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Increased Diagnostic Yield from Liver Biopsy in Suspected Nonalcoholic Fatty Liver Disease (NAFLD) Using Multiple Cores and Multiple Readings

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Abstract

Background and Aims—Liver biopsy is required to diagnose NASH in patients with suspected NAFLD but recent studies suggested significant sampling variability. We examined the relationship between histological findings and the number of cores and the number of readings in patients with suspected NAFLD undergoing percutaneous liver biopsy.

Methods—Fifty patients with suspected NAFLD had three cores of liver tissue obtained at the time of percutaneous liver biopsy. The diagnostic yield (percent with definite NASH) and other histological findings from two independent, blinded examinations of 1 core, 2 cores, and the composite of all three cores were assessed.

Results—Proportion with definite NASH was significantly lower in single core biopsy in comparison to 2 cores (37% vs. 57%, $p<0.001$) but it was not different between two cores and three cores (57% vs. 61%, $p=0.3$). Significantly lower severity in steatosis, lobular inflammation, hepatocellular ballooning and fibrosis were observed when 1 core biopsy was compared to multiple core biopsies. Compared to composite of two independent readings by the same pathologist, across 1, 2, and 3 core samples, single reading identified significantly lower proportion with definite NASH and had significantly lower steatosis, inflammation, ballooning, and fibrosis scores.

Conclusions—The widely used practice of single reading of a single core liver biopsy may be inadequate. More studies are needed to define optimal strategy of liver biopsy and histological examination to maximize liver biopsy yield in patients with suspected NAFLD.

Keywords

Sampling error; Liver biopsy; Non-alcoholic steatohepatitis

Introduction

Non alcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver disease in the United States.[1] It is now believed to be a component of metabolic syndrome and is associated with deposition of triglycerides in hepatocytes.[2, 3] A recent multiethnic

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population-based study showed that nearly a third of US adults have NAFLD.[4] The clinical spectrum of NAFLD ranges from benign hepatic steatosis to non alcoholic steatohepatitis (NASH), a progressive form of chronic liver disease resulting in cirrhosis, liver failure or hepatocellular cancer.[5-7] Because of their dichotomous natural history, there is a clinical need to distinguish NAFLD patients with steatohepatitis from those with simple steatosis alone. Although several recent studies that attempted to non-invasively identify NAFLD patients with advanced fibrosis have yielded encouraging, percutaneous liver biopsy remains the gold standard for establishing steatohepatitis and advanced fibrosis in patients with NAFLD.

Since the original description by Ludwig et al., in 1980, many histological features that characterize NASH have been described.[8-10] Several grading and staging systems have also been proposed to assess severity of certain histological features and for accurate diagnosis.[10, 11] More recently, the NASH Clinical Research Network has developed and validated the NAFLD Activity Score (NAS) for use in clinical trials to assess severity and treatment response.[12] In patients with NAFLD, NAS score of ≥ 5 strongly correlated with a diagnosis of "definite NASH" whereas NAS ≤ 3 correlated with a diagnosis of "not NASH".

Percutaneous liver biopsy is an important tool in the management of patients with chronic liver disease. However, concerns exist related to the sampling variability of liver biopsy samples and histological features.[13, 14] In advanced liver disease due to chronic hepatitis C, studies examining the sampling error showed variability by at least one stage in Ishak Fibrosis score in up to 38% of samples.[15-17] A similar concern for sampling error exists in the histological interpretation of NAFLD.[18, 19] In a study consisting of morbidly obese patients undergoing laparoscopic bariatric surgery, Merriman et al., have shown that significant variability exists between right and left lobe liver biopsies, and the diagnostic accuracy of right and left lobe liver biopsies combined was significantly higher than either right or left lobe biopsy samples alone.[20] In contrast, another study of right and left lobe liver biopsies obtained at bariatric surgery there existed minimal variability for severity of steatosis (kappa = 0.91), NAS ≥ 5 (kappa = 0.83), and fibrosis (kappa = 0.96). A higher degree of variability was however noticed with inflammation (kappa = 0.58) and ballooning necrosis (kappa = 0.73).[21] In a study of patients who had two core samples obtained from right lobe by percutaneous core approach, Ratziu et al., have shown significant variability in hepatocyte ballooning, lobular inflammation, and fibrosis between two biopsy samples.[22] Furthermore, authors reported that a diagnosis of steatohepatitis would have been missed in 25% of patients had only single liver core was obtained.[22] In order to further investigate issues related to sampling variability, we conducted a study in patients with suspected NAFLD who were undergoing percutaneous liver biopsy with two aims. First, we examined the relationship between number of liver biopsy cores ((1 vs. 2 vs. 3) and variability in histological findings and the diagnosis of NASH. Second, we examined the relationship between number of independent examinations by an expert hepatopathologist [single vs. two readings] and variability in histological findings and the diagnosis of NASH.

