Fibrosis Heterogeneity in Nonalcoholic Steatohepatitis and Hepatitis C Virus Needle Core Biopsy Specimens

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Key Words: Liver; Fibrosis; Nonalcoholic steatohepatitis; NASH; Hepatitis C virus; HCV; Scoring; Cirrhosis

DOI: 10.1309/EY72F1EN9XCB1KXX

Abstract

*We examined 46 nonalcoholic steatohepatitis (NASH) and 52 hepatitis C virus (HCV) biopsy specimens to determine the magnitude of fibrosis heterogeneity and minimum length for accurate fibrosis staging. Three fibrosis scores were recorded: lowest regional, highest regional, and most common overall. Mean specimen lengths were 1.6 and 1.8 cm in NASH and HCV, respectively (*P = .283*). Mean (highest minus lowest) fibrosis heterogeneity scores (highest regional fibrosis minus lowest regional fibrosis) were 3.7 and 2.0 in NASH and HCV, respectively (*P < .001*). Of 36 NASH specimens longer than 1.0 cm, 31 (86%) had the highest regional fibrosis in the deepest sampled parenchyma. Shorter specimens were associated significantly with greater fibrosis heterogeneity in NASH (coefficient, –1.3;* P < .001*) but not in HCV (*P = .901*). NASH specimens longer than 1.6 cm had significantly lower mean heterogeneity scores than specimens 1.6 cm or shorter (1.2 vs 3.4;* P = .012*). In NASH, fibrosis heterogeneity can be substantial and is greater than in HCV, and parenchymal injury, fibrosis, and healing might vary in different regions of the liver. The fibrosis stage in patients with NASH might not be assessed accurately in short specimens. Individual needle cores should be longer than 1.6 cm in NASH for accurate fibrosis staging.*

Hepatic fibrosis typically is viewed as a uniform process throughout the liver. The fibrosis stage in a needle core biopsy specimen is assumed to be representative of the fibrosis stage of the entire liver. This assumption might not hold true for all diseases. We noticed that regional fibrosis in nonalcoholic steatohepatitis (NASH) often is heterogeneous. In this situation, the fibrosis stage in the needle core biopsy specimen might not represent the fibrosis stage in the overall organ. In addition, thin, short, needle core biopsy specimens that might be adequate for fibrosis staging in hepatitis C virus (HCV) might be insufficient in NASH. This has become an important issue as an increasing proportion of biopsy specimens are short, small-bore-needle specimens, and serologic fibrosis assays are studied actively and some are marketed by commercial laboratories as viable surrogates to liver biopsy.[1-3]

We examined NASH and HCV needle core biopsy specimens to determine the magnitude of fibrosis heterogeneity. We further attempted to establish the minimum needle core length necessary for accurate fibrosis staging in NASH and HCV.

Materials and Methods

The study was reviewed and approved by the William Beaumont Human Investigations Review Committee (Royal Oak, MI; HIC No. E2004-065). The 2 biopsy groups were 46 consecutively accessioned NASH and 52 HCV study group liver needle core biopsy specimens matched for a similar degree of fibrosis (± 1 fibrosis score) with each NASH biopsy specimen. All patients were under the care of one of us (S.C.G.). All patients with NASH had the characteristic clinical, serologic, and morphologic features of nonalcoholic fatty

liver disease.[4-9] None had hepatitis B or C virus infections or autoimmune hepatitis. All 52 patients with HCV were positive for serum antibody and for HCV by reverse transcriptase–polymerase chain reaction. None of the HCV biopsy specimens revealed steatohepatitis or extensive steatosis.

