Prognostic Value of Ishak Fibrosis Stage: Findings from the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis Trial

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Studies of the prognostic value of Ishak fibrosis stage are lacking. We used multi-year follow-up of the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial to determine whether individual Ishak fibrosis stages predicted clinical outcomes in patients with chronic hepatitis C. Baseline liver biopsy specimens from 1050 patients with compensated chronic hepatitis C who had failed combination peginterferon and ribavirin were reviewed by a panel of expert hepatopathologists. Fibrosis was staged with the Ishak scale (ranging from 0 = no fibrosis to 6 = cirrhosis). Biopsy fragmentation and length as well as number of portal tracts were recorded. We compared rates of prespecified clinical outcomes of hepatic decompensation and hepatocellular carcinoma across individual Ishak fibrosis stages. Of 1050 biopsy specimens, 25% were fragmented, 63% longer than 1.5 cm, 69% larger than 10 mm^2, and 75% had 10 or more portal tracts. Baseline laboratory markers of liver disease severity were worse and the frequency of esophageal varices higher with increasing Ishak stage ($P < 0.0001$). The 6-year cumulative incidence of first clinical outcome was 5.6% for stage 2, 16.1% for stage 3, 19.3% for stage 4, 37.8% for stage 5, and 49.3% for stage 6. Among nonfragmented biopsy specimens, the predictive ability of Ishak staging was enhanced; however, no association was observed between Ishak stage and outcomes for fragmented biopsy specimens because of high rates of outcomes for patients with noncirrhotic stages. Similar results were observed with liver transplantation or liver-related death as the outcome. *Conclusion*: Ishak fibrosis stage predicts clinical outcomes, need for liver transplantation, and liver-related death in patients with chronic hepatitis C. Patients with fragmented biopsy specimens with low Ishak stage may be understaged histologically. (HEPATOLOGY 2010;51:585-594.)

Abbreviations: HALT-C Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis; HR hazard ratio; INR, international normalized ratio.

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Liver biopsy remains the accepted standard for histological assessment of liver disease activity and fibrosis, despite such limitations as sampling variability, potential complications of an invasive technique, and subjective scoring. Studies of the natural and treatment-modified histological progression of liver injury and fibrosis have relied on the adoption of uniform grading and staging criteria.[1] With the availability of semiquantitative measurements, investigators have been able to deduce relative rates of histological progression and to go well beyond qualitative information. Nevertheless, prospective studies of the prognostic value of staging of fibrosis—that is, of the correlation between the fibrosis stage and subsequent outcomes or complications of liver disease—are rare.[2] Among patients with established cirrhosis, clinical complications of chronic liver disease, such as ascites, variceal bleeding, hepatic encephalopathy, coagulopathy, and renal/electrolyte disorders, emerge progressively over time; however, it has not been shown whether the sequential stages of fibrosis (short of cirrhosis) identified by these grading systems predict a higher likelihood of the clinical consequences of chronic liver disease.

The Ishak staging system for fibrosis has not enjoyed the general popularity of other scoring systems.[3-7] A modification of the Knodell system (which lacks a stage 2), the Ishak system has more stages of fibrosis (0-6) than other systems. In recent years, the Ishak staging system has become widely used in clinical trials, especially in the United States. Because each Ishak fibrosis stage reflects more scarring than the preceding stage, clinicians and investigators assume that succession from one stage to the next represents progressively more advanced liver disease. Thus, patients with a higher stage should have an increasing risk of clinical outcomes if each score compared with its lower counterpart represents a significant difference in disease severity. However, the link between fibrosis stages and clinical outcomes has not been validated in a prospective trial.

The Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial provided an opportunity to follow prospectively more than 1000 patients for up to 6 years and to assess the association of Ishak fibrosis stage and clinical outcomes. Moreover, because no difference in clinical outcomes occurred between the maintenance peginterferon-treated and untreated control arms, the two arms could be combined, doubling the number of subjects for analysis. The demonstration of a stepwise increase in the frequency of clinical outcomes with each increase in histological fibrosis stage among patients who had not yet developed cirrhosis and patients with cirrhosis would help establish the validity of multiple fibrosis staging levels as reflected in the Ishak staging system.

Patients and Methods

The HALT-C Trial study design and main results have been described in detail.[8,9] Briefly, 1050 patients with compensated liver disease related to hepatitis C–related, who had not had a sustained virological response to an adequate course of peginterferon and ribavirin, were randomized to receive maintenance therapy with peginterferon alfa-2a in a dose of 90 μg/week or no treatment for $\frac{1}{2}$ years. Written informed consent was obtained from each patient, and the protocol had *a priori* approval by the institutional review committee of each participating center. All patients had local histological interpretation of protocol biopsy specimens by individual study pathologists followed by central reading (see below). Fibrosis was staged according to the Ishak fibrosis scale of 0 to 6.[1,7,10] In this system, a score of 2 is defined as fibrous expansion of most portal areas, with or without short fibrous septa; 3, fibrous expansion of most portal areas with occasional portal-to-portal bridging; 4, fibrous expansion of most portal areas with marked bridging (both portal-to-portal and portal-to-central); 5, incomplete cirrhosis characterized by marked bridging and occasional nodules; and 6, probable or definite cirrhosis. To enter the trial, a study participant had to have had a current or a past liver biopsy interpreted by the local clinical-site study pathologist as demonstrating an Ishak fibrosis score of 3 or greater. A subsequent central reassessment of the biopsy through a consensus evaluation at a multiheaded microscope by the pathology reading group (composed of pathologists from the individual centers) could result in a change of the stage. By the conclusion of the central reading process, 79 baseline biopsies were scored as stage 2; 53 of these biopsies had been scored as 3 or greater when interpreted at the local site, and 26 were from patients whose current biopsies were staged locally as 2 but who had had an earlier biopsy scored as stage 3 or greater. There was a slight systematic trend for down-staging with central reading. For example, 44.8% of biopsies were read as Ishak 5 to 6 locally as compared with 40.8% centrally. Consensus central reading was also employed for the year 2 and 4 protocol biopsies. The central, not the local, reading has been used to establish the fibrosis stage for all HALT-C trial manuscripts, including this one. According to the HALT-C Trial protocol, the biopsy required for study entry and stratification into the bridging fibrosis group versus the cirrhosis group could have been performed as long as 12 months before the baseline clinical visit and as long as 2 years before randomization for a few patients who had virological relapse after a full course of therapy.[8]

