Hepatology International (2019) 13:323–329
https://doi.org/10.1007/s12072-019-09945-4

ORIGINAL ARTICLE

Bi-lobar liver biopsy via EUS enhances the assessment of disease severity in patients with non-alcoholic steatohepatitis

Sandeep Khurana[1] · Waseem Butt[1] · Harshit S. Khara[1] · Amitpal S. Johal[1] · Sara F. West[1] · Zong-Ming E. Chen[2] · Andrea L. Berger[3] · David L. Diehl[1]

Received: 17 December 2018 / Accepted: 27 March 2019 / Published online: 16 April 2019

Abstract

Background In patients with non-alcoholic fatty liver disease (NAFLD), all-cause mortality increases with fibrosis stage. Liver biopsy (LB), performed predominantly in the right lobe, assesses fibrosis, however, right lobe LB may not be sufficient due to histological variation in different lobes. Endoscopic ultrasound (EUS) allows for biopsy of right and left liver lobes in the same setting.

Methods This retrospective study assessed for histologic variability amongst left and right liver lobe (L:R) specimens obtained via EUS at a tertiary care center. Between January 2012 and December 2015, 38 NAFLD patients underwent LB, in whom both lobes were sampled.

Results L:R agreement was near-perfect for steatosis ($\kappa = 0.816$, 95% CI 0.674, 0.958), good for ballooning ($\kappa = 0.740$, 95% CI 0.565, 0.916) and moderate for lobular inflammation ($\kappa = 0.401$ 95% CI 0.110, 0.692) and fibrosis ($\kappa = 0.473$, 95% CI 0.275, 0.672). Intra-observer variability assessed by blinded repeat slide readings was almost perfect for fibrosis and steatosis ($\kappa = 1$, 95% CI 1, 1 and $\kappa = 0.939$, 95% CI 0.881, 0.997 respectively) and substantial for lobular inflammation ($\kappa = 0.725$, 95% CI 0.584, 0.866). Only right lobe assessment underestimated fibrosis in 21%, inflammation in 13%, and steatosis and ballooning in 8% cases.

Conclusions These data indicate that in NAFLD, due to regional variation, EUS-guided bi-lobar LB improves assessment of disease activity and fibrosis.

Keywords Fatty liver · Endoscopic ultrasound · Liver biopsy · Histology · NAS score

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver disease in the western world. The spectrum of NAFLD includes steatosis alone,

Sandeep Khurana and Waseem Butt contributed equally.

✉ Sandeep Khurana
skhurana@geisinger.edu

Waseem Butt
wbutt@geisinger.edu

Harshit S. Khara
hskhara@geisinger.edu

Amitpal S. Johal
asjohal@geisinger.edu

Sara F. West
sfwest@geisinger.edu

Zong-Ming E. Chen
zechen@geisinger.edu

Andrea L. Berger
alberger@geisinger.edu

David L. Diehl
dldiehl@geisinger.edu

1 Department of Gastroenterology and Hepatology, Geisinger Medical Center, 100 N Academy Ave, Danville, PA 18702, USA

2 Department of Pathology, Geisinger Medical Center, Danville, PA, USA

3 Department of Biostatistics, Geisinger Medical Center, Danville, PA, USA

nonalcoholic steatohepatitis (NASH) and cirrhosis. The overall prevalence of NAFLD in United States in the general population is 10–46% and that of NASH is approximately 3–5% [1-3]. The diagnosis of NAFLD is suggested by the combination of a bright (hyperechoic) liver appearance on abdominal ultrasound, absence of heavy alcohol use, elevated serum aminotransferase levels and no other concomitant liver disease. The identification and assessment of its more severe form, NASH, may still require a liver biopsy [4].

There are several modalities available for obtaining liver tissue. The percutaneous route involves passing a biopsy needle either blindly (by percussion of liver span) or under image guidance via transabdominal ultrasound or CT scan [5]. A transjugular approach may be favored if the patient has coagulopathy, more than a trace of ascites, or if concomitant portal pressure measurements are required [6]. A laparoscopic approach has also been described, and is particularly valuable if done as part of a laparoscopic bariatric surgery [7]. Recently, it has been shown that endoscopic ultrasound-guided liver biopsy (EUS–LB) is safe, with tissue yields either equal or superior than percutaneous or transjugular routes [8, 9].