Patients and Methods

Study design

This study consisted of 50 patients with suspected NAFLD who had undergone percutaneous liver biopsy for clinical purposes but also consented to undergo additional core biopsy for investigational purposes. All these patients were seen by the senior author (NC) at Indiana University Hospital from July 2004 to June 2006. These patients were extensively evaluated to exclude competing etiologies including history of alcohol consumption. None of the subjects had ≥ 7 drinks per week of alcohol on average over the preceding 5 year period. At our institution, standard clinical practice for percutaneous liver biopsy is to obtain two cores of liver tissue from the left lobe using a single skin incision under ultrasound guidance. Typically,

patients receive mild conscious sedation with 25-50 mg of Fentanyl and 1-2 mg of Midazolam intravenously for the procedure. Liver biopsies are performed by invasive radiologists using an 18 gauge automated biopsy gun (Bard® Monopty®). Each pass is made using real time ultrasound guidance with the patient in suspended respiration. As part of an ongoing study approved by our institutional review board for storing human liver tissue for future research (liver tissue bank), all patients provided an informed consent to undergo a third pass during their liver biopsy procedure. This third pass was also made into the left lobe through the same skin incision but at a slightly different angle (research sample). Post procedure, all patients underwent 4 hour monitoring prior to their discharge. While the first two liver cores were placed in one bottle and sent to the pathology department in a single formalin bottle for tissue fixation (samples for clinical care), the third sample was flash frozen in liquid nitrogen and stored at -80° C for later use (research sample). All liver tissue was processed at the same histopathology lab and slides were stained with H&E and Masson trichrome stain. In essence, each participant had two sets of slides prepared with one set prepared from two core samples submitted for clinical purpose and another set prepared from third core sample made available for research purposes.

A single experienced hepatopathologist (OWC) examined liver biopsy slides 12-weeks apart on two separate occasions in a blinded fashion to score steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis using published NASH CRN criteria.[12] In addition, by pattern recognition, the pathologist assessed for steatohepatitis in each set of slides and categorized them as “definite”, “borderline” or “not”. This hepatopathologist was part of the pathology subcommittee of the NASH Clinical Research Network that developed and validated the NAFLD Activity Score (NAS) for use in NASH clinical trials. [12]

Data analysis

Several outcomes were measured in this study. First, we evaluated the effect of number of liver biopsy cores (single vs. two vs. three core samples) on the diagnostic yield of histological findings and overall diagnosis of “definite NASH”. Single core represented the research sample that was obtained with third pass, two cores represented clinical samples obtained with first two passes, and three cores represented composite of all three cores. Second, we measured the relationship between number of readings (first reading vs. composite of first and second readings) and diagnostic yield of histological findings and overall diagnosis of “definite NASH”.

P-values were nominal and were derived from ordered logistic regression (except for outcome of NASH activity score of 4 or more which used binary logistic regression) with robust variance estimation to account for within patient correlation due to either cores (for 1 vs 2 and 2 vs 3 core comparisons) or readings (for 1 vs 2 reading comparison). Coefficient of concordance (Kappa statistic) was calculated to assess the intraobserver variability in the interpretation of histological features. In a separate analysis, we examined sampling variability in histological findings according to length of liver biopsy obtained. Liver biopsy samples were grouped into < 10 mm, 10-14 mm, 15-25 mm, and > 25 mm in length. Four categories of biopsy length were treated as ordinal variables and a trend p-value was used to examine the relationship between biopsy length and liver biopsy findings. Statistical analyses used both Stata 9.0 (StataCorp, Stata Statistical Software: Release 9. College Station, TX: StataCorp LP, 2005) and SAS 8.0 (SAS Institute Inc., SAS/STAT User’s Guide, Version 8, Cary NC: SAS Institute Inc., 1999).

