POINT OF VIEW

Current indications of liver biopsy

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INTRODUCTION

Since the modifications introduced by Menghini in 1957 (1,2) regarding the technique for liver biopsy collection, the use of this procedure has become widespread, which has allowed a better understanding of the pathology and course of liver diseases, and the basing of our diagnoses on objective, pathologic grounds. Presently, liver biopsy is considered a procedure indicated: a) to establish the origin of abnormal liver tests; b) to assess activity extent and stage in chronic hepatitis; c) to evaluate alcohol-related liver disease; d) to study fever of unknown origin; e) in the diagnosis of multisystemic infiltrating and granulomatous diseases; f) to evaluate cholestatic conditions; g) in the diagnosis of neoplasm; h) in the assessment of drug-induced liver lesions; i) in the diagnosis of hereditary metabolic disease; j) in the assessment of response to therapy; k) in the evaluation of the liver following a liver transplantation; and l) to evaluate obscure jaundice, acute hepatitis, and hepatomegaly (3).

The etiologic identification of liver conditions using non-invasive methods has advanced a lot in recent years, and markers to reveal the extent and stage of liver disease have been sought. Such achievements have begun to question the absolute need for liver biopsy before treatment onset.

Indeed, most liver conditions are defined by histologic lesions; however, considering that the material obtained with this technique usually represents as little as 1/100,000 to 1/30,000 of the whole organ, representativity is arguable. Classic studies demonstrated that the information obtained from such biopsies was not reliable in the diagnosis of liver cirrhosis. For instance, Maharaj et al. (4) failed to identify this disease in 50% of patients. Abdi et al. (5) were more successful and recognized cirrhosis in 80% of cases. Poniachik et al. (6) histologically identified cirrhosis in only 68% of laparoscopic cirrhoses, and detected this disease in only 0.8% of cases overlooked by laparoscopy. The uncertainty of histologic diagnosis is also apparent when results obtained from two biopsies in one liver, one each from the right and left lobules, are compared, with 25-33% of cases showing relevant differences (7,8). A great part of diagnostic limitations regarding liver biopsy result from the small size of samples obtained by percutaneous puncture. Several studies demonstrating that diagnostic reliability depends on sample size have been reported in recent years. In a study including 161 liver biopsies, Colloredo et al. (9) found that the shorter or thinner the sample fragment, the more benign were inflammation extent and fibrosis stage interpreted to be. In the opinion of these authors, a correct assessment of histologic lesions required 11 portal spaces at least, and this was achieved only when biopsy samples were 2-cm long and 1.5-mm wide. Conclusions reached in the study by Badossa et al. (10) raised sample length to 2.5 cm, and Hohlund et al. (11) reduced it to 1.5 cm when using a 18g needle.

Difficulties derived from assessment subjectivity add to those mentioned on representativity. Various studies have shown great variability in lesion interpretation among pathologists (interobserver variability) and even in one single pathologist when assessing one sample at two different times (intraobserver variability). In a recent study, Petz et al. (12) showed that interobserver variability

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ty was 58%, and intraobserver variability was 56%. In 39-46% of cases, differences in activity extent reached 1 point, and two or more points in 12-17% of patients. Differences regarding stage assessment were 1 stage in 27-44% of patients, and 2 or more stages in 12-19% of patients. In the METAVIR study, samples were examined by 10 different pathologists, all of them experts in liver pathology, and while some lesions were assessed in a superimposable manner by all, other items revealed severe differences. These included erosive necrosis, activity, lobular necrosis, and Knodell index (13). In the study by Westin et al. (14), comparing the interpretations of lesions by three different pathologists, coincidences amounted to 84%; however, the authors were more tolerant in this study, and accepted differences up to 1 point as coincident diagnoses.

Obviously, liver biopsy is not a harmless test. It is usually painful (30%) and may originate serious complications (0.3%), including bleeding and biliary peritonitis, which may be even fatal (0.03%) (15,16). This precludes repeat procedures as frequently as desired to gain insight into disease progression or assess therapy response.