Several studies suggested that sampling variability may be a concern in the histological evaluation of various liver diseases [10-18]. Regarding NASH, regional variation in histological findings has been noted in some studies [19-22]. In clinical practice, most techniques to obtain liver tissue are via biopsy of the right lobe. Almost all ultrasound- or CT-guided liver biopsies are performed on the right lobe. Vast majority of liver biopsies obtained via transjugular route also sample the right lobe. Recent studies have indicated that in NAFLD, histological assessment of disease activity can predict prognosis. For example, two studies have shown that increase in fibrosis stage is associated with increase in all-cause and liver disease-related mortality [23, 24]. Therefore, accurate assessment of the disease activity, especially fibrosis, is key in assessing prognosis. In this regard, biopsies of more than one lobe of the liver are likely to enhance assessment of disease activity. EUS–LB can provide an opportunity to sample both right and left lobes of liver easily. Therefore, to determine the histological variation in disease activity in NASH patients, we compared left and right liver samples obtained via EUS–LB for steatosis, lobular inflammation, hepatocellular ballooning and fibrosis using NAS scoring criteria.

Methods

This retrospective study was approved by the Institutional Review Board. Between January 2012 and December 2015, 268 EUS–LBs were performed at our center, of which 43 patients were suspected to have NAFLD. Prior to biopsy, these patients were evaluated to exclude other etiologies including alcohol, viral hepatitis, iron overload, autoimmune, Wilson's disease and alpha-1 antitrypsin deficiency. Five patients were excluded from this analysis as these patients either did not have both left and right hepatic lobe sampling performed or had NAFLD activity score (NAS) < 3. This resulted in 38 patients for analysis.

EUS examination was performed in the left lateral decubitus position with a linear echoendoscope (GF-UC140P or GF-UCT180, Olympus America, Center Valley, Pennsylvania). Each patient underwent EUS–LB of both left and right lobes of liver with a 19-gauge EUS fine needle aspiration needle (19 g Expect, or 19 g Expect Flexible, Boston Scientific, Marlborough, MA). The left lobe was defined as that part of the liver seen by EUS from the upper stomach. The right lobe was defined as that part of the liver that is seen by EUS through the duodenal bulb. Liver cores obtained with EUS–LB were placed into formalin and transported to the surgical pathology laboratory for processing. Each biopsy was processed in the standard fashion and included a hematoxylin–eosin (H&E) stain, reticulin stain and Masson trichrome stain. Special stains, such as iron, rhodanine, and others, were ordered as needed.

The glass slides from the identified cases were pulled and "blinded" by affixing a piece of opaque tape over identifying information. The slides were then numbered in a random fashion. A single experienced gastrointestinal pathologist examined the liver biopsy slides twice, 4 weeks apart, in a blinded fashion. Assessment for steatosis, lobular inflammation, hepatocellular ballooning and fibrosis were made for each biopsy specimen. NAFLD activity score (NAS) was calculated by assigning points for each of these histologic features in a standard approach [22]. The slides were also quantitated for specimen length and number of complete portal triads.

38 patients had NAFLD activity scores (NAS) greater than or equal to 3 and readings from both the right and left lobes. For ten patients, the pathologist read more than one slide from a patient's left side or more than one slide from a patient's right slide. In these cases, a simple random sample of the slides was taken so that one slide from the right and one slide from the left was used for analysis.

Statistical analysis

Thirty-eight patients with a NAFLD activity score (NAS) greater than or equal to 3 were included in the analysis (a NAS score of 0–2 is not diagnostic of NASH, 3–4 is indeterminate and ≥ 5 is diagnostic of NASH). The agreement between left and right lobe individual NAS components was measured using Cohen's simple kappa coefficient and Cohen's weighted kappa coefficient, where kappa (κ)

of one indicates perfect agreement. $\kappa = 0.81$–1.00, almost perfect; $\kappa = 0.61$–0.80, substantial; $\kappa = 0.41$–0.60, moderate; $\kappa = 0.21$–0.40, fair; and $\kappa = 0.00$–0.20 indicates slight agreement [25].

