Indications of Liver Biopsy in the Era of Noninvasive Assessment of Liver Fibrosis

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Background: Liver biopsy (LB) has been used as diagnostic modality in liver diseases (LD). Over last two decades, there has been remarkable improvement in understanding of natural history, molecular diagnostics of viral hepatitis, genetic of LD, and also limitations of LB. There is current trend in avoiding LB in the management of various LDs. **Aim:** To determine utility of LB in clinical practice. **Material and Methods:** In a prospective study, 2413 patients of LD were followed up, 219 (9%) were acute, and remaining 2194 (90.9%) were chronic LD. Patients were evaluated by biochemical parameters, virological studies, and imaging endoscopy as and when required. LB was performed in 176 (7.2%) patients when no conclusion could be drawn from the noninvasive workup. Patients with platelet count $<50,000/\text{cm}^3$, ascites, and overt bleeding were excluded. Patients with international normalization ratio (INR) more than 1.5 were not excluded. No prophylactic use of fresh frozen plasma and platelet transfusion was done. There was no major complication related to the procedure. Indications for LB were as follows: cryptogenic LD 38 cases, hepatitis B infection 35, suspected autoimmune hepatitis 30, mass lesion in the liver and lymphoma 29, evaluation of portal hypertension 15, elevated liver enzymes 11, hepatitis C infection 9, and drug-induced LD 4, and miscellaneous 5 cases which were primary biliary cholangitis, primary sclerosing cholangitis, cholestatic LD, sarcoidosis, and amyloidosis. **Results:** LB changed the diagnosis in 55 (31.2%). These were cryptogenic LD in 24 cases, portal hypertension 15, elevated liver enzymes 11, and 5 others. In remaining, LB confirmed clinical diagnosis and helped in making management decisions. **Conclusion:** LB was required in 7.2% of patients with chronic LD. In 31.2% cases, LB changed the diagnosis. LB was a safe procedure even in presence of low platelet count and abnormal INR. (J CLIN EXP HEPATOL 2015;5:314–319)

First percutaneous liver biopsy (LB) was done in 1883.¹ Since then, it has been used as a primary tool for diagnosis and staging of liver disease (LD). LB provides qualitative information regarding structural integrity of liver such as type and degree of liver injury, host response to injury, diagnosis, and classification of tumors which cannot be obtained by other means like serology or imaging. Percutaneous LB can be performed safely with various types of needles with or without imaging guidance. It can be performed by transjugular route or peritoneoscopically. The risk associated with the procedure is low and acceptable. Traditionally, LB has been utilized for diagnosis, structural damage to the liver, prognosis, and evaluation of treatment response.^{2,3} With increased understanding of new bio-chemical tests, molecular tests, and

serum markers of fibrosis, the need for LB has gone down drastically.^{4,5} Currently, diagnostic use of LB is limited to the diseases like autoimmune LD (AIH), granulomatous LD, when multiple etiologies are suspected, abnormal liver tests of unknown etiology, cryptogenic LD, severe portal hypertension with no liver dysfunction, and focal liver abnormalities on imaging.⁶ In structural alterations, necroinflammatory activity can be ascertained by biochemical parameters like liver enzymes, serum HBV DNA, HCV RNA levels, and serum IgG levels in AIH. Fibrosis can be ascertained by various commercially available tests (fibrotest, fibrosure, fibrometer, fibrospect II, ELF and hepascrope), aspartate transaminases to platelet ratio index and various imaging techniques, which are ultrasound based (transient elastography, Acoustic radiation force impulse elastography (ARFI), magnetic resonance elastography).^{7–11} Simple, inexpensive, and reliable noninvasive means to assess disease severity in chronic viral hepatitis are being explored. Among the routine laboratory parameters such as decreased platelet count, increased AST/ALT ratios, prolonged prothrombin time (PT) are the earliest indicators of cirrhosis. Serum markers of fibrosis like products of collagen synthesis, procollagen III, amino terminal peptide, extra-cellular matrix enzymes like metalloproteinase 1, hyluronic acid, and laminin have limited accuracy in predicting fibrosis.^{12,13} Combination of biochemical markers, alpha 2 macroglobulin, apolipoprotein A1, hepatoglobulin, GGTP and total bilirubin (fibrotest), and ALT (actitest) have been