All the above questions the absolute value usually granted to liver biopsy in the diagnosis of liver disease, and hence the need for a risky, painful, anxiogenic, and costly test. Therefore, we should wonder whether this diagnosis may be reached without recourse to biopsy. Based on the study by Spycher et al. (17) we may answer that this is certainly possible on most occasions. In 365 patients these authors reported that biopsy confirmed the previous diagnosis in 84.4% of cases. Only in 6.8% of patients did biopsy demonstrate lesions other than those expected. Biopsy most commonly contributed to point at the nature of lesions (8.8%), besides the confirmation of suspected diagnoses, (8.8%) or to add further data (steatosis, siderosis, granulomas, etc.) of potential prognostic or therapeutic interest (10.5%). Similar conclusions were arrived at by other groups of authors (18-20). The presence of liver steatosis is a major factor in the progression of hepatitis C (21,22). Liver biopsy alone may assure on steatosis absence. Similarly, the presence of iron in the liver may only be detected with a liver biopsy. Its presence has been associated with disease progression and response to antiviral therapy (23,24). The frequency of previous diagnosis modifications by liver biopsy in this study is similar to that found by other authors (25-27) in their studies. Pre-biopsy diagnostic accuracy results from the fact that many tests are currently available for the diagnosis of liver disease with no need for biopsy. Such is the case of viral, autoimmune, and metabolic hepatitis, non-alcoholic fatty liver, primary biliary cirrhosis, primary sclerosing cholangitis, and commonly toxic-related liver disease. Interestingly, diseases unidentifiable without a biopsy procedure are those who less often may benefit from treatment (25).

Here I will review the need for liver biopsy in the assessment of patients with the three most common prob-

lems in our setting: a) viral hepatitis; b) alcohol-related liver disease; and c) non-alcoholic fatty liver.

LIVER BIOPSY FOR CHRONIC VIRAL LIVER DISEASE

Chronic hepatitis C

Arguments supporting liver biopsy in patients with chronic viral hepatitis include: a) liver biopsy allows a definitive diagnosis and rules out other conditions; b) assesses lesion significance and prognosis, and therapy urgency; and c) helps predict response to therapy. Several consensus meetings held during the late 1990s (26-31) recommended the routine performance of liver biopsy procedures before antiviral therapy onset. Such need was determined by a low response rate to antiviral therapy. However, ever since the conclusions of the first meeting were reported, voices questioning this need arose, and in the French Consensus Conference in 2002, as well as in the Guidelines issued by the American Association for the Study of Liver Diseases it was decided that liver biopsy was no mandatory requirement for treatment (32,33).

A relevant argument supporting liver biopsy in patients with chronic hepatitis C is that it allows the assessment of lesion significance, and hence disease prognosis and the urgency of therapy onset. No doubt, liver biopsy is the most effective means to assess the condition's process rate (necroinflammatory activity grade) and progression phase (stage). Biopsy is obviously warranted when decisive information regarding therapy may be obtained, which cannot be collected by other means. Treatment urgency and need will not be the same for advanced-stage disease (F3-F4) with severe necroinflammatory activity, and for minimal activity and fibrosis. The question is whether this information may be obtained without a biopsy. This is a topic that has drawn researchers' attention during the last decade. Patient symptoms and physical examination findings are of little value because they are only present when liver disease is already advanced. ALT will not reveal disease evolutionary stage; however, when elevated, it suggests a potentially progressive activity requiring treatment (34). In contrast, in patients with persistently normal ALT values lesions are considered usually as early, with no activity or tendency to progression (35-38). For such reasons, when ALT is elevated in patients with hepatitis C an antiviral therapy will be likely necessary regardless of histologic lesions. In contrast, when ALT is persistently normal biopsy usually reveals minimal changes (39) with little activity or progression (37,40-43), and thus both liver biopsy and treatment have been considered unnecessary. However, all studies show a small proportion of patients with normal ALT levels where biopsy reveals advanced lesions (34). Similarly, in some patients with elevated ALT (24.5%) (34) biopsy reveals scarcely evolved changes

(F0-F1), and antiviral therapy would be neither needed nor urgent. The study by Pradat et al. (34) found up to 13% of patients who despite showing normal transaminase levels had advanced fibrosis stages ($F \geq 2$) or even liver cirrhosis. These not always were inactive, scarring lesions, as even among those with an F1 stage 24% had moderate to severe necroinflammatory activity, and hence progressive disease requiring therapy. That is, if these patients had not undergone a biopsy because of their normal ALT, a significant amount of patients in need of treatment would have received none. In contrast, liver biopsy in patients with elevated ALT almost consistently showed $F \geq 1$ fibrosis stages, and $F \geq 2$ stages in 75% of cases. That is, in such cases patient treatment would be almost always warranted despite the absence of liver biopsy. The logical consequence of this study is that biopsy is likely not essential before treatment onset in patients with elevated ALT, but it is in patients with normal ALT values. If biopsy confirms that lesions and necroinflammatory activity are mild, therapy would not be required. On the contrary, therapy would be required for advanced stages and moderate to severe activity. Other studies advocate for treating patients with elevated transaminase levels even in the absence of an ensuing biopsy. In the study by Ghany et al. (44), ALT elevations beyond five times the upper normal limit were associated with a yearly fibrosis progression of 0.96 stages, which means that cirrhosis may develop in five years. Therefore, who may doubt that these patients should be treated, and that such decision may be made in the absence of a biopsy procedure? In the study by Marcellin et al. (45), this accelerated course of disease is also seen in patients with ALT values twice above normal.