Because of the difficulty in interpreting the severity of fibrosis correctly from an inadequate biopsy, we also ex-

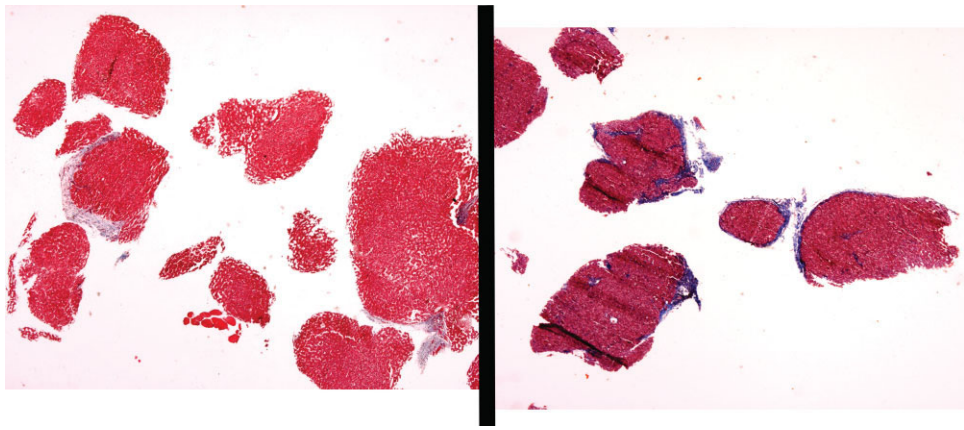

Fig. 1. Examples of fragmented biopsy specimens. Specimen on left, which only has fibrosis focally along the edge of one fragment, was interpreted as Ishak stage 3. Specimen on right has fibrosis enveloping several fragments and was interpreted as Ishak stage 6.

amined characteristics of biopsy quality. Biopsy length was determined at the time of central reading. For two ancillary studies, total biopsy area was measured on 1004 baseline biopsies and number of portal tracts recorded on 1002 biopsies. Biopsy fragmentation was determined by a single study pathologist, who judged as fragmented multiple small pieces of tissue with rounded contours, usually not more than 2 mm in size. When two biopsy passes had been made with one fragmented and the other not fragmented (of reasonable size), the biopsy was categorized as nonfragmented. Examples of fragmented biopsy specimens are shown in Fig. 1.

After the conclusion of the randomized phase, HALT-C participants continued scheduled semi-annual visits that included ascertainment of study outcomes. For this analysis, the first liver-related clinical outcome within 6 years of randomization was used as the primary endpoint. Approximately 80% of the original cohort had had an outcome, were followed for at least 6 years, or had been seen within 6 months before the data cutoff date of January 1, 2009. Because treatment had no impact on clinical outcomes,[9] we grouped treated and untreated control subjects together. The predefined primary clinical outcomes included an increase in Child-Turcotte-Pugh score to at least 7 on two successive study visits at least 3 months apart, ascites, encephalopathy, bleeding esophageal or gastric varices, hepatocellular carcinoma, or death. For these analyses, however, we excluded deaths that were considered unrelated to liver disease. Secondary outcomes included liver transplantation and liver-related death.

Statistical Methods. We compared rates of predefined liver-related clinical outcomes across Ishak fibrosis stages. Cumulative incidences over time of outcomes were calculated with Kaplan-Meier survival estimates and comparison of outcomes by fibrosis stage with the Cox proportional hazards model. Results are reported as hazard ratios (HRs) and 95% confidence intervals. Stage 2 and 3 cases were not compared by Cox analysis because of the small number of events for patients with stage 2 and because the model assumption of a constant HR across time could not be met. Analysis of variance, logistic regression, and the chi-squared test for trend were used to assess the relationship between biopsy characteristics, demographic, and laboratory variables. SAS version 9.1 was used for all analyses (SAS Institute, Cary, NC), and $P < 0.05$ was considered to be statistically significant. No adjustments were made for multiple comparisons.

Results

A total of 1050 patients were eligible to be followed for clinical outcomes. The mean (± standard deviation) time from biopsy to randomization was 269 ± 177 days, uniform across all Ishak fibrosis stages, except for Ishak stage 4, which was longer at 303 ± 175 days (Table 1). Similar proportions of patients were randomized to treatment across Ishak scores ($P = 0.55$). Several characteristics of the biopsy specimens were examined in addition to Ishak stage. Sixty-three percent of biopsy specimens were at least 1.5 cm long, 69% had an area of at least 10 mm^2, and 75% contained at least 10 portal triads; none of these biopsy characteristics varied substantially across Ishak score (test for trend, $P > 0.10$). Among the biopsy characteristics, fragmentation was associated most strongly with Ishak stage ($P = 0.0002$), occurring more commonly among patients with cirrhosis.

