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Rate of Progression of Hepatic Fibrosis in Patients with Chronic Hepatitis C: Results from the HALT-C Trial

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Abstract

Background & Aims—The gradual accumulation of hepatic fibrosis in chronic liver disease results in clinical complications. The rate of hepatic fibrosis score progression (RFSP) in predicting clinical outcomes was assessed by extending the 4-year HALT-C Trial to include preenrollment liver biopsies.

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The corresponding author had full access to all of the data and takes full responsibility for the veracity of the data and statistical analysis.

Scope of work:

John C. Hoefs, M.D.

Submitted biopsy slides from UCI, Organized the writing group, and directed the analysis. Dr. Hoefs led the group in the writing of the manuscript including the first version.

Mitchell L. Shiffman, M.D.

Obtained pre-study biopsy slides from all sites by sending for the slides, obtaining biopsies from his own site and organized them for eventual reading by the consensus histology evaluation panel of pathologists. He helped in the initial organization of the data and analysis plan. He was involved in the writing of the manuscript and critical evaluation of the data.

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He was a leader of consensus histology evaluation panel of pathologists and read with them all the pre-study biopsies. He helped in the initial organization of the data and analysis plan. He was involved in the writing of the manuscript and critical evaluation of the data.

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He was a part of the consensus histology evaluation panel of pathologists and read with them all the pre-study biopsies. He helped in the initial organization of the data and analysis plan. He was involved in the writing of the manuscript and critical evaluation of the data.

Jules L. Dienstag, M.D

He participated in designing the study and in obtaining prestudy liver biopsies from study patients at his clinical site; he also helped with the description of the analysis plan, organization of the manuscript, and critical evaluation of the data. He was involved in all portions of the writing of the manuscript.

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She did all the requested analysis and worked closely with Dr. Hoefs on the analysis plan. She was involved in all of the analysis, critical organization of the data, and statistical evaluation. She wrote the statistical section of the manuscript and was intimately involved with the critical evaluation of the data.

Methods—The RFSP was calculated from the linear regression slope of Ishak fibrosis score versus time in 457 patients with liver biopsies (≥10 mm length) prior to the HALT-C Trial (575 biopsies) plus 1101 on-study biopsies (total 1676 biopsies). Individual slopes were calculated if duration from first to last biopsy was >4 years.

Results—The RFSP as average fibrosis score versus average time in intervals (0–3 and >3 years prestudy, screening, month 24 and 48 on-study) in 455 patients in cohorts of baseline Ishak score ranged from 0.005 with Ishak score 2 to 0.124 with Ishak 6. The RFSP in individual patients (−0.35 to +0.97 Ishak Units/year) had a mean of 0.12 ± 0.23 in 344 patients with prestudy and onstudy biopsies (Group A) and 0.17 ± 0.22 in 169 with prestudy and screening biopsies (Group B). Patients with rapid fibrosis progression, (slope ≥0.2; 95 patients; 27.6%) had higher 7-year cumulative rates of non-HCC outcomes (46% versus 8%) and HCC (10% versus 3%) than slow progressors (slope <0.2; 249 patients, 72.4%) (P <0.0001). RSFP and screening Ishak score correlated independently (P <0.0001) with clinical outcomes in multivariate analysis.

Conclusions—Rapid RFSP in 26.7 % of HALT-C Trial patients correlated strongly with clinical outcomes.

Keywords

rate of progression; fibrosis; cirrhosis; hepatitis C; liver biopsy; Ishak score

Introduction

In chronic liver disease, the gradual accumulation of hepatic fibrosis results eventually in portal hypertension, hepatic dysfunction, and, ultimately, clinical complications (1–4). Theoretically, clinical outcomes such as ascites or liver-related death should correlate with both the prevalent stage of fibrosis and the rate of fibrosis accumulation over time; for such determinations, histologic stage can be measured by a number of different scoring systems (5–13). The limitations of histologic staging on liver biopsy resulting from reliance on categorical scores with narrow ranges, observer subjectivity, small biopsy size, and sampling variability is well established (5–13). Despite these limitations, the risk of clinical outcomes over 4 years was shown in the prospective Hepatitis C Antiviral Long Term Treatment Against Cirrhosis (HALT-C) Trial to correlate with baseline Ishak scores ranging from 2 to 6 (14), establishing the relationship between Ishak fibrosis stage and clinical outcomes.