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Abbreviations: AIH: autoimmune hepatitis; APRI: aspartate transaminases to platelet ratio index; CMV: cytomegalovirus; EBV: Epstein bar virus; HCC: hepatocellular carcinoma; HEV: hepatitis E virus; INCPH: idiopathic noncirrhotic portal hypertension; LB: liver biopsy; LD: liver disease; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; NRH: nodular regenerative hyperplasia
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shown to be accurate noninvasive markers in chronic viral hepatitis^{14,15}. If these findings are confirmed by large trials in various parts of the world, need for LB will go down. In spite of these advances, LB remains in perfect gold standard for assessing pre cirrhotic stage.

There are certain limitations for LB. It can be associated with abdominal pain in almost 30% patients while with severe complication, bleeding in 0.3% and death in 0.03% patients.^{6,16,17} Around 10–20% sampling errors are known in biopsy and there is 15–30% chance of underestimating cirrhosis in percutaneous biopsies. Intra- and inter-observer agreement is seen in 60–90% of cases.^{18,19} LB estimates the static mass of fibrosis and may not assess dynamic process of constant degradation, formation, and remodeling. Because of above-mentioned problems, there is reluctance in accepting LB by patients and many of the physicians. In spite of these limitations, the LB remains imperfect gold standard for assessing precirrhotic stage. To our knowledge, there is no single study in real life scenario which has assessed the need of LB. There has been lot of discussion about avoiding LB but still there is certain population of patients who need LB to ascertain etiology, grade of activity, progression, presence of other diseases, and risk factors for malignancy. Majority of studies on liver are based on individual disease group but the collective utility has not been studied. Hence, this study is planned to determine utility of LB in clinical practice.

MATERIAL AND METHODS

In a prospective, observational study, 2413 patients with suspected LD were seen at our center from January 2010 till 30 April 2012. Of these, 219 (9%) were acute while remaining 2194 were chronic LD. All patients were evaluated by biochemical parameters, virological studies, imaging, and endoscopy as and when required. Etiology of chronic hepatitis was nonalcoholic fatty LD 783 (35.6%), alcoholic LD 343 (15.6%), hepatitis B infection 341 (15.5%), hepatitis C infection 138 (6.2%), cryptogenic chronic LD 123 (5.6%), space occupying lesions of liver 108 (4.9%), autoimmune hepatitis 40 (1.8%), vascular disorders of liver 23 (1.04%), noncirrhotic portal hypertension 37 (1.6%), liver abscesses 58 (2.6%), drug-induced LD 29 (1.3%), infective hepatitis other than viral hepatitis 38 (1.7%), biliary diseases 55 (2.5%), hematological disorders involving liver 7 (0.3%), Wilson's disease 6 (0.2%), and miscellaneous 65 (2.9%) (Table 1). Of these, 11 patients fulfilled criteria of acute on chronic liver failure. There were 3 cases each of alcoholic cirrhosis, autoimmune hepatitis, and Wilson's disease. One case was of NASH and remaining were cases of drug-induced LD on background of NASH.

Liver cirrhosis was present in 508 (23.15%) cases of chronic LD. Etiology of cirrhosis was alcohol 179 (35.2%), HBV 64 (12.5%), HCV 74 (14.5%), autoimmune 18 (3.5%), nonalcoholic fatty LD 34 (6.6%), PBC 1 (0.5%),

Table 1 Etiology of Liver Disease (n = 2413).