All liver needle core biopsy specimens were obtained with a 1.9-mm-diameter Jamshidi needle in a single pass through the hepatic capsule by one of us (S.C.G.). None was the composite of several biopsy specimens obtained from multiple passes. Each needle core biopsy specimen was reviewed for specimen length, number of portal tracts, and fibrosis stage. Fibrosis was scored using a Masson trichrome stain. HCV biopsy specimens were scored using the Ishak fibrosis stage system,[10] and NASH biopsy specimens were scored using a modified Brunt fibrosis score.[11-13] The original 4 fibrosis scores of the Brunt system were expanded to a 6-tiered system to match the 6-tiered Ishak system **Table 1**.

Fibrosis Assessment and Fibrosis Heterogeneity Scores

Three fibrosis scores were recorded in each case: *lowest regional fibrosis,* comprising the parenchyma and portal tracts of 2 or more adjacent lobules; *highest regional fibrosis,* also spanning 2 or more adjacent lobules; and *most common overall fibrosis stage* **Image 1**. Fibrosis heterogeneity was compared with 2 calculated factors: (1) Highest – Lowest Fibrosis Heterogeneity Score and (2) Highest – Most Common Fibrosis Heterogeneity Score.

Associations between factors were analyzed by using the χ^2 comparison, Fisher exact test (2-tailed), and logistic regression. Statistical analysis was performed using the Systat statistics computer program, version 10.2 (Systat Software, Point Richard, CA).

Results

Needle Core Specimens

The mean biopsy specimen lengths were 1.6 cm (range, 0.4-3.3 cm; SD, 0.64 cm) and 1.8 cm (range, 0.6-3.2 cm; SD, 0.65 cm) in NASH and HCV specimens, respectively ($P = .283$).

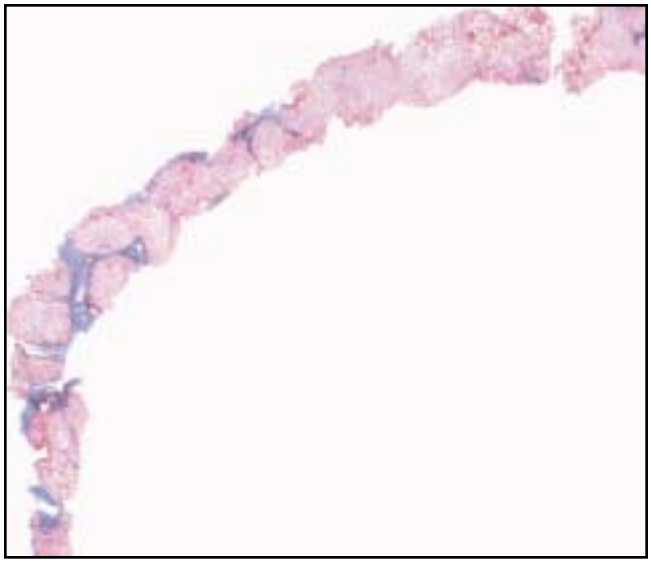

Image 1 Biopsy specimen of nonalcoholic steatohepatitis demonstrating the method of fibrosis score assessment. The Glisson capsule is on the right. The highest regional fibrosis is in the deepest parenchyma (Masson trichrome, ×1.1).

The mean numbers of portal tracts per specimen were 16.1 and 15.7 in NASH and HCV biopsy specimens, respectively ($P = .664$) **Table 2**. Of the 46 NASH and 52 HCV biopsy specimens, 7 (15%) and 13 (25%), respectively, had marked fibrosis or well-developed cirrhosis **Table 3**.

Fibrosis in NASH biopsy specimens was a heterogeneous process. Variation in the amount of regional fibrosis did not seem random. Of the 36 NASH liver biopsy specimens that were longer than 1.0 cm, 31 (86%) had a distinct gradient such that the regional fibrosis was lowest in the parenchyma subjacent to the Glisson capsule and the most severe bridging in the deepest sampled parenchyma (Image 1) **Image 2**, **Image 3**, and **Image 4**. In contrast, HCV biopsy specimens had a uniform pattern of fibrosis that did not change substantially across the length of the specimen **Image 5**. These qualitative differences were borne out by the fibrosis heterogeneity scores. The mean Highest – Lowest Fibrosis Heterogeneity Score was 3.7 in NASH biopsy specimens compared with 2.0