Mean ± standard deviation time from biopsy to enrollment laboratory testing was 94 ± 137 days and did not differ by Ishak fibrosis stage (test for trend, $P = 0.47$) (Table 1). Laboratory markers that reflect severity of liver disease (lower platelet count and albumin; higher aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio, bilirubin, and international normalized ratio (INR) and the frequency of esophageal varices were increased proportionately to increased Ishak stage ($P <$

Table 1. Patient and Biopsy Characteristics According to Ishak Fibrosis Stage; and Laboratory Information at Enrollment and Presence of Esophageal Varices According to Ishak Fibrosis Stage and Fragmentation

	Ishak Stage						*P* Value
	All	2	3	4	5	6	for Trend
Number of randomized patients	1050	79	355	188	231	197	
Mean days from baseline biopsy to randomization	269 (177)	277 (192)	273 (187)	303 (175)	245 (161)	253 (166)	0.05
Randomized to treatment	49.2%	51.9%	48.7%	50.5%	51.1%	45.7%	0.55
Biopsy characteristics							
Length ≥1.5 cm	62.9%	60.8%	67.6%	59.6%	62.8%	58.4%	0.11
Area ≥10 (n = 1004)	69.0%	70.5%	70.5%	66.1%	74.8%	61.5%	0.23
Portal triads ≥ 10 (n = 1002)	74.7%	65.3%	71.5%	84.2%	76.8%	72.6%	0.21
Fragmented (%)	23.5%	8.9%	22.5%	17.6%	29.9%	29.4%	0.0002
Laboratory information							
Days from biopsy to laboratory testing	94 (137)	108 (131)	90 (126)	113 (153)	78 (139)	95 (140)	0.47
All biopsies							
Platelet count (10^3/mm^3)	165 (66)	221 (59)	187 (62)	169 (59)	143 (60)	125 (51)	<0.0001
AST/ALT ratio	0.88 (0.29)	0.78 (0.26)	0.84 (0.28)	0.87 (0.28)	0.90 (0.29)	0.98 (0.32)	<0.0001
Total bilirubin (mg/dL)	0.79 (0.40)	0.68 (0.29)	0.71 (0.35)	0.78 (0.37)	0.85 (0.41)	0.92 (0.49)	<0.0001
Albumin (g/dL)	3.87 (0.39)	4.02 (0.31)	3.94 (0.35)	3.93 (0.35)	3.81 (0.40)	3.69 (0.46)	<0.0001
INR	1.04 (0.11)	0.99 (0.08)	1.02 (0.12)	1.02 (0.10)	1.07 (0.10)	1.08 (0.11)	<0.0001
Esophageal varices (%) (n = 1016)	25.7	10.5	13.5	23.1	34.5	45.3	<0.0001
Nonfragmented biopsies							
Platelet count** (10^3/mm^3)	171 (67)	225 (59)	196 (60)	172 (59)	148 (64)	121 (49)	<0.0001
AST/ALT ratio**	0.87 (0.30)	0.78 (0.26)	0.82 (0.27)	0.86 (0.29)	0.88 (0.29)	1.02 (0.34)	<0.0001
Total bilirubin (mg/dL)**	0.77 (0.38)	0.67 (0.29)	0.68 (0.34)	0.77 (0.36)	0.82 (0.36)	0.94 (0.46)	<0.0001
Albumin (g/dL)	3.89 (0.38)	4.03 (0.31)	3.96 (0.32)	3.94 (0.33)	3.84 (0.38)	3.68 (0.47)	<0.0001
INR	1.03 (0.11)	0.99 (0.08)	1.01 (0.11)	1.02 (0.10)	1.07 (0.10)	1.09 (0.11)	<0.0001
Esophageal varices (%)	24.0	11.4	10.3	22.5	34.2	47.1	<0.0001
Fragmented biopsies							
Platelet count (10^3/mm^3)	144 (57)	176 (47)	157 (61)	155 (61)	131 (50)	133 (54)	0.0006
AST/ALT ratio	0.90 (0.28)	0.72 (0.27)	0.88 (0.30)	0.88 (0.25)	0.95 (0.29)	0.91 (0.24)	0.10
Total bilirubin (mg/dL)	0.85 (0.46)	0.72 (0.27)	0.80 (0.38)	0.81 (0.41)	0.93 (0.50)	0.85 (0.56)	0.21
Albumin (g/dL)	3.82 (0.43)	4.01 (0.35)	3.89 (0.42)	3.87 (0.40)	3.76 (0.45)	3.74 (0.44)	0.007
INR	1.06 (0.12)	1.00 (0.08)	1.05 (0.15)	1.05 (0.10)	1.08 (0.10)	1.07 (0.11)	0.08
Esophageal varices (%)	31.1	0.0	24.4	27.3	35.3	41.1	0.009

Data are expressed as mean (SD) or percent.