Results

Selected clinical and demographic characteristics of fifty patients who participated in this study are shown in Table1. No serious complications following liver biopsy were noted. One patient complained of severe right shoulder pain soon after the liver biopsy. This patient did not have

hematoma or intraperitoneal bleeding on immediate abdominal imaging and was discharged home on the same day as his pain subsided within next several hours.

The mean ± S.D length of liver biopsy sample was 8 ± 3 mm for single core, 25 ± 5 mm for 2 cores, and 33 ± 6 mm for 3 cores combined (composite biopsy) **(**Table 2**)**. Intraobserver variability of various histological components for 1 core ranged from 0.39 for ballooning to 0.78 for steatosis, and for 2 cores it ranged from 0.56 for ballooning to 0.81 for presence of NASH **(**Table 3**)**.

Diagnostic yield of 1 vs. 2 vs. 3 liver core samples after the first reading

a. Compared to single core, histological assessment of two cores led to higher mean grades of lobular inflammation ($p<0.001$), hepatocellular ballooning ($p=0.02$) and NAS score ($p=0.005$) but not steatosis (0.9) or fibrosis **(**Table 4**)**. Compared to two cores, composite assessment of all three cores led to significantly higher mean grade of steatosis ($p<0.001$), lobular inflammation ($p=0.01$) and fibrosis ($p=0.01$) but not ballooning ($p=0.09$) or NAS score (=0.07) **(**Table 4**)**. Assuming 100% specificity and 100% positive predictive value for fibrosis with composite assessment of all three cores, single core had 60% sensitivity to diagnose cirrhosis and this increased to 80% when two cores were read **(**Table 5**)**.

b. The proportion of patients assessed to have “definite steatohepatitis” was significantly higher with two cores than single core assessment (37% vs. 57%, $p<0.001$). However, the proportion of patients with “definite steatohepatitis” was not significantly different between two cores and composite of all three cores (57% vs. 61%, $p=0.3$) **(**Table 4**)**. Assuming 100% specificity and 100% positive predictive value for diagnosing “definite NASH” with composite assessment of all three cores, single core had 64% sensitivity to diagnose definite NASH and it increased to 92% when two cores were read **(**Table 6**)**.

Diagnostic yield of single vs. composite of two independent readings

a. Across 1, 2, and 3 cores, composite of two independent readings had significantly higher steatosis ($p=0.03, 0.02, 0.008$ for 1, 2, and 3 cores respectively), lobular inflammation ($p<0.001, 0.006, 0.002$ for 1, 2, and 3 cores respectively), ballooning ($p<0.001, 0.001, <0.001$ for 1, 2 and 3 cores respectively), fibrosis ($p=0.02, 0.08, 0.005$ for 1, 2 and 3 cores respectively) and NAS > 4 ($p=0.006, 0.04, 0.06$ for 1, 2 and 3 cores respectively) **(**Table 4**)**.

b. Compared to single reading, composite of two independent readings led to higher proportion with a diagnosis of definite NASH in single core (37% vs. 45%, $p<0.001$), two cores (57% vs. 62%, $p=0.09$), and three cores (61% vs. 67%, $p=0.03$) **(**Table 4**)**.

Relationship between histological findings and length of liver biopsy

a. When samples were read single time, except for steatosis ($p=0.16$), there was a statistically significant relationship between length of liver biopsy available and all other histological variables examined, fibrosis stage ($p=0.04$), lobular inflammation ($p<0.001$), ballooning ($p=0.002$), NASH activity score (<0.001), and proportion with definitive NASH (<0.001) **(**Table 7**)**. For example, proportion of samples with definite NASH was only 29% in < 10 mm category whereas it was 65% in samples ≥ 25 mm in length.

b. For all length categories except for biopsies measuring ≥ 25 mm in length, two readings had higher scores than single reading for steatosis, fibrosis, lobular inflammation, and NAS score **(**Table 7**)**. Compared to single reading, two readings

identified a significantly higher proportion of samples with definite NASH in samples measuring < 10 mm in length ($p<0.0001$) but not in other length categories **(**Table 7**)**.