Acute liver disease	219 (9%)
Chronic liver disease	2194 (90.9%)
• Nonalcoholic Fatty liver disease	783 (35.6%)
• Alcoholic liver disease	343 (15.6%)
• Hepatitis B infection	341 (15.5%)
• Hepatitis C infection	138 (6.2%)
• Cryptogenic chronic liver disease	123 (5.6%)
• Space occupying lesions of liver	108 (4.9%)
• Autoimmune hepatitis	40 (1.8%)
• Vascular disorders of liver	23 (1.04%)
• Non-cirrhotic portal hypertension	37 (1.6%)
• Liver abscesses	58 (2.6%)
• Drug induced liver disease	29 (1.3%)
• Infective hepatitis other than viral hepatitis	38 (1.7%)
• Biliary diseases	55 (2.5%)
• Hematological disorders involving liver	7 (0.3%)
• Wilson's disease	6 (0.2%)
• Miscellaneous	65 (2.9%)

hematological disorders 6 (1.1%), Wilson's disease 3 (0.5%), cryptogenic 117 (23%), and miscellaneous 12 (2.3%).

Etiology of acute LD was as follows: hepatitis A 19 (9%) cases, HEV 81 (37%), HBV 17 (8%), drugs 15 (6.9%), and others where etiology was not specific 87 (39%). Out of these 2413 patients, LB was performed in 176 patients (7.2%) when no conclusion could be drawn from noninvasive workup. It was performed with biopsy gun under ultrasound guidance. Patients with platelet count <50,000, with ascites, and overt bleeding were excluded. Patients with international normalization ratio (INR) more than 1.5 were not excluded. No prophylactic use of fresh frozen plasma and platelet transfusion was done. Thirty-eight (21.5%) patients had platelet count ranging from 50,000 to 100,000. Twenty-eight (15.9%) patients had INR more than 1.5 (range 1.6–4). There was no major complication related to the procedure.

RESULTS

From total 2413 cases, 1766 (73.1%) were males and 647 (26.8%) females with male:female ratio 2.5:1. Mean age was 45 ± 15.34 years. LB was performed in 176 (7.2%) cases. Indications for LB were: cryptogenic LD 38 cases, hepatitis B infection 35, suspected autoimmune hepatitis 30 cases, mass lesion in the liver and lymphoma 29, evaluation of portal hypertension 15, elevated liver enzymes 11, hepatitis C infection 9, and drug-induced LD 4 and miscellaneous 5 cases which were primary biliary cholangitis, primary sclerosing cholangitis, cholestatic LD, sarcoidosis, and amyloidosis (Table 2). LB changed the diagnosis in 55 (31.2%) cases. These included cryptogenic LD 24 cases, portal

Table 2 Indications for Liver Biopsy (n = 176).

• Cryptogenic liver disease	38
• Hepatitis B	35
• Autoimmune hepatitis	30
• Mass lesion in the liver and lymphoma	29
• Evaluation of noncirrhotics portal hypertension	15
• Elevated liver enzymes	11
• Hepatitis C	09
• Drug induced liver disease	04
• Miscellaneous—PSC, PBC, Cholestatic liver diseases, Sarcoidosis and Amyloidosis	05

hypertension 15, elevated liver enzymes 11 and 5 others consisting of cholestatic LD, amyloidosis, and myeloproliferative disorders. In remaining 121 cases, LB confirmed the clinical diagnosis and helped in the treatment (Table 3). From 38 cryptogenic LD, LB suggested diagnosis of autoimmune hepatitis in 13 cases, nonalcoholic steatohepatitis (NASH) 9 cases, and Wilson's disease in 2 cases. All 13 cases of autoimmune hepatitis were negative for autoimmune serology. Two patients with diagnosis of Wilson's disease had ceruloplasmin levels just less than lower limit of normal. In all 15 cases of portal hypertension, mean ARFI value was 1.60 ± 0.35 m/s. LB did not show evidence of cirrhosis in these cases. Liver histology was nonspecific or near normal and these were cases of idiopathic non-cirrhotic portal hypertension (INCPH). All 11 cases of elevated liver enzymes were labeled as steatohepatitis on histology which were subsequently found to be NASH.