Table 1
Fibrosis Staging Systems for HCV and NASH Biopsy Specimens

Stage	Ishak System (HCV)	Modified Brunt System (NASH)
1	Portal fibrosis (some)	Slight perivenular or centrilobular pericellular fibrosis
2	Portal fibrosis (most)	Prominent perivenular or established centrilobular pericellular fibrosis
3	Occasional portal-portal fibrous bridges	Occasional central venule-portal fibrous bridges
4	Marked bridging fibrosis	Numerous fibrous bridges
5	Extensive bridging fibrosis or incomplete cirrhosis	Extensive bridging fibrosis or incomplete cirrhosis
6	Established cirrhosis	Established cirrhosis

HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis.

DOI: 10.1309/EY72F1EN9XCB1KXX

Table 2
Lengths of NASH and HCV Needle Core Biopsy Specimens*

Specimen Length (cm)	NASH (n = 46)	HCV (n = 52)
≤0.5	2 (4)	0 (0)
0.6 to ≤1.0	8 (17)	7 (13)
1.1 to ≤1.5	12 (26)	15 (29)
1.6 to ≤2.0	12 (26)	11 (21)
2.1 to ≤2.5	9 (20)	11 (21)
>2.5	3 (7)	8 (15)

HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis.
* Data are given as number (percentage).

Table 3
Most Common Fibrosis Stages in NASH and HCV Biopsy Specimens*

Stage†	NASH (n = 46)	HCV (n = 52)
1	19 (41)	8 (15)
2	10 (22)	20 (38)
3	7 (15)	8 (15)
4	3 (6)	3 (6)
5	3 (6)	6 (12)
6	4 (9)	7 (13)

HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis.
* Data are given as number (percentage). $P = .095$ for distribution across all fibrosis stages.
† Most common overall fibrosis stage.

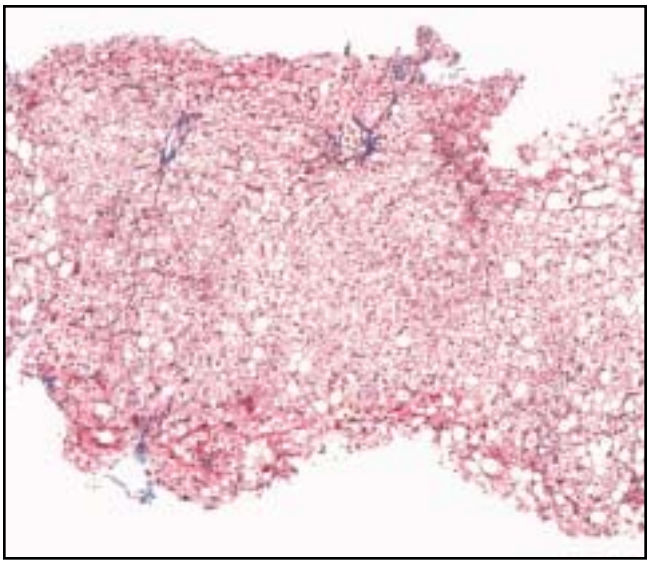

Image 2 High magnification of Image 1. Subcapsular parenchyma with focal, mild, perivenular fibrosis (Masson trichrome, ×10).