While a fibrosis score at one time point is informative, the change in score over time provides a dynamic assessment of histologic progression; however, measurement of the rate of fibrosis score progression is rendered difficult by the often long time required for changes in fibrosis to be detected. In several studies, investigators have attempted to assess changes in fibrosis over time (15–20). In the earliest study of fibrosis progression, the probable time of exposure to hepatitis C was estimated, at which time the fibrosis score was assumed to be 0; in addition, the rate of fibrosis progression was assumed to be linear over time, permitting the rate to be derived by dividing the METAVIR fibrosis score by the number of years since presumed acquisition of infection (2). In that study, the average rate of fibrosis progression was 0.1–0.2 METAVIR Units/year, and by 20 years of follow-up, cirrhosis had developed in 20% (2). Therefore, measuring actual fibrosis scores serially over time, without relying on assumptions about time of infection, would allow more accurate determinations of the rate of histologic progression.

The purpose of this paper was two-fold. First, we explored the progression of fibrosis score in the well characterized HALT-C Trial cohort by relying on a long observation period, maximized by extending the time of observation to include both prestudy and on-study

biopsies. Through that exploration, we determined the overall rate of fibrosis score change for the cohort as a whole and for each participant individually. We then investigated the association of this measure of fibrosis progression with subsequent development of clinical outcomes of hepatitis C.

Methods

The HALT-C Trial design and main results have been described (20, 21). A total of 1,382 patients with clinically compensated chronic hepatitis C who failed to respond in the past to interferon-based therapy were enrolled in the trial, conducted at 10 clinical sites. Patients who failed to achieve a sustained virological response during a preliminary lead-in phase of treatment with standard-dose peginterferon alfa-2a and ribavirin (n=1,050) were randomized to receive maintenance therapy with half-dose peginterferon alfa-2a (90 μg per week) or no treatment for 3½ years. Written informed consent from each subject and *a priori* approval by the institutional review committee of each participating center were obtained.

All patients underwent a liver biopsy at screening, and those who participated in the randomized trial were scheduled to undergo repeat biopsies at 24 months and 48 months after enrollment. All protocol biopsies were reviewed by study pathologists at their individual clinical sites, followed by central reading and a consensus staging by a panel of study pathologists (see below). Fibrosis was staged according to the Ishak fibrosis scale of 0 to 6 (11–13). Patients were eligible for enrollment in the trial if the individual clinical site pathologist assigned the screening biopsy an Ishak fibrosis score of ≥3 or, in rare cases in which the screening fibrosis score was 2, if a previous biopsy showed stage ≥3 fibrosis. A subsequent central reassessment of all biopsies through a consensus evaluation at a multiheaded microscope by the pathology reading group (composed of pathologists from all the individual centers and the central pathologist from the former Armed Forces Institute of Pathology) could result in a change of fibrosis stage; thus, in some cases, the local reading of ≥3 was restaged by group consensus to 2. In a previous report from the HALT-C Trial, we described the difficulty in interpreting the degree of fibrosis correctly in an inadequate biopsy specimen, and a correlation was observed between biopsy fragmentation (i.e., cirrhosis likely) and an increased frequency of clinical outcomes (14).

In order to maximize the number of biopsies and duration of observation available for calculation of fibrosis progression, we obtained as many prestudy biopsies as possible from HALT-C Trial participants, allowing us to go backwards in time to include previous biopsies and forward in time to include on-trial biopsies. All patients who had at least one prestudy biopsy before the screening biopsy provided additional written informed consent to have the slides of their previous biopsies retrieved and reviewed. We relied upon the central consensus reading to establish the fibrosis stage for all HALT-C Trial evaluations for prestudy, screening, year-2, and year-4 protocol biopsies.

Following the conclusion of the 24-week lead-in phase and the 3½-year randomized phase of the trial, HALT-C Trial participants continued scheduled semiannual visits for assessment of study outcomes. For this analysis, the first liver-related clinical outcome within 7 years of randomization was the primary endpoint. Because treatment in the trial had no impact on clinical outcomes (22), we grouped treated and untreated-control subjects together. The predefined primary clinical outcomes included an increase in Child-Turcotte-Pugh (CTP) score to ≥7 on two successive study visits at least 3 months apart, ascites, hepatic encephalopathy, bleeding esophageal or gastric varices, spontaneous bacterial peritonitis, hepatocellular carcinoma, or death (23). For these analyses, however, we excluded deaths that were unrelated to liver disease (23) and we considered hepatocellular carcinoma (HCC) as a separate outcome.