In remaining 121 cases, LB confirmed the clinical diagnosis and helped in the treatment. There were no major complications noted. Transient abdominal pain was present in 35 (19.8%). Protracted pain >2 h requiring analgesic injection was seen in 9 (5.1%) cases. None of the cases had bleeding and there was no mortality.

DISCUSSION

Liver fibrosis is a dynamic process characterized by deposition of collagen. Accurate determination of fibrosis is

essential for assessing liver damage and managing treatment. Before 1970s, LB was used almost exclusively as diagnostic tool.^{2,3} Then serology became well-established method for diagnosis of viral hepatitis. Imaging such as abdominal ultrasonography and CT scan were established around 1980s as noninvasive methods for liver fibrosis.^{20,21} In 1990s, LB was still required in patients of HCV infection taking interferon therapy. By then, liver transplantation was also established.^{22–24} Currently, non-invasive tests which are simple to perform are widely available and reproducible have replaced LB.^{7,10–12,25} Ultrasound-based imaging can differentiate between fibrosis stages III–IV from 0 to II but cannot differentiate stage 0–II or between III and IV. It has been shown that stage 2 fibrosis has highest misclassification rate on noninvasive techniques.^{7,26,27} In spite of this till today LB is used in suspected cases of autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cholangitis, NASH, cryptogenic cirrhosis, infections such as CMV or EBV, and liver tumors not well defined by imaging techniques.⁶

With this reference, a study was planned to determine utility of LB in day-to-day hepatology practice. From 2413 patients of LD, 2194 were chronic LD and biopsy was performed in 176 (8%) cases. The most common indication for LB was cryptogenic LD seen in 38 (21.5%). In spite of recent advances in diagnostic technology, cryptogenic LD or cryptogenic cirrhosis remains common clinical dilemma in which etiology of LD is not known. Prevalence of cryptogenic cirrhosis varies from 5% to 30%.^{28–30} Determining etiology is challenging task. Neither imaging nor serology is helpful in such cases. LB is the only modality which can give some clue to etiology of LD. Factors that may be found on the LB which could affect the approach and management of these patients are severity of inflammation, degree of fibrosis, cholestasis, iron content, and few other additional features. Some of the possible etiologies for cryptogenic LD include occult viral hepatitis, chronic alcoholism, burnt out AIH, and NASH. Non B, non C hepatitis is an important cause of post-transfusion hepatitis which can remain silent for many years and present as cryptogenic cirrhosis.^{31–33} Clinicopathological correlation has shown that NASH and autoimmune hepatitis are leading causes of cryptogenic cirrhosis.^{34–37} Though asymptomatic autoimmune hepatitis is not known, previously unrecognized cirrhosis has been described, especially in patients with acute presentation of autoimmune hepatitis.³⁸ Positive antinuclear antibody is a feature of autoimmune hepatitis, however, it is a nonspecific finding and frequently seen in patients with NASH and even cryptogenic cirrhosis.^{39,40} Obesity and diabetes mellitus associated with NASH can be asymptomatic and can progress to cirrhosis. Loss of fatty infiltration has been described in patients of NASH progressing to cirrhosis. Hence, absence of typical histological features may lead to diagnosis of cryptogenic LD.

Table 3 Change in Diagnosis after Liver Biopsy.

Category where diagnosis was changed: 55 (31.2%) cases	
Cryptogenic	24 (63.1%)
Portal hypertension (noncirrhotic)	15 (100%)
Elevated liver enzyme	11 (100%)
Others (cholestatic liver disease, amyloidosis, myeloproliferative disorder)	05 (100%)

Portal inflammation, lymphocytes, plasma cells, few eosinophils, interface activity, and rosettoid arrangement of hepatocytes suggest diagnosis of autoimmune hepatitis. LB is useful in diagnosis, grading, and staging the disease. It helps to monitor treatment response in patients of autoimmune hepatitis. LB can also differentiate autoimmune hepatitis from DILI and helps in diagnosis of overlap syndrome.^{37,41–45}