Discussion

There is a dichotomy in the natural history of patients with NAFLD. The patients with simple fatty liver appear to have benign natural history whereas those with steatohepatitis can progress to cirrhosis and liver failure. Imaging studies can establish the presence of fatty liver but liver biopsy is essential to identify patients with NASH. Due to the lack of a definite and conclusive noninvasive test, liver biopsy continues to play a critical role in the management of patients with NAFLD. A recently published randomized controlled of diet and pioglitazone and an accompanying editorial highlighted that only liver histology can serve as a valid primary outcome for NASH therapeutic trials.[23, 24] Furthermore, liver histology is critical in determining whether an individual with NAFLD is eligible to participate in therapeutic trials. For example, many ongoing clinical trials of NASH specify that NAS score ≥ 4 is one of the eligibility criteria ***(Clintrials.Gov)***. But liver biopsy is prone for sampling variability and such sampling variability may have many confounding effects in the clinical management of patients with NAFLD as well as conducting therapeutic trials. Sampling variability may influence patients' eligibility to participate in available clinical trials and may have important implications for sample size calculations and for the interpretation of histological end points.

Our study confirmed earlier observation by Ratziu et al., that in patients with suspected NAFLD, liver samples obtained via percutaneous biopsy carry significant risk of sampling variability.[22] As previous studies have suggested that two core samples may have higher diagnostic yield than a single core, we have addressed the logical next question if three core samples would lead to even higher diagnostic yield. When compared to two cores, histological assessment of three cores led to higher fibrosis scores but not ballooning, NASH diagnosis or NAS score. In other words, if the objective of a liver biopsy is to establish a diagnosis of NASH in patients with suspected NAFLD, then obtaining two core samples would be sufficient. However, if the objective is to establish advanced fibrosis then three cores will identify greater proportion of patients with advanced fibrosis than two cores or single core.

A novel finding of our study is the observation that two readings of the same specimen by the same experienced pathologist would lead to greater diagnostic yield than single reading. In order to confirm this observation, our samples were read separately twice by a community-based general pathologist, and two readings consistently led to greater diagnostic yield than the first reading alone (data not shown). The reasons for this phenomenon are unclear but it does not appear to be related to experience or the learning curve. Interestingly, two readings of the single core had generally comparable diagnostic yield to that of two cores read single time. As adopting two separate readings into clinical practice will have practical implication, we believe that our findings should be confirmed independently.

It can be argued that sampling variability is a manifestation of length of the liver tissue rather than number of cores available for histological assessment. Our study showed that there is a clear relationship between length of the liver sample available and histological findings. But length of the liver biopsy samples and number of cores (passes made) are obviously interrelated with more tissue available from multiple passes. If recommendations were to be made regarding minimal amount of tissue required to maximize liver biopsy yield in patients with suspected NAFLD, it may be more practical to establish criteria in terms of number of core samples needed rather than length of biopsies because it may be more difficult to measure the length of fresh liver biopsy samples.

Some aspects of our study require further discussion. First, the average length of our single core was only 8 mm with only 28% of single core measuring 10 mm or longer in length. If sampling variability is a manifestation of length of the liver tissue available for histological examination, then shorter length of our single cores may have exaggerated its inferiority relative to two and three cores. Although 8 mm length is assumedly shorter for a single core, many single core liver samples obtained in the community practice are less than 10 mm in length. For example, in 50 consecutive patients who had percutaneous liver biopsy done at an outside facility prior to their presentation to our clinic, average length of liver biopsy was only 9 mm with nearly 60% measuring less than 10 mm in length. Second, our biopsies were obtained from left lobe of the liver and thus may not be extrapolated to biopsies obtained from the right lobe. It has been our institutional policy over a decade to obtain left lobe liver biopsies under ultrasound guidance wherever possible to minimize multiple passes through the intercostal muscles. Finally, as our second reading was done 12 weeks after the initial reading, it is unknown if a second reading performed at a much shorter interval (e.g., one hour) would have the same effect.

Most studies that examined sampling variability in patients with suspected NAFLD involved laparoscopic liver biopsies obtained at the time of bariatric surgery. Findings from such studies may not be extrapolated to NAFLD patients undergoing percutaneous liver biopsies in an outpatient setting. To our knowledge, the study by Ratziu et al., is the only other study conducted on NAFLD patients undergoing percutaneous liver biopsies.[22] However, our study is a significant extension to their study not only because we included 3 cores but also because we assessed the effect of multiple readings on sampling variability.

In summary, in patients with suspected NAFLD undergoing percutaneous ultrasound guided left lobe liver biopsies, two core and three core liver biopsy samples have significantly higher diagnostic yield than single core liver biopsy samples. Two cores are as good as three cores for diagnosing NASH but three cores identify a greater proportion of patients with advanced fibrosis. Two readings of the same sample by the same experienced pathologist lead to greater diagnostic yield than single reading alone. Our data suggest that current practice of single reading of single core biopsy is sub-optimal for histological characterization in patients with suspected NAFLD. More studies are needed to define optimal strategy of liver biopsy and histological examination to maximize liver biopsy yield in patients with suspected NAFLD.