Results

Demographic data for the 38 patients is provided in Table 1. The mean patient age was 50 years, 23 patients (60.5%) were female. Mean BMI was 34.6. Regarding risk factors for NASH, 20 patients (52.6%) were hypertensive, 12 patients (31.6%) were diabetic and 22 patients (57.9%) had hyperlipidemia. The median ALT was 74 U/L normal range (10–50), median AST was 65 U/L normal range (10–50). Total length of biopsy specimens was 12–133 mm mean (30.1 mm for left and 23.2 mm for right); complete portal triad counts were 5–68 mean (12.7) for left and 6–29 mean (8.7) for the right. All specimens had adequate sample for histological diagnosis and assessment of fibrosis. See Fig. 1.

Table 1 Demographic and Lab Data

Characteristic	Values
Age, mean (SD)	50.0 (11.3)
Sex, *n* (%)	
Female	23 (60.5%)
Male	15 (39.5%)
Race, *n* (%) White	38 (100.0%)
Ethnicity, *n* (%)	
Hispanic/Latino	2 (5.3%)
Not Hispanic/Latino	36 (94.7%)
BMI,* mean (SD)	34.6 (7.5)
Comorbidities, *n* (%)	
Hypertension	20 (52.6%)
Diabetes Mellitus	12 (31.6%)
Hyperlipidemia	22 (57.9%)
Laboratory parameters	
HbA1C,* mean (SD)	6.4 (1.3)
ALT,* median (IQR)	74 (52, 119)
AST,* median (IQR)	65 (45, 105)
Cholesterol, mean (SD)	206.9 (51.5)
Direct Bilirubin, median (IQR)	0.2 (0.1, 0.2)
Glucose, median (IQR)	109 (97, 166)
HDL,* mean (SD)	47.3 (16.6)
LDL,* mean (SD)	119.7 (48.8)
Total bilirubin, median (IQR)	0.5 (0.3, 0.7)
Triglycerides, mean (SD)	47.3 (16.6)

BMI body mass index, *HbA1C* hemoglobin A1C, *LT* alanine aminotransferase, *AST* aspartate aminotransferase, *HDL* high density lipoprotein, *LDL* low density lipoprotein

Agreement between right and left lobes was substantial for steatosis weighted ($\kappa = 0.816$, 95% CI 0.674, 0.958) and ballooning weighted ($\kappa = 0.740$, 95% CI 0.565, 0.916), moderate for lobular inflammation weighted ($\kappa = 0.401$, 95% CI 0.110, 0.692) and fibrosis weighted ($\kappa = 0.473$, 95% CI 0.275, 0.672). The agreement between left and right NAS scores was also moderate weighted ($\kappa = 0.606$, 95% CI 0.394, 0.818). Both simple and weighted κ values are shown in Table 2.

Intra-observer agreement for two readings of the same slides 4 weeks apart was strong for steatosis weighted ($\kappa = 0.939$, 95% CI 0.881, 0.997), lobular inflammation weighted ($\kappa = 0.725$, 95% CI 0.584, 0.866) and fibrosis weighted ($\kappa = 1.000$, 95% CI 1.000, 1.000) but not for ballooning weighted ($\kappa = 0.609$, 95% CI 0.451, 0.768). The agreement between first and second readings of NAS scores was also moderate weighted ($\kappa = 0.695$, 95% CI 0.563, 0.828). Both simple and weighted κ values are shown in Table 3.

Tables 4, 5 indicate that the probability of agreement between both lobes for steatosis was 84%, for inflammation 74%, ballooning 82%, and NAS score 74%. Fibrosis had a 63% probability of agreement. However, examination of left liver biopsy specimen enhanced the steatosis score in three cases, inflammation score in five cases, ballooning in three cases and overall NAS score in four cases. Very importantly, examination of left liver specimen enhanced the fibrosis stage in eight cases.