0.0001 for all these variables). These trends were examined further according to whether the biopsy specimen was fragmented. Patients with fragmented biopsy specimens had lower platelet counts than those with nonfragmented biopsy specimens ($P < 0.0001$). In addition, the decline in platelet count with increasing Ishak stage was steeper among patients with nonfragmented biopsy specimens than among patients with fragmented biopsy specimens (test for interaction, $P < 0.0001$). AST/ALT ratio ($P = 0.06$), total bilirubin ($P = 0.02$), and INR ($P = 0.001$) were higher, and albumin ($P = 0.17$) was slightly lower for the fragmented than nonfragmented biopsy specimens, but the interaction with Ishak score was not statistically significant for AST/ALT ratio ($P = 0.09$), bilirubin ($P = 0.05$), and albumin ($P = 0.32$), yet was for INR ($P = 0.01$). The prevalence of esophageal varices was higher for patients with fragmented (31.1%) than nonfragmented biopsies (24.0%) ($P = 0.001$). As was the case for platelets and INR, the prevalence of varices was higher in patients with fragmented than with nonfragmented biopsy specimens across Ishak stages, especially for Ishak stages 2 and 3 (test for interaction *P*-value = 0.02).

Prognostic Significance of Ishak Fibrosis Score on Primary Outcomes. The cumulative 6-year incidence of a first clinical outcome was 27.0% for all patients and ranged from 5.6% for Ishak stage 2 to 49.3% for Ishak stage 6 (Table 2 and Fig. 2A). For patients with an Ishak stage of 6, clinical events began to occur shortly after randomization and rose by approximately 8% per year (Fig. 2A). A progressive lag in events was observed for the patients with lower-stage disease, most pronounced for patients with an Ishak fibrosis stage of 2, none of whom had an event until nearly 5 years after randomization (and only three after that). When the rate of clinical outcomes was compared for each Ishak stage with the rate for the next lower stage, the outcome rate was statistically signif-

Table 2. Cumulative 6-Year Incidence (and Hazard Ratios for Fragmentation) of First Clinical Outcome by Baseline Ishak Fibrosis Stage According to Biopsy Characteristic and Treatment Assignment

	Ishak Fibrosis Stage					
	2	3	4	5	6	*P* Value for Trend
	Cumulative Incidence of 1st Outcome (N with Outcomes)					
All biopsies	5.6% (3)	16.1% (47)	19.3% (31)	37.8% (74)	49.3% (83)	<0.0001
Fragmentation						
Nonfragmented (NF)	4.0%	10.2%	16.2%	35.6%	51.1%	<0.0001
Fragmented (F)	16.7%	35.3%	33.5%	43.0%	45.2%	0.05
F versus NF: HR* (95% CI) & *P*-value	–	3.9 (2.2–6.8) <0.0001	2.1 (0.95–4.5) 0.07	1.4 (0.9–2.2) 0.18	0.9 (0.5–1.4) 0.52	–
Time randomized from biopsy	Cumulative incidence of 1st outcome					
<200 days	7.2%	22.2%	13.5%	41.4%	49.9%	<0.0001
≥200 days	4.2%	9.9%	24.1%	33.4%	48.4%	<0.0001
Randomized						
Treatment	3.1%	19.2%	17.8%	32.8%	50.2%	<0.0001
Control	8.2%	13.0%	20.5%	43.1%	48.6%	<0.0001
Biopsy length						
≥1.5 cm	3.5%	13.7%	15.4%	27.5%	50.9%	<0.0001
<1.5 cm	8.6%	21.3%	24.5%	53.5%	47.0%	<0.0001
Biopsy area						
Area ≥ 10 mm^2	3.1%	15.8%	18.5%	31.1%	49.1%	<0.0001
Area < 10 mm^2	11.9%	15.4%	22.1%	43.6%	48.8%	<0.0001
Portal triads						
≥10	2.9%	15.3%	18.8%	34.1%	47.0%	<0.0001
<10	11.1%	17.3%	23.8%	48.8%	59.6%	<0.0001

*Hazard ratio. Ishak 2 not compared because of too few outcomes.

icantly higher for Ishak 6 compared with Ishak 5, for Ishak 5 compared with Ishak 4, but not for Ishak 4 compared with Ishak 3 (top of Fig. 3). Treatment assignment had no effect on the results.

Effect of Fragmentation on First Primary Outcome. The same analysis of outcomes was performed according to whether the biopsy specimen was fragmented. The cumulative, 6-year incidence of clinical liver disease outcomes according to baseline Ishak fibrosis stage and fragmentation is shown in Fig. 2B (nonfragmented) and C (fragmented). Overall, patients with fragmented biopsy specimens were more likely to have clinical outcomes than patients with nonfragmented biopsy specimens (HR = 1.9; 95% confidence interval = 1.4-2.4); however, this observed difference in clinical outcomes was confined to patients with Ishak fibrosis stages 3 and 4. Thus, at Ishak stages less than 5, fragmentation was associated with increased rates of outcomes, a finding not seen in patients with cirrhosis, stages 5 and 6 (test for interaction, $P < 0.0001$). In the comparison of rates of outcomes between one Ishak stage and the next lower stage, the hazard ratios were statistically significant for patients with nonfragmented biopsy specimens (Fig. 3) (test for trend, $P < 0.0001$). Specifically, when the rate of clinical outcomes was compared for each Ishak fibrosis stage with the rate for the next lower stage, the outcome hazard ratio for each comparison was higher for patients with nonfragmented biopsy specimens than with fragmented biopsy specimens (Fig. 3). If the biopsy specimen were fragmented, only a borderline association of Ishak stage with outcomes was observed (test for trend, $P = 0.05$). This association was a result of a modest prognostic value (HR = 1.6; 95% confidence interval = 1.004-2.4, $P = 0.05$) for the presence of cirrhosis (Ishak stage 5 or 6) relative to the presence of less severe fibrosis (Ishak stage 4 or less).