The concept of NASH is given by pathologist Ludwig. He described LB features similar to alcoholic LD who do not consume excess alcohol.⁴⁶ The evolution of natural history of NASH progressing to cirrhosis and hepatocellular carcinoma (HCC) came into picture while evaluating liver biopsies. Liver ultrasonography is basic and simple modality for diagnosis of fatty LD but its sensitivity is low as it detects steatosis when >30% of hepatocytes are involved.⁴⁷ MRI has good sensitivity but cost and availability are its major restrictions.⁴⁸ Presence of inflammation and early fibrosis can be seen only on liver histology. Hence, LB is considered to be gold standard for diagnosis of NASH. It is the only mean to separate NASH from simple fatty liver.^{6,7}

In present study, from 38 (21.5%) cryptogenic LD, biopsy changed the diagnosis in 24 (63%). Of these, 13 were autoimmune hepatitis, 9 cases of NASH and 2 Wilson's disease. Another group of patients in which LB helped in diagnosis was elevated liver enzymes of unknown etiology. From 11 patients of elevated liver enzymes of unknown etiology, liver histology showed features of steatohepatitis with early fibrosis. It has been established that LB is indicated when clinical and serological data do not suggest increased transaminases or hepatomegaly. Steatohepatitis is the common cause for asymptomatic raised transaminases in serological marker negative patients. While evaluating these patients, almost 25% of them show presence of liver-related autoantibodies in their blood.^{39,40} At this situation LB is essential to differentiate NAFLD from autoimmune hepatitis.⁴⁹

LB was found to be most useful in cases of noncirrhotic portal hypertension. There were 15 cases with portal hypertension having normal liver function tests, workup for chronic LD was negative, and ultrasonography had suggested cirrhosis. All of them underwent LB which confirmed absence of cirrhosis. Various conditions leading to noncirrhotic portal hypertension include hepatic vein obstruction, portal vein obstruction, schistosomiasis, congenital hepatic fibrosis, biliary cirrhosis, venoocclusive disease, and nodular regenerative hyperplasia (NRH).⁵⁰ In India important cause of intrahepatic portal hypertension is INCPH.⁵¹ INCPH is also known to be common cause of cryptogenic intrahepatic portal hypertension in which liver function is well preserved. Incidence of INCPH in India ranges from 5% to 23% while in western countries it is 3–5%.⁵² Clinically, NCPH accounts for 10–30% of all cases of variceal bleed all over world including India.⁵³ On

abdominal ultrasonography, liver is nodular. Portal veins are patent and it mimics cirrhosis on CT scan. At present there is no single test that is diagnostic of INCPH. LB is the only means by which these can be differentiated. Liver histology features in these patients are nonspecific. They range from normal histology to portal fibrosis, phleboscrosis, and sinusoidal dilatation to NRH. LB helps to rule out cirrhosis as the cause of portal hypertension. It is essential to diagnose this condition since it has better outcome and prognosis than liver cirrhosis.

With availability of serological and noninvasive techniques, LB is not required for diagnosis of hepatitis B and C infection but is recommended if treatment decision is taken on the basis of fibrosis and fibroscan or biomarkers have equivocal results. LB is not mandatory before starting treatment in chronic HCV as highly effective treatment is available as contrast to this in chronic HBV infection where treatment may be life long, and liver histology may be of prime importance in patients who do not have clinically advanced disease.⁵⁴ In hepatitis B infection, LB may be of help in immune tolerant phase. It also identifies associated conditions such as steatosis, iron overload, or overlap syndromes.

In other LDs such as hemochromatosis and Wilson's disease, biopsy is required for diagnosis and assessment of iron and copper content in the liver respectively.^{55,56} Need for the LB for diagnosis of mass lesions in the liver cannot be undermined.^{57,58} For diagnosis of HCC, biopsy is not required when alpha feto protein and imaging techniques confirmed the diagnosis. In atypical lesions, biopsy is required for diagnosis of HCC.