Discussion

Our data indicate that in patients with NAFLD, (a) bi-lobar liver biopsies enhance the assessment of disease activity, especially fibrosis—the key indicator of prognosis, (b) there is significant variation amongst the two lobes for assessment of inflammation and fibrosis, which underscores the importance of sampling both lobes for histological assessment instead of just the right lobe, and (c) EUS guidance provides means to sample both liver lobes at the same setting and thus can be readily applied in clinical practice.

Although NASH is suspected based on fatty liver on imaging and elevated aminotransferases in the absence of significant alcohol use or other chronic liver disease, for diagnosis, liver biopsy remains the gold standard. Analysis of liver biopsies in various chronic liver disease states has shown that regional variation in disease severity is a concern in interpreting results. For example, Skripenova et al. [17] and Siddique et al. [18] showed that histological variation in grading and staging exists in chronic hepatitis C. Similarly, a case report showed variability in disease distribution in Wilson's disease with more copper accumulation in the right than the left lobe of liver [26]. Another study highlighted the

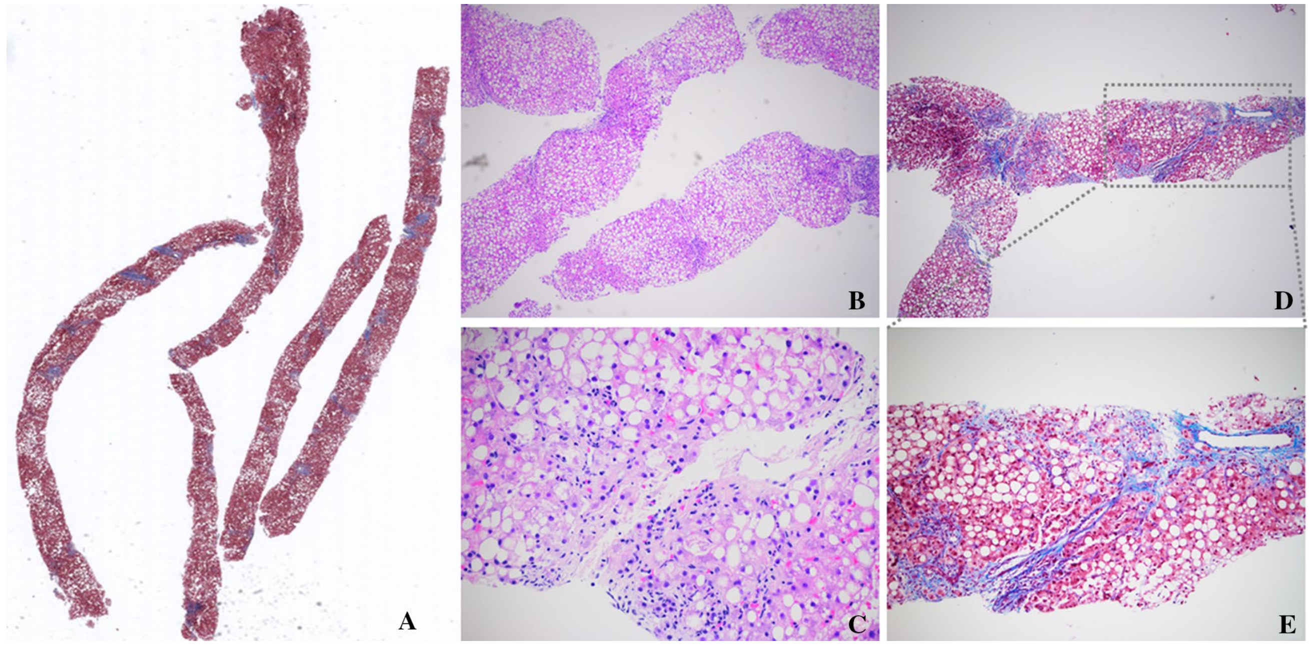

Fig. 1 Photomicrograph of liver sections from EUS–LB are shown. **a** Low-powered (4X) view indicates multiple trichrome-stained (blue) biopsy samples. **b** 20X view of H&E-stained section indicates severe hepatic steatosis. **c** A 200X view of H&E-stained section is shown. **d** 20X view shows a trichrome-stained section. **e** A 100X view of section shown in panel D illustrates hepatic fibrosis (blue)