The need for biopsy to decide therapy is based on its capacity to discern between different disease stages and the various inflammatory activity grades, mainly between F0-F1 and $F \geq 2$. Is such distinction possible in the absence of liver biopsy? When chronic hepatitis C is advanced and cirrhosis has already developed, laboratory parameters may probably show changes raising suspicion of this lesion. Such is the case with an AST/ALT ratio above 1 (40,45,46), and with decreased platelet rates or prothrombin activity (47), which in various studies predict the presence of liver cirrhosis. These are signs with low sensitivity even though their specificity may be high.

In the last few years special attention has been paid to the search for markers able to identify liver disease stage, and hence patients most in need for etiologic therapy. Various extracellular matrix breakdown or synthesis byproducts, and a number of enzymes involved in matrix metabolism have been assessed in this respect [laminin, procollagen type-III amino-terminal peptide (PIIINP), hyaluronic acid, collagen IV, metalloproteinases (MMP-1, MMP-2, MMP-9), tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2), etc.]. Amongst these, those with seemingly a higher predictive value for fibrosis include (SE, 10-88%; SP, 59-100%) and laminin (SE 57-80%; SP, 78-85%) (48). PIIINP sensitivity (SE) oscillat-

ed from 24 to 89%, and specificity (SP) from 38 to 88% (48). Despite this, results obtained with the use of single markers are not satisfactory, and thus marker combinations have been sought to better discriminate between the various stages of chronic hepatitis. Several markers have been proposed, and overall it may be concluded that these allow to adequately distinguish early from advanced stages in chronic hepatitis, but poorly discriminate between intermediate stages. For example, combining MMP-2, collagen IV 7S fragment, and hyaluronic acid may reveal absent or minimal fibrosis with an SE of 68% and SP of 73% (48). Multivariate models have allowed to recognize up to 94% of patients with liver cirrhosis (49). An age- and sex-adjusted index including α_2 -macroglobulin, haptoglobin, total bilirubin, GGT, and apolipoprotein A1 (*FibroTest*) [$4.467 \times \log \alpha_2$ -macroglobulin (g/L) - $1.357 \times \log$ haptoglobin (g/L) + $1.017 \times \log$ GGT (U/L) + $0.0281 \times \text{age (years)} + 1.737 \times \log$ bilirubin ($\mu\text{mol/L}$) - $1.184 \times \text{apolipoprotein A1 (g/L)} + 0.301 \times \text{sex (female = 0; male = 1)}$ - 5.540] (50) was highly capable in excluding moderate and severe fibrosis grades (F2, F3, F4) [negative predictive value (NPV) when lower than 0.31 was 91%], that is, patients requiring therapy. Similarly, when this index is above 0.72 said fibrosis grades may be predicted (PPV, 76%) with great accuracy. By adding serum ALT to *FibroTest* these same authors developed an additional index (*ActiTest*) for the recognition of necroinflammatory activity extent. When necroinflammatory activity is mild, this index is lower (0.26 ± 0.20) than for moderate (0.5 ± 0.24) or severe (0.59 ± 0.17) activity. *ActiTest* NPV and PPV are 85 and 77%, respectively, when cut-offs are 0.36 and 0.60, respectively (51). Forns et al. proposed another simple model that also allows the exclusion of advanced fibrosis stages. This model considers platelet count, blood GGT and cholesterol rates, and age [$7.811 - 3.131 \times \ln$ platelets ($10^9/\text{L}$) + $0.781 \times \ln$ GGT (U/L) + $3.467 \times \ln$ age (years) - 0.014 cholesterol (mg/dl)] (52). More recently the European Group for the Study of Liver Fibrosis developed another index also including age, hyaluronic acid, PIIINP, and TIMP-1, which has proven highly capable of discriminating liver fibrosis extent both in viral hepatitis and alcohol-related liver disease, as well as in non-alcoholic liver steatosis [$-0.014 \times \ln$ age (years) + $0.616 \times \ln$ hyaluronic acid (ng/ml) + $0.586 \times \ln$ PIIINP (ng/ml) + $0.472 \times \ln$ TIMP-1 (ng/dl) - 6.38]. NPV for indices lower than 0.063 in the exclusion of advanced fibrosis stages was 94.9%, and PPV for indices higher than 0.56 in the acknowledgment of advanced fibrosis was 83.3% (53). Finally, a system recognizing the amount of fibrous tissue based on organ rigidity (*Fibroscan*) was developed to identify liver fibrosis stage. Its usefulness has been recently assessed by two research teams (54,55), and it seemingly discriminates adequately between patients with and without cirrhosis. For rigidity values higher than 14.6, PPV is 78% and NPV is 97%. The optimum value to differentiate absent or mild fibrosis (F0, F1) from advanced fibrosis was 8.8,