It is general concept that liver cirrhosis is hypocoagulable state in which there is increased risk of bleeding. In order to assess bleeding risk, platelet count, PT, and INR are routinely done before LB. Patients are frequently subjected to transfusion of plasma or platelets before invasive procedure or LB. In fact, PT measures only procoagulant activity and fails to detect changes in anticoagulants. Thrombin generation time is also normal in patients with cirrhosis. There is no recommendation regarding prebiopsy testing for coagulation or use of prophylactic transfusion.⁶ It has been also proved in various studies that conventional tests for coagulation measure only procoagulant factors and do not estimate actual in vivo hemostatic balance.^{59,60} Tripodi et al.^{61,62} have emphasized that the concept of coagulation tests before performing LB is misleading. Abnormal bleeding after LB is a random event which can not be predicted by routine tests for coagulation. In cirrhosis, effective balance is maintained between procoagulants and anticoagulants. Decreased procoagulation is accompanied by reduced naturally occurring anticoagulants. In this study, none of the patients had bleeding nor did they receive plasma or platelet infusion. We have previously reviewed coagulation in decompensated liver cirrhosis and concluded that coagulation correction is not required prior to LB.⁶³

In conclusion, 7.2% of patients with chronic LD required LB. In 31.2% cases, LB changed the diagnosis. It was a safe procedure even in presence of low platelet and abnormal INR.

CONFLICTS OF INTEREST

The authors have none to declare.