Table 2 Right/Left Agreement of Kappa coefficients and their 95% confidence intervals

Right/Left Agreement	*n*	Kappa	95% Confidence Interval for Kappa	
Steatosis				
Simple kappa	38	0.763	0.586	0.939
Weighted kappa	38	0.816	0.674	0.958
Lobular Inflammation				
Simple kappa	38	0.381	0.076	0.687
Weighted Kappa	38	0.401	0.110	0.692
Ballooning				
Simple kappa	38	0.696	0.495	0.898
Weighted Kappa	38	0.740	0.565	0.916
Fibrosis				
Simple kappa	38	0.410	0.189	0.632
Weighted Kappa	38	0.473	0.275	0.672
NAS Score				
Simple kappa	38	0.549	0.315	0.783
Weighted Kappa	38	0.606	0.394	0.818

Table 3 Intraobserver agreement between two readings (Kappa coefficients and their 95% confidence intervals)

Reading 1/Reading 2 Agreement	n	Kappa	95% Confidence Interval for kappa	
Steatosis				
Simple kappa	76	0.921	0.846	0.996
Weighted kappa	76	0.939	0.881	0.997
Lobular Inflammation				
Simple kappa	76	0.700	0.539	0.861
Weighted kappa	76	0.725	0.584	0.866
Ballooning				
Simple kappa	76	0.586	0.426	0.746
Weighted kappa	76	0.609	0.451	0.768
Fibrosis				
Simple kappa	76	1.000	1.000	1.000
Weighted kappa	76	1.000	1.000	1.000
NAS Score				
Simple kappa	76	0.653	0.502	0.804
Weighted kappa	76	0.695	0.563	0.828

importance of variable distribution of disease in terms of grading and staging in primary sclerosing cholangitis [16].

Similarly, in different lobes of liver, NASH appears to have variable disease activity. Ratziu, et al showed that agreement between two percutaneously obtained samples from disparate areas of the right lobe of liver was good for steatosis, ballooning and fibrosis but less so for lobular inflammation [19]; Another study comparing left and right liver biopsy specimens obtained during surgeries showed similar findings [21]. However, Merriman et al showed that agreement between left and right lobes of liver tissue specimens obtained intra-operatively were similar for steatosis

Table 4 Probability of agreement between both lobes for steatosis, inflammation, ballooning and NAS scoring

Right Lobe	Left Lobe				Total
	0	1	2	3	
Steatosis					
0	1	0	0	0	1
1	0	11	2	0	13
2	0	1	13	1	15
3	0	0	2	7	9
Totals	1	12	17	8	38
Lobular Inflammation					
0	0	1	0		1
1	1	22	4		27
2	0	4	6		10
Totals	1	27	10		38
Ballooning					
0	4	1	0		5
1	3	16	2		21
2	0	1	11		12
Totals	7	18	13		38
NAS score	0–2	3–4	≥ 5		
0–2	2	2	0		4
3–4	1	12	2		15
≥ 5	0	5	14		19
Total	3	19	16		38

and fibrosis but not for ballooning and lobular inflammation [20].

Our results indicate that distribution of steatosis and ballooning was similar between left and right lobes, but there was variability for lobular inflammation and fibrosis. Reading of biopsies, separated by 1 month, was performed to assess whether these differences were attributable to intra-observer variability. Our results indicate (Table 3) that there was good agreement for most histological features except for ballooning, for which agreement was moderate. The observed differences in lobular inflammation and fibrosis between left and right lobes may have significant clinical implications. Accurate assessment of these parameters in various regions of liver are critical to assess the effects of newer therapies being developed that target hepatic inflammation and fibrosis.