An additional analysis of fragmentation was based on paired protocol biopsies. For 547 patients without cirrhosis on the entry biopsy, the odds ratio for cirrhosis on the next biopsy was 1.82 ($P = 0.009$) for patients with fragmented baseline biopsy specimens relative to patients without fragmented baseline biopsy specimens when controlling for initial stage.

Other Markers of Biopsy Quality. Other markers of biopsy quality did not have an appreciable influence on the results. Thus, in general, patients with longer biopsy specimens, larger biopsy areas, or more numerous portal tracts did not have different rates of outcomes than patients with lower-quality biopsy specimens (Table 2). The only exception was biopsy length among patients with an Ishak fibrosis stage 5; in this single subset, patients with biopsy length of less than 1.5 cm had cumulative outcomes of 53.5% versus a cumulative incidence of 28.5% among patients with longer biopsy specimens ($P = 0.0003$). Unlike fragmentation, these measures of biopsy

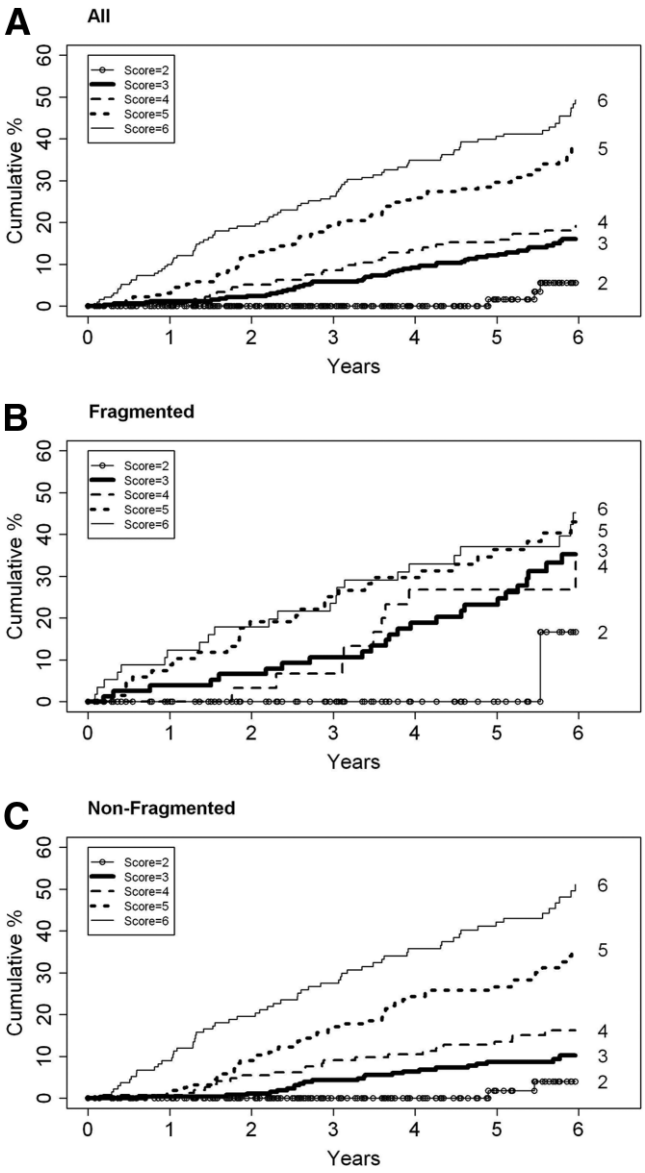

Fig. 2. Cumulative, 6-year incidence of first clinical liver disease outcome according to baseline Ishak fibrosis stage. (A) All (nonfragmented and fragmented) biopsy specimens, (B) nonfragmented biopsy specimens, and (C) fragmented biopsy specimens.

quality did not interact significantly with Ishak stage in relation to outcomes.

Prognostic Significance of Ishak Fibrosis Stage for Liver Transplantation or Liver-Related Death. Nearly half of all first clinical events (48.3%) were increases in Child-Turcotte-Pugh score to 7 or more, primarily achieved through deterioration in laboratory components of the score (albumin, bilirubin, and prothrombin time/INR). To assess the impact of Ishak fibrosis stage on secondary clinical outcomes of liver transplantation and liver-related deaths, the same analyses were performed as described previously for primary clinical outcomes. Sixty-eight patients underwent liver transplantation, and another 58 patients died of liver disease without liver transplantation (half of end-stage liver disease and half of hepatocellular carcinoma). The cumulative 6-year incidence of these outcomes was 14.3%. As was the case for primary clinical outcomes, there was a strong association between successive Ishak fibrosis stages and liver-related death and liver transplantation (Fig. 4A; Table 3). In individual comparisons between fibrosis stages, there was a statistically significant difference in secondary clinical outcomes between Ishak 4 (marked bridging but no nodules) and 5 (bridging with occasional nodules), but not for 3 (occasional bridging) versus 4 or 5 versus 6 (cirrhosis) (Fig. 3, lower half). As with the primary outcomes, there was an effect of fragmentation on the association between Ishak stage and outcome (Fig. 4B, C) (test for interaction, $P = 0.0005$). This effect was most striking with Ishak stage 3; for this subgroup, if the biopsy was fragmented, the frequency of liver-related death or liver transplantation was nearly as high as that for patients with stage 6.