and a PPV and NPV of 88% and 56% were obtained, respectively. In general, these methods manage to distinguish advanced from initial fibrosis stages, but discrimination between intermediate stages is poor. They are particularly unreliable to differentiate between stages F1 and F2, and hence to separate patients requiring treatment from those who do not need it. Castéra's group suggests that liver biopsy may be disregarded when FibroTest and Fibroscan results are in agreement (55). This situation develops in 70 to 80% of patients. In such cases, liver biopsy results are usually consistent with those obtained with these two noninvasive tests (55). Therefore, it may be too early to affirm that liver biopsy may be dispensed with in the workup of patients more in need of therapy; however, tests are being developed that bring hope that such identification will be possible in the forthcoming years.

Another argument for biopsy performance in patients with chronic hepatitis C is that it helps *predict whether patients will respond to antiviral therapy*. There is enough evidence to confirm that patients with more advanced lesions are less likely to respond to treatment when compared to patients with milder lesions (48). However, patients with advanced lesions are precisely those more in need for treatment, as they will more easily develop liver cirrhosis. Therefore, the decision to treat patients cannot be solely based on biopsy results. According to these, patients with early lesions should receive treatment given they are more likely to respond. Patients with advanced lesions should also be treated in view of their greater risk for liver cirrhosis.

From all the above, the indication of liver biopsy for hepatitis C should be currently applied on an individual basis. For instance, *biopsy may be disregarded*: a) in case of *patient refusal* (32,33). It is no longer acceptable that therapy is conditioned by biopsy results, and therefore treatment cannot be denied based on the availability of a histology study; b) in case of *treatment delay*. In such cases, biopsy, if needed, will be performed as close as possible to treatment onset; c) in patients infected by *genotypes 2 and 3*. In these patients treatment is mandatory no matter the lesion's nature, since probabilities for sustained viral response are in excess of 70%; d) when therapy indication is *not liver disease itself* [infected healthcare staff, infected mothers-to-be, cryoglobulinemia, HCV/HIV coinfection (32)]. In the latter case antiviral therapy is recommended regardless of liver lesions to prevent side-effects from dual anti-HCV and anti-HIV medication; e) *non-invasive tests clearly suggest lesion type*. For example, several tests are consistent regarding fibrosis stage assessment; and f) when *ALT is elevated*, mainly above five-fold the normal value. In contrast, liver biopsy is *indicated and of help* for decision making when: a) the patient is infected by *genotype-1* hepatitis C; b) when *ALT is normal* or scarcely elevated; c) when *non-invasive tests are inconclusive* regarding disease stage; d) the *patient wishes* treatment to be based on precise knowledge regarding lesion severity; e) in the pres-

ence of factors *predicting poor response to treatment* (very high viral load; obesity; insulin resistance); and f) *before treatment withdrawal* in a patient with no biopsy when tolerance is poor. In the latter two instances, treatment may be spared when lesions are mild. Obviously, when antiviral medications sufficiently effective against infection by HCV most resistant genotypes become available, liver biopsy indications for chronic hepatitis C will dramatically decrease.

Chronic hepatitis B

Liver biopsy has been recommended in chronic hepatitis B before antiviral therapy onset in order to confirm the chronic hepatitis B diagnosis, to identify other causes of liver disease, and to assess necroinflammatory activity grade and disease stage (56,57). The latter issues are of prognostic interest (58), may determine therapy, and ultimately may predict response to treatment (59). However, a chronic hepatitis B diagnosis may be reached in the absence of biopsy. Indeed, such diagnosis is warranted for patients remaining HBsAg-positive for more than six months, with high HBV-DNA levels [(above 10^5 copies/ml (20,000 IU/mL) or, under selected circumstances, 10^4 copies/ml (2000 IU/mL)] and inflammatory activity. Several studies have shown that such viral load allows differentiation between patients with inactive disease who do not require therapy, and patients with active disease in need of treatment (56,60,61). Serum ALT elevations are considered a marker of necroinflammatory activity (62). In this infection, liver biopsy should be warranted when the information it may provide may impact therapeutic decisions.