Therefore, we argue that to accurately assess the disease activity, different regions of the liver may be biopsied. EUS-guided sampling of both lobes of the liver is readily accomplished with EUS–LB, compared to more invasive approaches of bilateral percutaneous or surgical biopsy. The efficacy and safety of EUS–LB has been well documented. In a seminal study, 22 patients underwent left lobe-EUS–LB using a 19-gauge FNA needle. The median specimen length was 36.9 mm with a median of 9 complete portal tracts (CPTs) [27]. A multicenter study of 110 cases of EUS–LB using 19-gauge FNA needle reported diagnostic yield of 98% with median aggregate length of 38 mm and median CPTs of 14 [9]. In this study, liver biopsies were obtained from left, right, or both lobes. There was only one complication of self-limited bleeding in patient who was coagulopathic and had thrombocytopenia, which was managed conservatively.

In clinical practice, EUS–LB is being increasingly used and is effective and safe. Tissue yields are excellent and reproducible. Expertise with EUS-guided FNA is a requirement, and the procedure is currently being performed exclusively by endosonographers. There are many potential advantages of EUS–LB other than just the ability to easily sample widely separated areas of liver. For example, in patients who require an EGD or EUS in addition to liver biopsy, EUS–LB is cost saving. Since EUS–LB is performed using conscious or deep sedation (in our center is always performed in propofol-sedated patients), EUS–LB is well-tolerated, particularly in anxious adults and children. To refine EUS–LB technique, our group has conducted several prospective randomized studies. 19G EUS–FNB needle gives the best samples. We have found that a single needle pass with three needle “throws” gives excellent specimen yields. Priming the needle with saline or heparin also enhances the yield [28]. Patients with coagulopathy or significant ascites are excluded from EUS–LB, and liver biopsy in these cases is accomplished by the transjugular route.

Table 5 Probability of agreement between both lobes for fibrosis

Fibrosis	Left Lobe					Total
	0	1	2	3	4	
Right lobe						
0	0	1	0	0	0	1
1	0	12	3	0	0	15
2	0	3	11	2	1	17
3	0	1	2	1	1	5
4	0	0	0	0	0	0
Total	0	17	16	3	2	38

Our study demonstrates that bi-lobar liver sampling is easily accomplished by EUS, and this sheds light on regional distribution of the histologic manifestations of NASH. A strength of the study is that it utilized repeat, blinded reading of the biopsies by a single expert pathologist. A possible limitation of the study is that it comprised a relatively small sample size. However, our sample size is comparable or larger than other studies that assessed the variation in disease activity in bi-lobar liver biopsy specimens.

In summary, sampling of both left and right lobes of the liver by EUS–LB demonstrated that lobular inflammation and fibrosis showed regional variation, whereas steatosis and ballooning were more evenly distributed. Overall NAS scores were similar between lobes, likely due to the relatively high weight assigned to the steatosis component of the score. Single lobe liver biopsy is generally adequate if an overall NAS score is required. However, biopsies of both left and right lobes of liver can provide valuable information regarding individual components of the NAS score, and especially fibrosis—the key indicator of prognosis.

Funding None.

Compliance with ethical standards

Conflict of interest Sandeep Khurana, Waseem Butt, Harshit S. Khara, Amitpal S. Johal, Sara F. West, Zong-Ming E. Chen, Andrea L. Berger, David L. Diehl declare that they have no conflict of interest.

Ethical standard This retrospective study was approved by the Institutional Review Board.