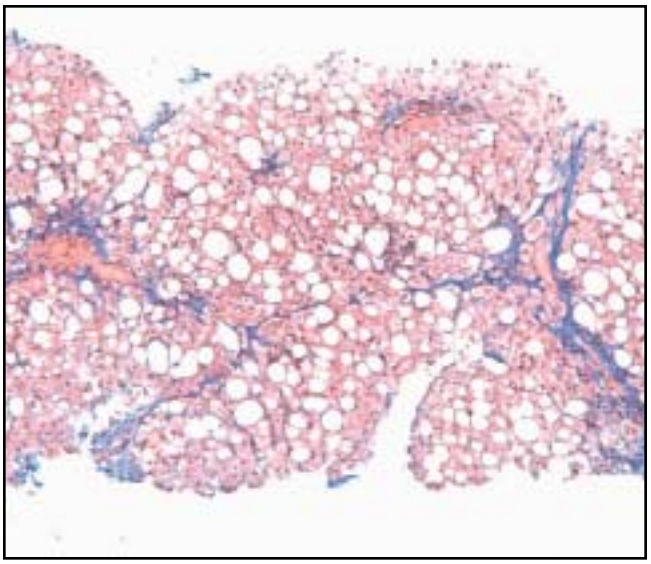

Image 3 High magnification of Image 1. Parenchyma from the midbiopsy region. There is bridging fibrosis (Masson trichrome, ×10).

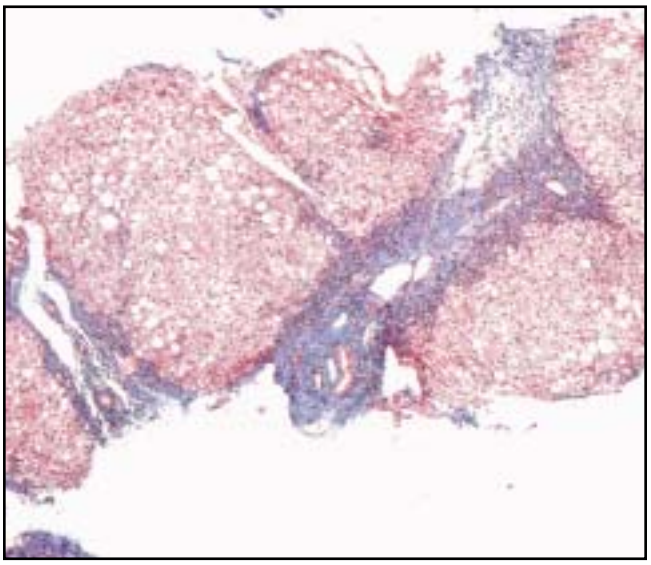

Image 4 High magnification of Image 1. Parenchyma from the deepest sampled parenchyma. There is well-developed cirrhosis (Masson trichrome, ×2.5).

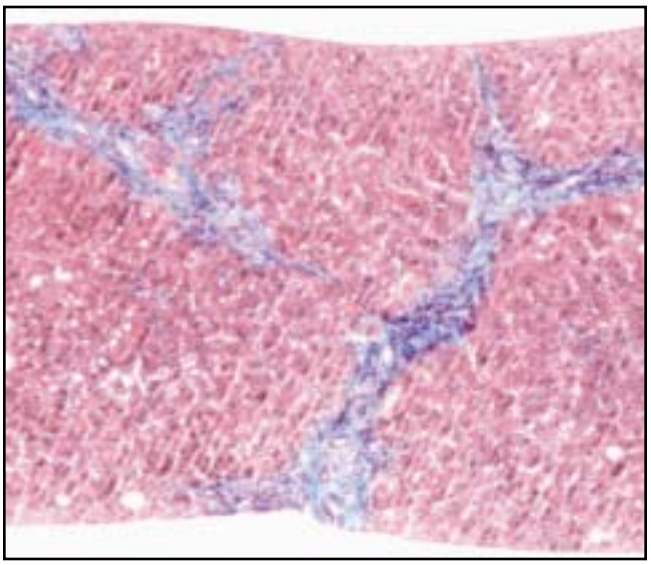

Image 5 Hepatitis C virus. The fibrosis score is uniform across the length of the biopsy specimen (Masson trichrome, ×20).