Chronic infection by HBV may impact patients in various ways, resulting in differing diagnostic and therapeutic attitudes.

In some cases patients are HBsAg carriers only. HBeAg is negative, anti-HBe antibodies are positive, HBV-DNA load is very low or non-detectable, and blood ALT levels are normal. These patients are *chronic, inactive HBsAg carriers*. In them, biopsy is not warranted and neither is treatment. In cases when these patients underwent a biopsy procedure, a lack of necroinflammatory activity was confirmed. Nothing but surveillance every 6 to 12 months should be attempted in these patients.

Other patients, besides being HBsAg-positive, are HBeAg carriers; blood HBV-DNA levels are fairly high ($>10^7$ - 10^{10} copies/ml or 2×10^6 to 2×10^9 IU/mL), and serum ALT levels are normal. These patients are *immunotolerant* to HBV. While some controversy exists on what should be done in such cases, it is considered that they should undergo neither biopsy nor treatment. Biopsy usually reveals the presence of minimal, if any, necroinflammatory activity (56,63), and antiviral therapy is commonly ineffective (59). These patients should be periodically followed every 6 to 12 months for loss of immunotolerance. This will be recognized from increased transaminase levels.

Some patients with active chronic hepatitis B are *HBeAg carriers*, and have negative anti-HBe antibodies, blood HBV-DNA levels higher than 10^5 copies/mL ($> 20,000$ IU/mL) and high transaminase titers. These patients would require antiviral therapy. However, response to therapy relates to blood ALT levels. For elevations smaller than 2 times the upper normal limit, response probabilities with HBeAg (+) to anti-HBe (+) seroconversion are low (5%). In contrast, when ALT rises 2- to 5-fold, seroconversion is reached in 26%, and for elevations in excess of 5 times the upper normal limit response rates go up to 64% of cases (59). Given this, antiviral therapy with no need for liver biopsy is warranted for ALT values above 2-fold the upper normal limit. If a biopsy is performed, it will confirm the presence of necroinflammatory activity, and the progressive nature of disease. In contrast, for ALT elevations smaller than 2-fold, response probabilities with HBeAg seroconversion are scarce, and indefinite antiviral therapy will likely be required. On the other hand, inflammatory activity is usually low. Hence, therapy should be warranted in terms of lesion progression before onset, and this can only be assessed with a biopsy. If biopsy reveals that grade and stage remain below 2, treatment can wait. If these histologic markers reveal an active, progressive disease, antiviral therapy is mandatory. Therefore, a biopsy should be performed for serum ALT levels smaller than 2-fold the upper limit of normal. Liver biopsy is not essential for values higher than this limit, and patients may be treated without it. If seroconversion is not achieved by treatment, a liver biopsy should be performed prior to indefinite maintenance, in order to assess whether lesion severity warrants it. Appropriate interpretation of such biopsies requires having in mind that antiviral therapy transiently improves lesions, even if it will not reach viral eradication (64).

A final group of patients with chronic hepatitis B lack HBeAg and carry anti-HBe antibodies, as do inactive HBsAg carriers, but blood HBV-DNA and ALT rates are high. Blood HBV-DNA levels associated with disease activity are usually lower than those seen in HBeAg (+) chronic hepatitis B (62). In such cases, cut-off viremia to discriminate active from inactive disease is 10^4 copies/mL (2000 IU/mL). In these chronic hepatitis forms, normal transaminase levels do not ensure disease inactivity, and thus ALT levels not always help assess what patients should receive treatment and what patients should not (62). However, if viremia is higher than 10^4 copies/mL (2000 IU/mL) and serum ALT levels are greater than 2-fold the upper limit of normal, disease may be presumed to be active, and patients should receive indefinite therapy with no need for liver biopsy. In contrast, if blood ALT titers are smaller than twice the normal value, or within the normal range, but viral load is greater than the aforementioned level, disease activity or progression cannot be excluded (65,66). A liver biopsy is required to assess this and then establish therapy needs. If

biopsy reveals moderate or severe activity or a stage equal to or greater than F2, indefinite treatment is indicated. Treatment withdrawal and the development of antiviral drug resistance are automatically followed by an increase first in viral load and then in transaminase levels (67).

Fibrosis stage recognition using non-invasive methods has also been attempted in chronic hepatitis B. Markers evaluated include the abovementioned FibroTest. When this test provides values below 0.20, severe fibrosis may be fairly safely excluded (NPV, 92%). In contrast, when FibroTest provides values above 0.80, advanced fibrosis is a sure issue (F2-F4) (PPV, 92%) (68). If such data are confirmed by other groups, and there is no particular interest in knowing hepatitis stage, liver biopsy could be dispensed with in nearly 50% of patients with chronic hepatitis B, and restricted to patients with FibroTest results between 0.20 and 0.80. Between these limits, this test cannot appropriately discriminate lesion stage.