REFERENCES

- Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med*. 2001;344:495–500.
- Sherlock S. Aspiration liver biopsy, technique and diagnostic application. *Lancet*. 1945;ii:397.
- Reuben A. Just a second. *Hepatology*. 2003;38:1316–1320.
- Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. *Hepatology*. 2006;43:S113–S120.
- Rockey DC. Non-invasive assessment of liver fibrosis and portal hypertension with transient elastography. *Gastroenterology*. 2008;134:8–14.
- Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology*. 2009;49:1017–1044.
- Bota S, Herkner H, Sporea I, Salzi P, Sirli R, Neghina AM. Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis. *Liver Int*. 2013;33:1138–1147.
- Fontana RJ, Lok ASE. Noninvasive monitoring of patients with chronic hepatitis C. *Hepatology*. 2002;36:S57–S64.
- Imbert-Bismut F, Ratzu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet*. 2001;357:1069–1075.
- Wai CT, Greenon JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38:518–526.
- Poynard T, Imbert-Bismut F, Ratzu V, et al. Biochemical markers of liver fibrosis in patients infected by hepatitis C virus: longitudinal validation in a randomized trial. *J Viral Hepat*. 2002;9:128–133.
- Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology*. 2004;127:1704–1713.
- El-Mezayen HA, Habib S, Marzok HF, Saad MH. Diagnostic performance of collagen IV and laminin for the prediction of fibrosis and cirrhosis in chronic hepatitis C patients: a multicenter study. *Eur J Gastroenterol Hepatol*. 2015;27:378–385.
- Salkic NN, Jovanovic P, Hauser G, Brcic M. FibroTest/Fibrosure for significant liver fibrosis and cirrhosis in chronic hepatitis B: a meta-analysis. *Am J Gastroenterol*. 2014;109:796–809.
- Anastasiou J, Alisa A, Virtue S, Portmann B, Murray-Lyon I, Williams R. Noninvasive markers of fibrosis and inflammation in clinical practice: prospective comparison with liver biopsy. *Eur J Gastroenterol Hepatol*. 2010;22:474–480.
- Westheim BH, Østensen AB, Aagenæs I, Sanengen T, Almaas R. Evaluation of risk factors for bleeding after liver biopsy in children. *J Pediatr Gastroenterol Nutr*. 2012;55:82–87.
- Kirnap M, Akdur A, Haberal Reyhan N, et al. Evaluation of safety and efficacy of liver biopsy following liver transplant. *Exp Clin Transplant*. 2015;13(Suppl. 1):312–314.
- Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol*. 2002;97:2614–2618.
- Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology*. 2003;38:1449–1457.
- Sanford NL, Walsh P, Matis C, Baddeley H, Powell LW. Is ultrasonography useful in the assessment of diffuse parenchymal liver disease. *Gastroenterology*. 1985;89:186–191.
- Harbin WP, Robert NJ, Ferrucci Jr JT. Diagnosis of cirrhosis based on regional changes in hepatic morphology: a radiological and pathological analysis. *Radiology*. 1980;135:273–283.
- Dienstag JL. The role of liver biopsy in chronic hepatitis C. *Hepatology*. 2002;36(5 Suppl. 1):S152–S160.
- Hübscher S. Diagnosis and grading of liver allograft rejection: a European perspective. *Transpl Proc*. 1996;28:504–507.
- Banff Working Group. Demetris AJ, Adeyi O, et al. Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology*. 2006;44:489–501.
- Tai DI, Tsay PK, Jeng WJ, et al. Differences in liver fibrosis between patients with chronic hepatitis B and C: evaluation by acoustic radiation force impulse measurements at 2 locations. *J Ultrasound Med*. 2015;34:813–821.
- Bacchetti P, Boylan R. Estimating complex multi-state misclassification rates for biopsy-measured liver fibrosis in patients with hepatitis C. *Int J Biostat*. 2009;5. <http://dx.doi.org/10.2202/1557-4679.1139> [Article 5].
- Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2014;40:1209–1222.
- Caldwell S. Cryptogenic cirrhosis: what are we missing. *Curr Gastroenterol Rep*. 2010;12:40–48.
- Caldwell SH, Oelsner DH, Iezzoni JC, Hespeneide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology*. 1999;29:664–669.
- Sanjeevi A, Lyden E, Sunderman B, Weseman R, Ashwathnarayan R, Mukherjee S. Outcomes of liver transplantation for cryptogenic cirrhosis: a single-center study of 71 patients. *Transpl Proc*. 2003;35:2977–2980.
- Yang X, Tang XP, Lei JH, Luo HY, Zhang YH. A novel stop codon mutation in HBsAg gene identified in a hepatitis B virus strain associated with cryptogenic cirrhosis. *World J Gastroenterol*. 2003;9:1516–1520.
- Anvari FA, Alavian SM, Norouzi M, Mahabadi M, Jazayeri SM. Prevalence and molecular analysis of occult hepatitis B virus infection isolated in a sample of cryptogenic cirrhosis patients in Iran. *Oman Med J*. 2014;29:92–96.
- Carreño V, Bartolomé J, Castillo I, Quiroga JA. Occult hepatitis B virus and hepatitis C virus infections. *Rev Med Virol*. 2008;18:139–157.
- Clark JM, Diehl AM. Nonalcoholic fatty liver disease: an under-recognized cause of cryptogenic cirrhosis. *JAMA*. 2003;289:3000–3004.
- Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology*. 2002;123:134–140.
- Gasser DJ, Garcia H, Tanaka K, Reinus JF. Corticosteroid-responsive cryptogenic chronic hepatitis: evidence for seronegative autoimmune hepatitis. *Dig Dis Sci*. 2007;52:2433–2437.
- Amarapurkar DN, Patel ND. Spectrum of autoimmune liver diseases in western India. *J Gastroenterol Hepatol*. 2007;22:2112–2117.
- Panayi V, Froud OJ, Vine L, et al. The natural history of autoimmune hepatitis presenting with jaundice. *Eur J Gastroenterol Hepatol*. 2014;26:640–645.
- Adams LA, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol*. 2004;99:1316–1320.
- Cho DH, Choi MS, Kim DH, et al. A prospective study on the prevalence and clinical significance of autoantibodies in patients with suspected nonalcoholic fatty liver disease. *Korean J Hepatol*. 2005;11:261–267.

