 2006;187:1184-1187.

[51] Khuroo MS, Wani NA, Javid G, Khan BA, Yattoo GN, Shah AH, et al. Percutaneous drainage compared with surgery for hepatic hydatid cysts. N Engl J Med 1997;337:881-887.

[52] Hsiung PL, Hardy J, Friedland S, et al. Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy. Nat Med. 2008;14:454–458.

[53] Hoffman A, Goetz M, Vieth M, et al. Confocal laser endomicroscopy: technical status and current indications. Endoscopy. 2006;38:1275–1283.

[54] Ray K. Imaging: confocal endomicroscopy enables deeper in vivo imagingof human liver. Nat Rev GastroenterolHepatol 7: 417, 2010.

[55] Becker V, Wallace MB, Fockens P, et al. Needle-based confocal endomicroscopyfor in vivo histology of intra-abdominal organs: first results ina porcine model (with videos). GastrointestEndosc. 2010;71: 1260–1266.

[56] Goetz M, Deris I, Vieth M et al. Near-infrared confocal imaging during minilaparoscopy:a novel rigid endomicroscope with increased imaging planedepth. J Hepatol. 2010; 53: 84–90.

[57] Ngo Y, Munteanu M, Messous D, et al. A prospective analysis of the prognostic value of biomarkers (FibroTest) in patients with chronic hepatitis C. Clin Chem. 2006; 52: 1887–96.

[58] Imbert-Bismut F, Messous D, Thibault V, et al. Intra-laboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and references ranges in healthy blood donors. ClinChem Lab Med. 2004;42: 323–333.

[59] Boursier J, Vergniol J, Sawadogo A, et al. Thecombination of a blood test and FibroScan improves the non-invasivediagnosis of liver fibrosis. Liver Int 2009;29:1507–1515.

[60] Kim SU, Choi GH, Han WK, et al.What are 'true normal' liver stiffness values using FibroScan?: a prospectivestudy in healthy living liver and kidney donors in South Korea.Liver Int 2010;30:268-274.

[61] Das K, Sarkar R, Ahmed SM, Mridha AR et al.''Normal'' liver stiffness measure (LSM) values are higher in both leanand obese individuals: a population-based study from a developing country.Hepatology. 2012;55:584-593.