To summarize liver biopsy indications in chronic hepatitis B, it may be said it is recommended when transaminase levels are low –below 2-fold normal– and viral load is greater than 10^5 copies/ml (20,000 IU/ml) if HBeAg is positive, and 10^4 copies/ml (2000 IU/ml) if HBeAg is negative. Biopsy is not required for inactive HBsAg carriers or immunotolerant individuals. Therapy may be initiated in chronic hepatitis patients –with either negative or positive HBeAg– without a previous liver biopsy when transaminase levels are high.

LIVER BIOPSY IN ALCOHOLIC LIVER DISEASE (ALD)

The need for liver biopsy in ALD has been based on its potential to establish a diagnosis, and to assess the presence of lesions with prognostic value.

Studies performed in the 1970s showed that, among drinkers with suspected ALD, liver biopsy revealed a failed diagnosis and causes other than alcohol for hepatic changes in 20% of patients (69). However, the study by Levin et al., including patients who drank less than 80 g alcohol/day, was performed when there were still no means for hepatitis C recognition, and included patients with transaminase levels that had not improved following withdrawal for several months. In 1988, Talley et al. found that pre-biopsy, clinical-laboratory diagnosis was correct for only 71% of alcoholics, with a specificity of 79% and a sensitivity of 90%. Nevertheless, errors would have been less common should laboratory workup have been more exhaustive (70). In 1989, Van Ness and Diehl (26) showed that sensitivity and PPV in the pre-biopsy diagnosis of ALD were 91 and 88%, respectively, whereas specificity and NPV were 96 and 97%, respectively. That is, according to the latter results, the presence or absence of ALD may be fairly certainly predicted based on clinical and laboratory criteria alone. However, while al-

coholic liver disease may be fairly confidently diagnosed without recourse to liver biopsy, the latter may be required for diagnostic certainty (26,71-73) in atypical clinical-laboratory profiles, when a parameter raises suspicion of a different etiologic factor, and when liver changes do not regress to normal after 3 to 6 months of alcohol withdrawal.

Liver biopsy is no doubt the most reliable test to identify lesions with prognostic value, specifically those of alcoholic hepatitis (AH). Sorensen et al. (74) found that the presence of AH, lipogranulomas, and Mallory bodies is of prognostic value and predicts the development of liver cirrhosis after 13 years in 50% of cases. Similarly, Marbet et al. (75) showed that AH severity, fibrosis stage, presence of sclerosing hyaline necrosis, and central vein sclerosis predicted progression to cirrhosis in upcoming years. The study by Chedid et al. (76), based on the analysis of 281 alcoholic subjects, found that 2-year survival was only 58% when biopsy revealed AH, 49% when there was liver cirrhosis, and 35% for concurrent cirrhosis and AH. In almost all studies AH is a first-order prognostic factor. However, the clinical-laboratory diagnosis of AH is unreliable. Various studies have shown this diagnosis to be correct in only 10 to 15% of patients (77,78). Difficulties in recognizing AH are particularly high in cirrhotic patients. In such cases, encephalopathy, ascites, reduced prothrombin activity, and increased bilirubin may result from cirrhosis, not AH. In addition, recognizing its presence is important to initiate specific treatments (79-81). Therefore, a diagnosis of AH most often requires a liver biopsy. However, many of these patients have severe coagulation disturbances, and hence biopsy may be particularly dangerous or contraindicated. In such cases recourse to a transjugular biopsy may be had. When using aspirating needles, the yield of this biopsy is lower when compared to modern, TruCut-like needles (82,83). Using the latter, average sample length is 1.2 cm (0.2-2.0 cm), and portal space count is 8.8 (1-21 portal spaces). Therefore, samples obtained through the transjugular route may be adequate for histologic assessment in 71% of patients. Also for AH non-invasive methods helping in the recognition of these lesions in patients with ALD have been sought. In 80 patients with liver cirrhosis and clinically suspected AH, Castéra et al. (84) found that laminin and collagen IV recognized the presence of this lesion with a sensitivity of 90 and 87%, respectively, and a specificity of 77 and 77%, respectively. This sensitivity and specificity of laminin regarding AH has been also confirmed by Annoni et al. (85). PIIINP and collagens I and III seem to be of lower diagnostic value regarding AH.