Complexities of the HALT-C Trial Design That Could Have Affected the Results. Two design issues of the HALT-C Trial that could have affected the results were examined: (1) the difference in time between the initial biopsy and the beginning of observation for clinical outcomes and (2) randomization assignment to treatment or control. From the date of the initial baseline biopsy, the time when observation for clinical outcomes began varied widely, ranging from 10 to 918 days. Because only patients who did not have an outcome between the baseline biopsy and randomization could be followed for clinical outcomes, it could not be determine directly whether the variable delay between biopsy and randomization could have affected the results, and patients with cirrhosis did have a trend to shorter biopsy-to-randomization interval (test for trend, $P = 0.05$, Table 1). When outcomes were compared for patients whose randomization date was either more than 200 or 200 or fewer days (close to the median of 198 days), the trend to more frequent outcomes with increasing Ishak stage was not affected by the time to randomization (Table 2). Overall, when controlled for Ishak stage, patients with longer times between the baseline biopsy and randomization had a statistically insignificant lower rate of outcomes (HR, 0.79; 95% confidence interval, 0.61-1.03; $P = 0.08$) than patients with shorter biopsy-to-randomization times. This observation was most apparent for patients with Ishak 3 stage (Table 2); however, in light of the fact that the rate of clinical outcomes within a year of randomization for Ishak stage 3 was only 1.7%, the delay before randomization was unlikely to have substantially affected the results. Most important, no marked evidence was apparent for an interaction of time to randomization with Ishak stage on outcomes ($P = 0.08$). Similarly, because randomization was based on fibrosis stratum (before cirrhosis fibrosis group versus cirrhosis group),

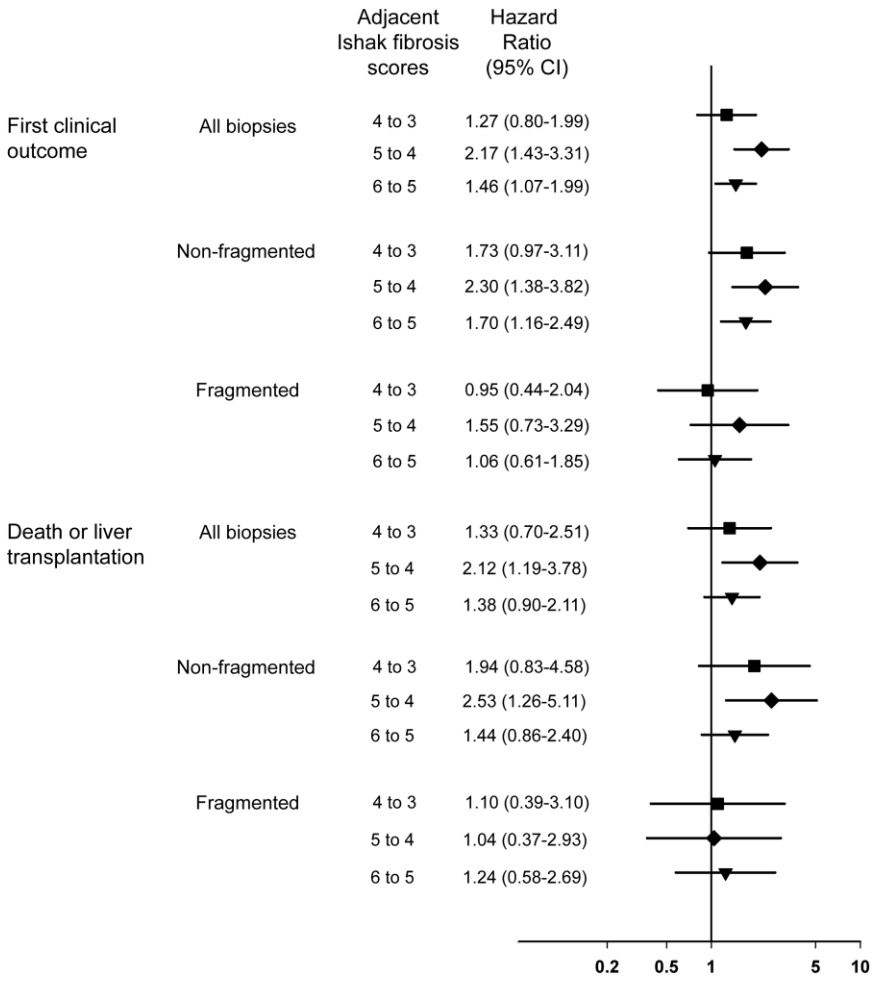

Fig. 3. Hazard rate ratios and 95% confidence intervals for clinical outcomes over 6 years for Ishak fibrosis stage relative to the next lower stage.

no statistically significant difference in Ishak stages was observed between treated and untreated patients (Table 1, $P = 0.55$), and treatment had no effect on outcomes ($P = 0.75$). Thus, treatment did not appear to influence the relationship between Ishak fibrosis stage and outcomes (test for interaction, $P = 0.53$).

Discussion

In designing this study, we pursued two principal questions: (1) Do the individual Ishak fibrosis stages have prognostic value? and (2) Does consideration of the presence of liver biopsy tissue fragmentation enhance the staging of bridging fibrosis? Our analysis indicates that the answer to both questions is yes.