41. Czaja AJ, Freese DK, American Association for the Study of Liver Disease. Diagnosis and treatment of autoimmune hepatitis. *Hepatology*. 2002;36:479–497.
42. de Boer YS, van Nieuwkerk CM, Witte BI, Mulder CJ, Bouma G, Bloemena E. Assessment of the histopathological key features in autoimmune hepatitis. *Histopathology*. 2015;66:351–362.
43. Czaja AJ. Autoimmune hepatitis—approach to diagnosis. *Med Gen Med*. 2006;23(8):55.
44. Lewis JH. Diagnosis: liver biopsy differentiates DILI from autoimmune hepatitis. *Nat Rev Gastroenterol Hepatol*. 2011;8:540–542.
45. deLemos AS, Foureau DM, Jacobs C, Ahrens W, Russo MW, Bonkovsky HL. Drug-induced liver injury with autoimmune features. *Semin Liver Dis*. 2014;34:194–204.
46. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo clinic experience with a hitherto unnamed disease. *Mayo Clin Proc*. 1980;55:434–438.
47. Bril F, Ortiz-Lopez C, Lomonaco R, et al. Clinical value of liver ultrasound for the diagnosis of nonalcoholic fatty liver disease in overweight and obese patients. *Liver Int*. 2015. <http://dx.doi.org/10.1111/liv.12840> [Epub ahead of print].
48. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol*. 2013;10:666–675.
49. Nalbantoglu IL, Brunt EM. Role of liver biopsy in nonalcoholic fatty liver disease. *World J Gastroenterol*. 2014;20:9026–9037.
50. Goel A, Madhu K, Zachariah U, et al. A study of aetiology of portal hypertension in adults (including the elderly) at a tertiary centre in southern India. *Indian J Med Res*. 2013;137:922–927.
51. Madhu K, Avinash B, Ramakrishna B, et al. Idiopathic non-cirrhotic intrahepatic portal hypertension: common cause of cryptogenic intrahepatic portal hypertension in a Southern Indian tertiary hospital. *Indian J Gastroenterol*. 2009;28:83–87.
52. Schouten JN, Garcia-Pagan JC, Valla DC, Janssen HL. Idiopathic noncirrhotic portal hypertension. *Hepatology*. 2011;54:1071–1081.
53. Sarin SK, Khanna R. Non-cirrhotic portal hypertension. *Clin Liver Dis*. 2014;18:451–476.
54. Amarapurkar D. Role of liver biopsy in management of chronic hepatitis B and chronic hepatitis C. *Trop Gastroenterol*. 2007;28:67–68.
55. Turlin B, Deugnier Y. Evaluation and interpretation of iron in the liver. *Semin Diagn Pathol*. 1998;15:237–245.
56. Stromeyer FW, Ishak KG. Histology of the liver in Wilson's disease: a study of 34 cases. *Am J Clin Pathol*. 1980;73:12–24.
57. Pittman ME, Brunt EM. Anatomic pathology of hepatocellular carcinoma: histopathology using classic and new diagnostic tools. *Clin Liver Dis*. 2015;19:239–259.
58. Kim H, Park YN. Role of biopsy sampling for diagnosis of early and progressed hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol*. 2014;28:813–829.
59. Segal JB, Dzik WH, Transfusion Medicine/Hemostasis Clinical Trials Network. Paucity of studies to support that abnormal coagulation test results predict bleeding in the setting of invasive procedures: an evidence-based review. *Transfusion*. 2005;45:1413–1425.
60. Caldwell SH, Hoffman M, Lisman T, et al. Coagulation disorders and hemostasis in liver disease: pathophysiology and critical assessment of current management. *Hepatology*. 2006;44:1039–1046.
61. Tripodi A, Caldwell SH, Hoffman M, Trotter JF, Sanyal AJ. Review article: the prothrombin time test as a measure of bleeding risk and prognosis in liver disease. *Aliment Pharmacol Ther*. 2007;26:141–148.
62. Tripodi A, Mannucci PM. Abnormalities of hemostasis in chronic liver disease: reappraisal of their clinical significance and need for clinical and laboratory research. *J Hepatol*. 2007;46:727–733.
63. Amarapurkar PD, Amarapurkar DN. Management of coagulopathy in patients with decompensated liver cirrhosis. *Int J Hepatol*. 2011;2011:695470.