Liver biopsy is the safest test to assess ALD stage. However, non-invasive markers allowing the assessment of disease stage with no need for biopsy have also been searched in this condition. PIIINP, collagens I, III and IV, laminin, and YKN-40, a growth factor belonging to the bacterial chitins class, all correlate with fibrosis, inflammation and necrosis extent in ALD, and have been suggested as markers for fibrosis stage; however, their diag-

nostic utility has not been well assessed (86-91). YKN-40 and PIIINP are associated with the presence of liver fibrosis, but stage discrimination is poor. Despite this, increased serum levels for these markers are associated with poorer prognosis (90,91). Poynard et al. showed that prothrombin activity, GGT, and apolipoprotein A1 (*PGA index*) also fairly confidently predict cirrhosis in liver biopsy samples. They applied this index in 333 alcoholic patients, and showed that, when smaller than two, cirrhosis possibilities were nil, and normal or minimal-change liver possibilities were 86%. In contrast, when this index was above 9, cirrhosis probabilities were 86%, and normal or minimal-change liver probabilities were 0% (92). When used in patients with ALD, *FibroTest*, developed by this same team led by Poynard to assess liver fibrosis stage, showed a sound discrimination between F0-F1 patients and F2-F4 patients, and thus, in these authors' view, this test may contribute to a reduction in liver biopsy needs (93). An algorithm by Rosemberg et al. (53) also effectively discriminates between patients with mild fibrosis and patients with advanced fibrosis or cirrhosis. Sensitivity and NPV for this index in the recognition of F3 and F4 stages were both 100%.

From all the above, it may be concluded that liver biopsy is not essential in the diagnosis of ALD or the recognition of most advanced liver fibrosis stages, but it is for AH identification and when accurate data on disease stage are wanted.

LIVER BIOPSY IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Non-alcoholic fatty liver disease (NAFLD), a most common lesion in the West, represents a range of lesions from simple fatty liver to so-called non-alcoholic steatohepatitis (NASH).

Liver biopsy is considered the *gold standard* in the diagnosis of this condition; as the latter is defined by strong histologic criteria (liver steatosis, ballooning degeneration, inflammation in the absence of alcohol abuse) (94), liver biopsy allows to differentiate this liver disease from other liver conditions resulting in unexplained laboratory changes, and is the only test to separate simple fatty liver from NASH.

While ultrasonography has been known for years to identify liver steatosis (95,96), the usefulness of imaging techniques is limited. Saadeh et al. (97) showed that the minimum amount of liver steatosis detectable by ultrasonography or axial tomography was 33%. For such steatosis extent, these tests' sensitivity was 100% and 93%, respectively, and PPV was 62 and 76%. That is, these imaging techniques only detect moderate or severe fatty degeneration. Laboratory tests are also unreliable in the assessment of this condition (98). Its presence may be presumed in the face of abnormal liver tests with no other etiologic laboratory markers in a patient with metabolic

syndrome (obesity, type-II diabetes mellitus, hypertension, hypertriglyceridemia). In a study including 81 patients with no etiologic markers for liver disease, biopsy showed the presence of simple steatosis in 41 and of steatohepatitis in 26 (99). That is, most patients of this kind have NAFLD. These possibilities are even greater when ultrasonography shows a glossy liver. In such cases, biopsy is not needed to confirm the diagnosis of NAFLD. However, when suspected NASH is to be confirmed, having recourse to liver biopsy is mandatory. Ultrasonographic or radiographic signs of steatosis are absent for non-severe extents of fat deposition (100), and NAFLD may exist in the absence of metabolic syndrome signs. NAFLD is found in 2.4% of thin subjects (101).

Even when there is a fair number of patients in whom liver biopsy would be unnecessary to diagnose NAFLD, this procedure would certainly be warranted to *differentiate simple fatty liver from NASH*. This differentiation is relevant as fatty liver is considered a benign disease with a very low potential for progression, whereas NASH is more prone to progress to cirrhosis and result in complications. For instance, the study by Teli et al. (102) states that no patient with simple liver steatosis developed more severe lesions during 11.5 years on average. These same results were reported by Ratzius et al. (103). In contrast, NASH progresses to more advanced stages in 43% of patients and cirrhosis in 14%. Powell et al. (104) found that patients progressing to cirrhosis were those with fibrosis in their initial biopsy. In the study by Harrison et al. this progression was witnessed in one third of their patients (105). This prognostic meaning has been reported by other authors. In the study by Matteoni et al., after 10 years of follow-up liver cirrhosis was found in 4% when steatosis was the initial lesion, and in 20% when NASH was the initial lesion (106). To this date we have no reliable means to differentiate whether lesions restrict themselves to simple steatosis or there is already NASH in a patient with NAFLD. In the study by Saadeh et al. (97) none of the assessed imaging tests could differentiate this, and no NASH-defining histologic changes were identified using these techniques. Angulo et al. (107) also found no factors allowing to differentiate NASH from simple steatosis. In the study by Dixon et al. (108) more advanced NAFLD stages were found to be associated with higher insulin resistance indices, high blood pressure, and elevated ALT. More recently, Sumida et al. (109) showed that serum thioredoxin, a marker for oxidative stress, is significantly higher in patients with NASH (60.3 ng/ml) *versus* those with simple steatosis (24.6 ng/ml). However, the true diagnostic utility of this test remains to be revealed. Thus, even though biopsy may be in some cases unnecessary for the diagnosis of NASH, it is needed to assess whether a liver lesion corresponds to simple, unimportant steatosis or else consists of progressive NASH.