Numerical scoring systems for evaluating liver biopsy specimens came into widespread use in clinical trials in the 1990s as new treatments for hepatitis B and C were developed. The four systems used most often are the Knodell score,[3] which has only three stages of fibrosis (portal, bridging, and cirrhosis), the Batts-Ludwig and Scheuer,[4,5] with four stages (portal, periportal, septal with nodularity, and cirrhosis) and METAVIR,[6] with four stages (portal, few septa, numerous septa, and cirrhosis). The Ishak system,[7] with six stages of fibrosis, has been used less often, but, because of its finer distinctions of fibrosis and architectural remodeling, we chose it as the primary system for the HALT-C Trial. Therefore, the current analysis addressed the issue of whether, in fact, the Ishak fibrosis staging system provided clinically useful prognostic information.

More than 100 papers have been published on the natural history of and prognostic factors in patients with cirrhosis resulting from hepatitis C and other liver diseases.[11] Only a few studies have incorporated numerical scoring systems to assess fibrosis progression in repeated liver biopsies to estimate rates of fibrosis progression to cirrhosis in hepatitis C.[11-17] To our knowledge, however, in only one study have investigators examined the utility of a contemporary fibrosis scoring system (the Ishak system) along with other clinical and laboratory factors in predicting clinical outcomes in patients with chronic hepatitis C.[18] In that single-region study, 131 clinically compensated patients with advanced fibrosis, defined as Ishak stages 4 (marked bridging) to 6 (cirrhosis), were followed

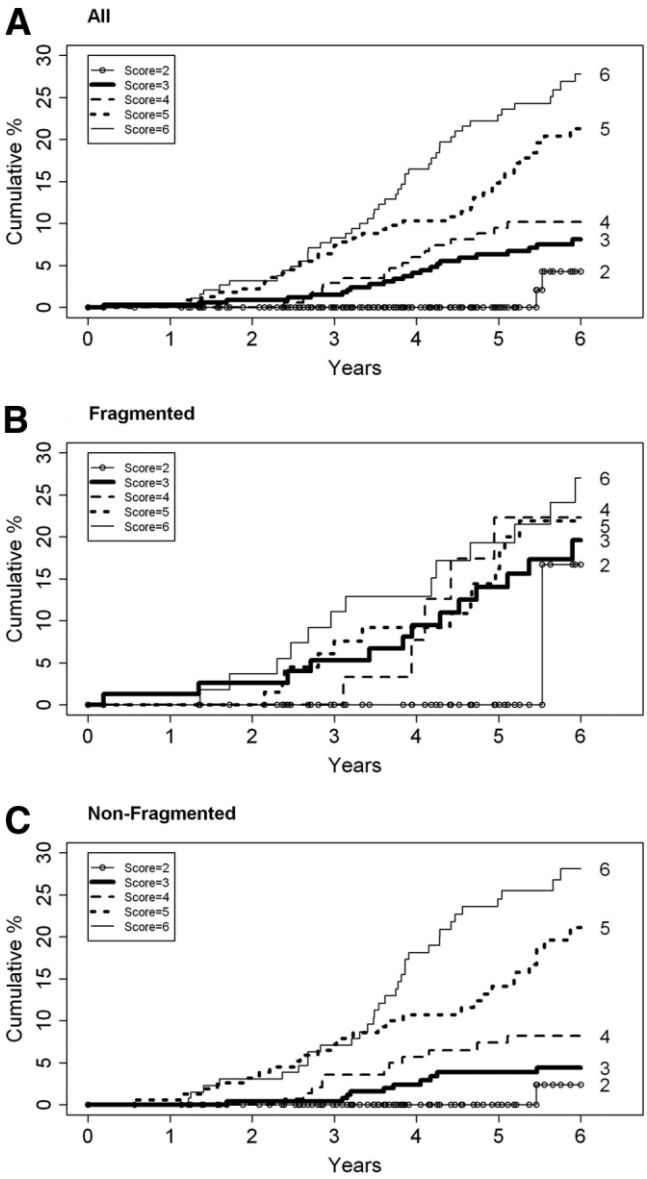

Fig. 4. Cumulative incidence of transplantation or liver related death according to baseline Ishak fibrosis stage. (A) all (nonfragmented and fragmented) biopsy specimens, (B) nonfragmented biopsy specimens, and (C) fragmented biopsy specimens.

for a median of 51 months, during which 25% died or underwent liver transplantation. The authors did not find an association of Ishak stage with prognosis, but the sample was much smaller than that of the HALT-C Trial (for example, only 26 patients had Ishak fibrosis stage 4), and deaths were not restricted to those related to liver disease. In contrast, in the current study of 1050 patients who were followed clinically for up to 6 years, we found statistically significant differences between successive Ishak stages 4 through 6 for the likelihood of developing clinical outcomes. Overall, when all study patients were assessed, we observed no significant difference in clinical outcomes between patients with stages 3 (occasional bridging) and 4 (marked bridging); however, in patients with baseline biopsies interpreted as stage 3, those with fragmented biopsy specimens had both indicators of poorer liver function and portal hypertension and much higher rates of outcomes than patients with nonfragmented stage 3 biopsy specimens. A reasonable inference is that many of the fragmented stage 3 biopsy specimens were understaged and actually cirrhotic. Excluding fragmented biopsy specimens from the analysis, we found that the difference in outcomes between stages 3 and 4 was much closer to achieving statistical significance (Fig. 3). We also found that for patients without a reading of cirrhosis on the entry biopsy, the likelihood of having cirrhosis diagnosed on subsequent biopsy was higher if the first biopsy was fragmented. These findings reflect the difficulties for pathologists in interpretation of biopsy specimens that are not intact cores. The pathologist cannot assume that fragmentation equals cirrhosis; rather, he or she evaluates the amount of collagen present as well as other subtle features of cirrhotic remodeling. In very small and fragmented biopsy specimens, there may be very little of either.