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in HCV specimens ($P < .001$) **Table 4**. The mean Highest – Most Common Fibrosis Heterogeneity Score also was significantly greater in NASH than in HCV biopsy specimens (2.3 vs 0.6, $P < .001$).

In NASH biopsy specimens, the most common fibrosis score approached the lowest regional fibrosis score in short biopsy specimens and shifted to become closer to the highest regional fibrosis score in long needle core biopsy specimens **Figure 1**. Shorter needle core biopsy specimens were associated significantly with a greater Highest – Most Common Fibrosis Heterogeneity Score in NASH biopsy specimens (coefficient, –1.3; $P < .001$) but showed no association or trend in HCV biopsy specimens ($P = .901$).

We defined *minimal fibrosis heterogeneity* as a Highest – Most Common Fibrosis Heterogeneity Score of 2 or less. The shortest NASH biopsy specimen length with minimal fibrosis heterogeneity was 1.6 cm **Table 5**. By using this cut-point length, NASH biopsy specimens longer than 1.6 cm had a significantly lower mean fibrosis heterogeneity score than biopsy specimens 1.6 cm or shorter (1.2 vs 3.4; $P = .012$). There was no significant difference in mean fibrosis heterogeneity scores of HCV biopsy specimens that were shorter or longer than 1.6 cm (1.3 vs 1.6; $P = .548$).

Table 4
Fibrosis Heterogeneity in NASH and HCV Liver Biopsy Specimens

	Mean Fibrosis Heterogeneity Scores		
Factor	**NASH**	**HCV**	***P***
Highest – lowest regional	3.7	2.0	<.001
Highest regional – most common	2.3	0.6	<.001

HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis.

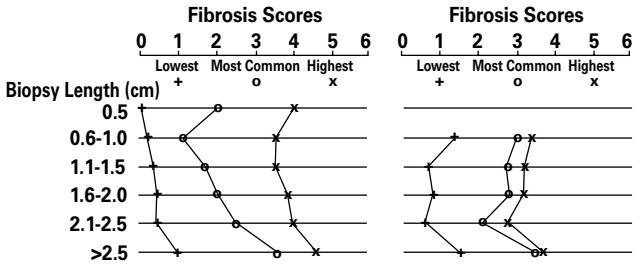

Figure 1 Left, In biopsy specimens of nonalcoholic steatohepatitis, the most common fibrosis score was closer to the lowest regional fibrosis score in shorter biopsy specimens and shifted to be closer to the highest regional fibrosis score in longer biopsy specimens. Right, There was no change in the relationships of the most common fibrosis score in biopsy specimens of hepatitis C virus.

Discussion

The fibrosis stage in liver needle core biopsy specimens traditionally has been considered to be representative of fibrosis of the entire liver. This view probably derives from the large collective experience of evaluating needle core biopsy specimens from patients with viral hepatitis and from seeing the uniformity of fibrosis in cross-sectional organ slices from autopsied cirrhotic patients. Viral infection, the immunologic reaction, and resultant fibrosis are relatively uniform processes throughout the liver. This assumption does not seem to hold true uniformly in NASH. We found that fibrosis in NASH frequently was heterogeneous. Fibrosis scores in the parenchyma at opposite ends of the needle core biopsy specimens often were markedly different. These results are similar to the observations made 25 years ago in an autopsy study that compared the findings in postmortem liver needle biopsy specimens with those seen on standard autopsy liver sections.[14]

The mechanisms that produce a heterogeneous pattern of fibrosis in NASH are unknown. Factors operating at the regional, lobular (acinar) level might have greater impact on fibrogenesis in NASH than in viral hepatitis. One potential regional factor is the level of intrahepatic free fatty acids. Free fatty acids damage cell membranes and injure mitochondria, leading to decreased mitochondrial respiration.[15] Peroxisomal free fatty acid oxidation eventuates in reactive oxygen molecules initiating lipid peroxidation, releasing of malondialdehyde, a stellate cell collagen stimulus factor.[15-17]

A second potential regional factor is the oxygen saturation of sinusoidal blood. Sinusoidal blood oxygen levels are related directly to the proportion of blood contributed by capsular arterioles and ratio of systemic to portal system blood through portal tracts. Oxygen levels in sinusoidal blood can vary substantially between regional areas of the liver.[18-21] Parenchyma supplied by blood with relatively lower concentrations of oxygen might generate more reactive oxygen species, resulting in greater amounts of collagen production.