Statistics

Patients with the same Ishak score on the screening biopsy were grouped together and referred to as cohorts with screening Ishak score of 2, 3, 4, 5 or 6. We evaluated the group progression rate in all valid patients by calculating the average fibrosis score at average time intervals prestudy and on-study; we used prestudy intervals of 0–3 and >3 years for calculation of group slopes. The group progression rates were calculated in cohorts of screening Ishak score as the least squares regression slope (group slope) of average fibrosis to average time interval.

To provide an overall subject-specific estimate of fibrosis progression and to account for the differences in timing of the prestudy biopsies, we computed a subject-specific measure of change in fibrosis score per year (slope) based on least squares regression methods of fibrosis score versus time. We used descriptive statistical methods to determine the minimal interval required for individual slope calculation (distribution of slope versus time interval from first to last liver biopsy) (appendix). Based on these analyses, we defined valid slopes to be those based on biopsies that spanned at least a 4-year period and for which the first prestudy Ishak score was <6 (further increase not possible). All patients with individual slope calculation had at least one prescreen liver biopsy but a variable number of on-study biopsies ranging from 1 to 3, as long as they met criteria for >4 years between first and last biopsy. The individual slope was computed for group-A patients (subjects with prestudy, screening, and/or on-study biopsies) prior to the first clinical outcome (all on-study liver biopsies were obtained prior to the first clinical outcome) and for group-B patients (subjects with prestudy and screening biopsies but no on-study biopsies). We then divided the Group-A participants with valid slopes into rapid and slow progressors (≥0.2 fibrosis units/year versus <0.2 fibrosis units/year). To test the association of this categorization on other participant characteristics, we used analysis of variance (continuous measures) or Chi-square test of homogeneity (categorical measures). To explore the association of rate of progression with outcomes, we used time-to-event methods: Kaplan-Meier life-table analysis and Cox proportional hazards regression analysis. All analyses were carried out with SAS statistical software (SAS Institute, Inc., Cary, NC).

Results

PreStudy Liver biopsies

Of the 1,382 patients enrolled in the HALT-C Trial, 545 had at least one biopsy prior to the screening biopsy (725 biopsies); however, only 457 patients had at least 1 prestudy biopsy of ≥10 mm in length (575 biopsies, Table 1), a requirement for inclusion (Figure 1 flow diagram). Among the 575 biopsies ≥10 mm in length, 30 were fragmented. Of the 457 patients with at least one valid prestudy biopsy, 455 had a valid screening biopsy (see flow diagram). Of these 457 patients, 397 nonresponders were assigned randomly to the treatment or control groups, and they contributed in addition to the 575 pre-study biopsies, 455 screening biopsies, 343 biopsies at 24 months and 303 at 48 months, for a total of 1676 biopsies >10 mm in these patients. The number of days between the valid 575 prestudy liver biopsies and corresponding screening liver biopsies ranged from 60 to 8,900 days (2 months to almost 24 years). The mean time between the prestudy and screening biopsies was 1,409 days (4 years) and median 1,114 days (3 years).

Fibrosis and inflammation over time

Table 2 shows average fibrosis scores and mean time of the prestudy biopsies as well as screening, month-24, and month-48 Ishak scores in all 455 patients (2 patients did not have a valid ≥10 mm screening biopsy). Prestudy biopsies were obtained at irregular times prior to screening, and, therefore, for this analysis, we calculated the mean time for biopsies at 0–3

years and >3 years before the screening biopsy. For patients in each cohort of screening Ishak score, we calculated the mean interval from prescreening biopsies to the screening biopsy (prestudy biopsies assigned a negative time and on-study biopsies a positive time) and the mean Ishak score for each time point before and on-study. The group slope in Table 2 was calculated as the least squares fit of mean Ishak score and mean time for each Ishak score cohort; group slope increased as a function of higher screening Ishak scores. Ishak inflammation scores decreased significantly ($P < 0.0001$) in all screening Ishak fibrosis cohorts from prestudy to month 24 and month 48 during the study, with the total percent mild (Ishak inflammation score 0–6): 18% of patients baseline and prestudy, 40% month 24, and 59% month 48, although the inflammation scores in randomized-treated subjects decreased significantly ($P < 0.0001$) more than in randomized-untreated subjects.