A confident histological diagnosis of cirrhosis requires remodeled vascular architecture as assessed by the observation of nodules of hepatocytes surrounded by fibrous tissue.[19] In the absence of this direct criterion, fragmentation of a biopsy specimen obtained by suction biopsy technique has been recognized to provide strong suggestive evidence that the

Table 3. Cumulative 6-Year Incidence and Hazard Ratios of Liver Transplantation or Liver-Related Death According to Ishak Fibrosis Stage and Biopsy Fragmentation

	Ishak Stage					
	2	3	4	5	6	*P* Value for Trend
	Cumulative Incidence of Transplant or Death (N with Outcomes)					
All biopsies	4.3% (2)	8.1% (23)	10.2% (16)	21.3% (40)	27.8% (45)	<0.0001
Nonfragmented (NF)	2.4%	4.4%	8.2%	21.1%	28.1%	<0.0001
Fragmented (F)	16.7%	20.0%	22.3%	21.9%	27.0%	0.31
F versus NF: HR* (95% CI)	–	4.4 (1.9–10.1)	2.4 (0.8–7.0)	1.1 (0.6–2.1)	0.9 (0.5–1.7)	–
and *P* value		0.0004	0.10	0.83	0.79	

*Hazard ratio. Ishak 2 not compared because too few outcomes.

patient may have underlying cirrhosis[20]; therefore, especially in this setting, correlation with clinical and laboratory findings can help establish a clinical-pathological diagnosis of advanced liver disease with probable cirrhosis.[19,21,22] To prevent bias in clinical trials, however, histological reviews must be blinded to clinical information and laboratory findings, and different biopsy techniques from various centers cannot be avoided. Accordingly, even though a fragmented biopsy is suspicious for cirrhosis, unless the specimen contains at least some fragments surrounded by a thin rim of fibrous tissue, the patient's fibrosis stage will be underscored. The fact that specimen fragmentation introduces a systematic source of error in liver biopsy interpretation emphasizes the need for use of standardized biopsy techniques in clinical trials to avoid this problem. For studies of fibrosis, liver biopsies performed with cutting biopsy needles have been shown to be superior to those performed with suction needles and are more likely to yield the correct diagnosis of cirrhosis.[23-25]

The current findings have an important implication for the design of clinical trials of new forms of therapy for hepatic fibrosis and remodeling. The six-stage Ishak system distinguishes between early or incomplete cirrhosis and established or advanced cirrhosis, but the other histological scoring systems do not. Our results show that the difference between early or incomplete (stage 5) cirrhosis and established (stage 6) cirrhosis is clinically significant; therefore, in trials of antifibrotic therapy, the Ishak system can be considered superior to other systems that do not include this distinction.

The implications of our findings extend to clinical practice. When reporting the results of liver biopsy specimens obtained from patients with chronic hepatitis C, many pathologists provide histological stage on a limited scale of only 0 to 4; biopsy specimens consistent with cirrhosis, whether early or advanced, are categorized as being stage 4 in the scoring systems in most common use. In clinical practice, whether fibrosis is staged on a limited 4-point or more finely discriminative 6-point scale, a more meaningful and precise pathology report should include an accompanying narrative that states whether the cirrhosis is early or incomplete or established, which are more precise distinctions that carry prognostic implications for the patient. In such clinical settings, the narrative is superior to and conveys more meaningful information than a simple numerical stage.

Although our data allow us to conclude that, in the absence of tissue fragmentation, Ishak fibrosis stages of 3, 4, 5, and 6 represent clinically progressive liver disease, a limitation of this study is the small number of patients with Ishak stages of 2 or less. In the HALT-C Trial, only patients with advanced hepatic fibrosis were enrolled to include a population in which clinical outcomes could be anticipated during the period of monitoring; the rate of clinical progression in patients with milder degrees of fibrosis would have been too low for this assessment. Therefore, studies of serial biopsies over time in patients with mild fibrosis will be necessary to determine whether prognostic information can be derived from early as well as late Ishak fibrosis stages. Nevertheless, in the HALT-C trial, patients with Ishak fibrosis stage 2 had no clinical outcomes until nearly 5 years after randomization (Fig. 2) and closer to 6 years after the baseline biopsy.

Thus, in patients with advanced hepatic fibrosis, the Ishak fibrosis stage provides prognostically meaningful distinctions between and among stages in technically adequate, nonfragmented biopsy specimens. Attempts to optimize specimen quality and to avoid tissue fragmentation will improve the accuracy and value of fibrosis scoring. Alternatively, if fragmentation has occurred and is accompanied by pertinent clinical and laboratory findings, then there is a high likelihood of cirrhosis (stage 6). Such patients need more accurate assessments of disease severity and appropriate follow-up surveillance for development of hepatic decompensation and hepatocellular carcinoma.

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