Table 5
Fibrosis Heterogeneity and Needle Core Biopsy Specimen Length

	Needle Core Biopsy Length (cm)	
Fibrous Heterogeneity Score*	**Minimum**	**Mean**
0	1.8	2.0
1	1.8	2.2
2	1.6	1.9
3	0.9	1.4
4	0.5	1.0
5	0.6	0.8
6	0.5	1.1

* Score for Highest Regional Fibrosis Stage – Most Common Overall Fibrosis Stage.

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A third potential regional factor is the sinusoidal blood levels of antioxidants received from the gut. Antioxidant molecules, such as vitamin E, function in stoichiometric relationships to inactivate reactive oxygen and other reactive molecules. Parenchyma closer to the hilum or with a higher proportion of postprandial portal blood might allow fewer reactive oxygen species to form. We observed that the highest regional fibrosis score was in the deepest region of the sampled parenchyma, farthest from the capsule in 31 (86%) of 36 needle biopsy specimens that were longer than 1 cm. Fibrosis in NASH seems to occur at different rates in subcapsular hepatic parenchyma than in parenchyma, which is closer to the hilum.

Additional support for the central role of regional factors in NASH-related fibrosis comes from 2 studies by Wanless et al[22] and Wanless and Shiota.[23] Photographs of hepatitis B virus–induced cirrhosis regression by these authors showed a markedly heterogeneous pattern of fibrosis, which is unlike the usual, homogeneous pattern of cirrhosis seen in untreated hepatitis B virus–related cirrhosis.[22] Collagen degradation and removal, by its nature, occurs at the cellular level. The presence of fibrosis heterogeneity between different regions of the liver is evidence that regional factors are central to hepatic fibrosis. Wanless and Shiota[23] also recently proposed a model of hepatic injury in NASH of terminal venular damage, fibrosis, and collagen degradation. Regional, lobular-level factors that can eventuate in terminal (central) venule-portal fibrous bridges are central to this concept.

The heterogeneous nature of fibrosis in NASH raises several issues. First, fibrosis heterogeneity might underlie the poor correlation between morphologic features and progression.[24-30] Fibrosis stage scoring might be more difficult in NASH than in HCV and likely is associated with greater levels of interobserver variation. Second, which fibrosis score most accurately reflects overall hepatic function is unknown. NASH is common in morbidly obese patients who elect to undergo bariatric surgery.[31-35] This group of patients is at increased risk of postoperative stress–associated impaired hepatic function or hepatic failure compared with nonobese patients. It is possible that the lowest regional fibrosis score, especially if fibrosis is extensive throughout the length of the biopsy specimen, might better indicate an increased risk of postoperative stress–associated impaired hepatic function than the highest or most common overall fibrosis score. It might be prudent to provide the highest-regional and most common hepatic fibrosis scores in NASH liver biopsy reports until these issues are clarified. Finally, fibrosis heterogeneity in NASH raises the question of the viability of a commercial serologic fibrosis assay, similar to those being marketed for HCV.[1-3]

We found that single-pass needle core biopsy specimens should be than longer than 1.6 cm in NASH cases for accurate staging of fibrosis. Shorter needle core specimens might lead to inaccurate fibrosis stage scores owing mostly to underscoring.[36] These results are similar to those in studies of needle core biopsy specimens in viral hepatitis.[37-39] One recent study demonstrated that the width and length of the biopsy specimen affected the accuracy of histologic activity and fibrosis scores.[36]

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