References

1. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140(1):124–131
2. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34(3):274–285
3. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988–1994. Am J Epidemiol. 2013;178(1):38–45
4. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis. 2004;24(1):3–20
5. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. American Association for the Study of Liver D Liver biopsy. Hepatology. 2009;49(3):1017–1044
6. Kalambokis G, Manousou P, Vibhakorn S, Marelli L, Cholongitas E, Senzolo M, et al. Transjugular liver biopsy–indications, adequacy, quality of specimens, and complications–a systematic review. J Hepatol. 2007;47(2):284–294
7. Chisholm J, Seki Y, Toouli J, Stahl J, Collins J, Kow L. Serologic predictors of nonalcoholic steatohepatitis in a population undergoing bariatric surgery. Surg Obes Relat Dis. 2012;8(4):416–422
8. Pineda JJ, Diehl DL, Miao CL, Johal AS, Khara HS, Bhanushali A, et al. EUS-guided liver biopsy provides diagnostic samples comparable with those via the percutaneous or transjugular route. Gastrointest Endosc. 2016;83(2):360–365
9. Diehl DL, Johal AS, Khara HS, Stavropoulos SN, Al-Haddad M, Ramesh J, et al. Endoscopic ultrasound-guided liver biopsy: a multicenter experience. Endosc Int Open. 2015;3(3):E210–E215215
10. Baunsgaard P, Sanchez GC, Lundborg CJ. The variation of pathological changes in the liver evaluated by double biopsies. Acta Pathol Microbiol Scand A. 1979;87(1):51–57
11. Abdi W, Millan JC, Mezey E. Sampling variability on percutaneous liver biopsy. Arch Intern Med. 1979;139(6):667–669
12. Labayle D, Chaput JC, Albuisson F, Buffet C, Martin E, Etienne JP. Comparison of the histological lesions in tissue specimens taken from the right and left lobe of the liver in alcoholic liver disease (author's transl). Gastroenterol Clin Biol. 1979;3(3):235–240
13. Maharaj B, Maharaj RJ, Leary WP, Cooppan RM, Naran AD, Pirie D, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. Lancet. 1986;1(8480):523–525
14. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol. 2002;97(10):2614–2618
15. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38(6):1449–1577
16. Olsson R, Hagerstrand I, Broome U, Danielsson A, Jarnerot G, Loof L, et al. Sampling variability of percutaneous liver biopsy in primary sclerosing cholangitis. J Clin Pathol. 1995;48(10):933–935
17. Skripenova S, Trainer TD, Krawitt EL, Blaszyk H. Variability of grade and stage in simultaneous paired liver biopsies in patients with hepatitis C. J Clin Pathol. 2007;60(3):321–324
18. Siddique I, El-Naga HA, Madda JP, Memon A, Hasan F. Sampling variability on percutaneous liver biopsy in patients with chronic hepatitis C virus infection. Scand J Gastroenterol. 2003;38(4):427–432
19. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128(7):1898–1906
20. Merriman RB, Ferrell LD, Patti MG, Weston SR, Pabst MS, Aouizerat BE, et al. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. Hepatology. 2006;44(4):874–880
21. Larson SP, Bowers SP, Palekar NA, Ward JA, Pulcini JP, Harrison SA. Histopathologic variability between the right and left lobes of the liver in morbidly obese patients undergoing Roux-en-Y bypass. Clin Gastroenterol Hepatol. 2007;5(11):1329–1332
22. Vuppalanchi R, Unalp A, Van Natta ML, Cummings OW, Sandrasegaran KE, Hameed T, et al. Effects of liver biopsy sample length and number of readings on sampling variability in nonalcoholic Fatty liver disease. Clin Gastroenterol Hepatol. 2009;7(4):481–486
23. Hagstrom H, Nasr P, Ekstedt M, Hammar U, Stal P, Hultcrantz R, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017;67(6):1265–1273
24. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology. 2017;65(5):1557–1655

25. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313–1321
26. Faa G, Nurchi V, Demelia L, Ambu R, Parodo G, Congiu T, et al. Uneven hepatic copper distribution in Wilson's disease. J Hepatol. 1995;22(3):303–308
27. Stavropoulos SN, Im GY, Jlayer Z, Harris MD, Pitea TC, Turi GK, et al. High yield of same-session EUS-guided liver biopsy by 19-gauge FNA needle in patients undergoing EUS to exclude biliary obstruction. Gastrointest Endosc. 2012;75(2):310–318
28. Mok SRS, Diehl DL, Johal AS, Khara HS, Confer BD, Mudireddy PR, et al. A prospective pilot comparison of wet and dry heparinized suction for EUS-guided liver biopsy (with videos). Gastrointest Endosc. 2018;88(6):919–925