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Table 1

Selected Demographic and biochemical parameters of the subjects (n=50)

Age (Yrs, Mean ± S.D)	46.8 ± 9.9
Female (%)	50
Caucasian (%)	98
BMI (kg/m^2) (%)	33.2 ± 6.2
Diabetes Mellitus (%)	40
Dyslipidemia (%)	42
Hypertension (%)	32
AST (Mean ± S.D) U/L	72 ± 52
ALT (Mean ± S.D) U/L	81 ± 47

Table 2
Lengths of Different Liver Biopsy Samples

	1 core†	2 cores†	3 cores†
Biopsy size (mm)			
< 10	72%	0	0
10-19	28%	6%	0
20-29	0	81%	23%
≥ 30	0	13%	77%
Mean ± S.D.	8 ± 3	25 ± 5	33 ± 6
Median	8	24	33
Range	2-18	11-39	20-46

† 1 core represents sample obtained from 3rd pass; 2 cores represent samples obtained from first 2 passes; 3 cores represent composite of samples obtained from all 3 passes

Table 3

Intra-reader agreement (weighted kappa) on classification of histologic components according to number of cores

Histologic component (categorization)*	One core† (n=50) Weighted kappa (95% CI)	Two cores† (n=50) Weight kappa (95% CI)
Steatosis (0-3)	0.78 (0.65 - 0.91)	0.77 (0.62 - 0.92)
Lobular inflammation (0-3)	0.39 (0.17 - 0.61)	0.56 (0.27 - 0.84)
Ballooning (0-2)	0.63 (0.46 - 0.79)	0.68 (0.51 - 0.84)
Fibrosis (0-4)	0.74 (0.63 - 0.85)	0.80 (0.69 - 0.90)
NAS (0-8)	0.57 (0.44 - 0.69)	0.61 (0.49 - 0.74)
NAS > 4 (0-1)	0.71 (0.51 - 0.90)	0.60 (0.37 - 0.83)
NASH (0-2)	0.70 (0.55 - 0.84)	0.81 (0.65 - 0.96)

*See reference 13 for details of categorization

†1 core represents sample obtained from 3rd pass; 2 cores represent samples obtained from first 2 passes;

Table 4

Variation in classification of features of NASH activity and fibrosis by the number of available cores and readings

	1 core†		2 cores†		3 cores†		P¶ 1 vs. 2 cores	P¶ 2 vs. 3 cores
	Single reading	Two readings*	Single reading	Two readings*	Single reading	Two readings*		
Steatosis								
% Grade 0	12	12	12	10	4	4		
% Grade 1	48	42	52	46	46	33	0.86	<**0.001**
% Grade 2	32	34	21	29	33	44		
% Grade 3	8	12	15	15	17	19		
Mean Grade	1.36	1.46	1.38	1.48	1.62	1.77		
P-value 1vs. 2 readings		0.03		0.02		0.008		
Lobular Inflammation								
% Grade 0	26	0	2	0	2	0		
% Grade 1	48	58	51	40	38	23	<**0.001**	**0.01**
% Grade 2	24	40	45	58	57	74		
% Grade 3	2	2	2	2	2	2		
Mean Grade	1.02	1.44	1.47	1.62	1.60	1.79		
P-value 1vs. 2 readings		<0.001		0.006		0.002		
Ballooning								
% Grade 0	64	47	48	33	41	24		
% Grade 1	26	33	35	43	39	44	**0.02**	0.09
% Grade 2	10	20	17	24	20	31		
Mean Grade	0.46	0.73	0.70	0.91	0.78	1.07		
P-value 1vs. 2 readings		<0.001		0.001		<0.001		
Fibrosis								
% stage 0	34	26	23	21	17	6		
% stage 1-2	44	50	58	56	56	62	0.37	**0.01**
% stage 3-4	22	24	19	23	27	31		
Mean stage	1.32	1.50	1.46	1.54	1.75	1.94		
P-value 1vs. 2 readings		0.02		0.08		0.005		
NASH Activity Score (range 0-8)								
% with ≥ 4	36	49	59	67	65	73	**0.005**	0.07
Mean score	2.84	3.55	3.57	3.98	3.76	4.33		
P-value 1vs. 2 readings		0.006		0.04		0.06		
Presence of NASH								
% Not	43	27	17	15	17	11	<**0.001**	0.31
% Borderline	20	29	26	23	22	22		
% Definite	37	45	57	62	61	67		
P-value 1vs. 2 readings		< **0.001**		**0.09**		**0.03**		