As with liver disease with other etiologies, prognosis is also defined by *stage*, which in turn depends on *liver fibrosis grade*. Biopsy is a definitive tool in defining NASH

stage. Means to obtain this information without having recourse to biopsy have been explored. However, not many are available, less so the useful ones. The study by Angulo et al. (107) reports that age, diabetes mellitus, and AST/ALT ratio predict severe fibrosis in patients with NASH; however, they do not tell patients with severe fibrosis from those with mild fibrosis. Even though the study by Sorbi et al. (98) reports that AST/ALT ratio increases with liver fibrosis extent in NASH, the study by Mofrad et al. (110) shows that many patients with advanced liver disease or cirrhosis exhibit normal transaminases. More recently, Lédíngheñ et al. (111) showed that advanced fibrosis grades were significantly associated with transaminase rates above two times normal. Fibrosis markers have also been used in NASH to assess disease stage (112,113). According to data provided by Sakugawa et al. (113), the combination of collagen-IV 7S domain (> 5.0 ng/ml) and hyaluronic acid (> 50 ng/ml) detects advanced fibrosis with a sensitivity of 54.2%, a specificity of 92.2%, a PPV of 83.9%, and a NPV of 72.8%. The algorithm suggested by Rosenberg et al. (53), which includes hyaluronic acid, PIIINP, TIMP-1, and age, allows to recognize these same stages in NASH with a sensitivity of 89%, a specificity of 96%, a PPV of 80%, and a NPV of 98%. If these results are confirmed, the need for liver biopsy in these patients would be questioned.

Those who question the need for liver biopsy in these patients, besides the already noted general reasons (pain, risk, concern about sample representativity), base their concerns in the scarce probabilities that biopsy results will force a therapy change. In most patients with hypertransaminasemia and no cause to warrant it, a manageable disease is rarely found (114). This is particularly true in NASH, as we lack therapies with proven efficacy for this condition. In the near future we may possibly have proven-efficacy therapies available; till then, we shall keep on recommending these patients just what we advised before biopsy availability: "alcohol withdrawal, weight loss, increased exercise, and avoidance of potentially hepatotoxic drugs" (98). The few therapeutic implications of biopsy results in NASH should be reported to patients before they accept the procedure.

To summarize the indication of liver biopsy in NAFLD, we must point out that no consensus has thus far been reached among those involved in this topic. It is widely accepted that biopsy is the surest test for NAFLD diagnosis, to differentiate simple fatty liver from NASH, and to establish disease stage. Therefore, biopsy cannot be disregarded in controlled studies where patient diagnosis must be assured. In clinical practice, biopsy indication will have to be individualized, and consider issues to be solved (115). Ramesh and Sanyal have suggested that biopsy should be performed in patients with abnormal transaminase levels of unknown etiology and no metabolic syndrome. For patients with metabolic syndrome biopsy will be necessary only if it may yield information allowing therapy changes (116). If in the future we have

effective, well tolerated therapies available that may be administered to all patients with this disease, biopsy will likely be unnecessary. If such therapies only benefit a particular patient subgroup, biopsy will be necessary to establish whether our patient belongs to the subgroup where therapy is indicated.

In summary, if we wish to have enough information to decide a treatment regimen as required by a particular patient, biopsy indication will have to be made on an individual basis, and be likely dispensed with on many occasions. If, on the contrary, we wish to assume no risks regarding our decision, and want to be absolutely sure about our diagnosis, know the presence of other factors associated with the primary etiologic factor (virus, fat, iron, etc.), or accurately establish liver lesion stage and necroinflammatory activity extent, there will be no choice but to obtain a liver biopsy sample with the appropriate length, thickness, and number of portal spaces. Currently available non-invasive tests are not sensitive enough to provide all these data.

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