*Two readings represented composite of first and second readings

†1 core represents sample obtained from 3rd pass; 2 cores represent samples obtained from first 2 passes; 3 cores represent composite of samples obtained from all 3 passes

¶p-values for single reading

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Table 5

Diagnostic yield of one versus two cores in staging liver fibrosis as compared to three cores in patients with suspected NAFLD (n=50)

	Fibrosis from 1 core					Fibrosis from 2 cores				
	0	**F1**	**F2**	**F3**	**F4**	**0**	**F1**	**F2**	**F3**	**F4**
Fibrosis from 3 cores										
None (Stage 0)	8	0*	0*	0*	0*	8	0*	0*	0*	0*
Mild (Stage F1)	4	10	0*	0*	0*	1	13	0*	0*	0*
Moderate (Stage F2)	2	3	8	0*	0*	1	2	10	0*	0*
Bridging (Stage F3)	1	0	0	7	0*	1	1	1	5	0*
Cirrhosis (Stage F4)	0	1	0	1	3	0	1	0	0	4
Kappa (95% CI)	0.73 (0.59-0.88)							0.78 (0.63-0.94)		
Diagnostic Yield†										
Mild			10/14 =71%			13/14 = 93%				
Moderate			8/13 = 62%			10/13= 77%				
Bridging			7/8 = 88%			5/8= 62%				
Cirrhosis			3/5 = 60%			4/5 = 80%				

*Equal to 0 by definition. Specificity and positive predictive value are forced to be 100%.

†Percent of three cores correctly classified

Table 6

Diagnostic yield of one versus two cores in diagnosing NASH as compared to three cores in patients with suspected NAFLD (n=50)

	NASH from 1 core			NASH from 2 cores		
	No	**Borderline**	**Definite**	**No**	**Borderline**	**Definite**
NASH from 3 cores						
No	8	0*	0*	8	0*	0*
Borderline	6	5	0*	0	10	0*
Definite	5	5	18	0	2	26
Kappa (95% CI)		0.53 (0.35-0.71)			0.97 (0.87-1.00)	
Diagnostic Yield†						
Borderline		5/11= **45%**			10/10= **100%**	
Definite		18/28= **64%**			26/28= **92%**	

*Equal to 0 by definition. Specificity and positive predictive value are forced to be 100%

†Percent of three cores correctly classified

Table 7

Relationship between histological findings and length of liver biopsy

	< 10 mm (n=36)			10-14 mm (n=14)			15-24 mm (n=26)			25+ mm (n=23)			Trend P-value (single reading)
	Single reading	Two readings	P-value: 1 vs 2 readings	Single reading	Two reading	P-value: 1 vs 2 readings	Single reading	Two reading	P-value: 1 vs 2 readings	Single reading	Two reading	P-value: 1 vs 2 readings	
Fibrosis Mean Stage	0.78	1.09	0.001	1.08	1.31	0.06	0.92	1.23	0.005	1.04	1.17	0.08	0.04
Steatosis Mean Grade	1.19	1.50	0.002	1.69	2.00	0.02	1.27	1.58	0.003	1.57	1.78	0.05	0.16
Lobular Inflammation Mean Grade	0.97	1.62	<0.0001	1.15	1.62	0.009	1.32	1.60	0.006	1.61	1.61	1.00	0.0002
Ballooning Mean Grade	0.42	0.97	<0.0001	0.46	0.77	0.03	0.62	0.88	0.02	0.83	1.05	0.05	0.002
NASH Activity Score													
% with ≥ 4	31	59	0.0003	46	77	0.03	42	58	0.03	78	82	0.42	0.001
Mean Score	2.58	3.79	<0.0001	3.31	4.15	0.002	3.25	3.88	0.002	4.00	4.27	0.09	<0.0001
Presence of NASH													
% none	51	21	<0.0001	31	15	0.12	16	12	0.09	13	9	0.30	<0.0001
% borderline	20	15		23	38		28	24		22	22		
% definite	29	64		46	46		56	64		65	70		