Rate of fibrosis score progression in individual subjects

The individual RFSP slope represents the average change in fibrosis score per year by least squares regression analysis and is limited in accuracy by the time interval and the number of data points. We analyzed (appendix) the relationship of the interval between first and last biopsy and the accuracy of the calculated slope and selected 4 years as the minimum interval for calculation of RFSP slope. The patients included in the remainder of the analysis are those with at least one prestudy liver biopsy, an interval of ≥4 years between the first and last liver biopsy, and a first prestudy Ishak fibrosis score <6 (38 patients Ishak 6), because further increase was not possible; 344 patients with fibrosis progression slopes covering the entire study period (both prestudy and on-study biopsies) (Group A) met these criteria, and only 169 patients with fibrosis progression slopes covering only prestudy biopsies inclusive of the screening biopsy (Group B) met these criteria (Figure 1).

Associations of individual fibrosis progression slopes with clinical outcomes—The initial prestudy biopsy was evaluated for the relationship of fibrosis stage, excluding Ishak 6, on the first biopsy to individual slope distribution. The initial biopsy showed Ishak 5 cirrhosis in 46/344 (14.4 %) group-A patients and 13/169 (7.7 %) of group-B patients. The mean ± SD, range, and median progression slope in group A for patients with cirrhosis on the prestudy biopsy were 0.01 ± 0.20, −0.57 to +0.45, and 0.05 and for patients with precirrhotic fibrosis on the prestudy biopsy 0.13 ± 0.23, −0.35 to +1.28, and 0.10. The mean ± SD, range, and median RFSP slope in group B for patients with cirrhosis were 0.05 ± 0.17, −0.32 to +0.24, and 0.12 and for patients with precirrhotic fibrosis on the prestudy biopsy 0.18 ± 0.22, −0.32 to +0.97, and 0.15. The majority of patients did not have cirrhosis on the first biopsy, but 40% did have cirrhosis at the time of baseline screening, consistent with relatively rapid progression in a sizable proportion of patients. Those who did have cirrhosis on the initial biopsy must have had relatively slow clinical progression to qualify for the study by remaining free of hepatic decompensation at the time of screening. Based on these progression-slope distributions, we defined rapid RFSP to be a slope of ≥0.2 Ishak fibrosis Units/year. The selection of the cut-point was determined by the upper quartile of the distribution of slopes. This resulted in “fast” progression being defined as progression of 1 Ishak stage in less than 5 years and “slow” progression as progression of 1 stage in ≥5 years.

In Table 3, focusing on Group A (prestudy and on-study biopsies), we compared rapid and slow progressors. No difference was detected in mean Ishak score for the earliest prestudy biopsy at a similar average prestudy time between the two groups (Table 3); however, rapid progressors had significantly ($P < 0.0001$) more advanced chronic liver disease at study baseline than slow progressors and more frequent laboratory evidence of substantial hepatic injury (higher AST, ALT, and AFP), despite a nonsignificant correlation with histologic inflammation. In Group B (prestudy biopsies only), we found that similar clinical variables correlated with rapid progression (data not shown). In a logistic regression analysis with

progression (rapid or slow) as the dependent variable, we identified independent associations between rapid progression and baseline AST (P = 0.0002), AFP (P = 0.005), and creatinine (P = 0.03) (data not shown).

Clinical outcomes in Group-A patients were found a median of 2 years after the last on-study liver biopsy and up to 6 years after the last biopsy. The 7-year cumulative rate of clinical outcomes (excluding HCC) in rapid progressors was 46%, compared to 8% in slow progressors, as determined by Kaplan-Meier life-table analysis (P <0.0001) (Figure 2a). Similarly, in a Cox proportional hazards analysis, we found that the hazard rate ratio (HR) of clinical outcomes for the rapid versus slow progressors was 7.15 (95% *CI: 3.82 – 13.4)*, P <0.0001).

The 7-year cumulative rate of HCC was 10% in rapid progressors, compared to 3% in slow progressors (P = 0.006) (Figure 2b). In a Cox proportional hazards analysis, we found that the hazard ratio of HCC for rapid versus slow progressors was 3.79 (95% *CI: 1.34 – 10.5*, P = 0.01).

In a previous analysis of HALT-C Trial data, Ghany et al (23) developed a model to predict clinical outcomes based on baseline patient variables including liver biopsy fibrosis score, the HALT-C Trial clinical predictors model. We evaluated whether the RFSP slope would contribute significantly to the predictive power of that model. First, we applied the HALT-C Trial clinical predictors model to the subset of patients in our analysis, and, then, we added the measure of rapid versus slow progression to that model (Table 4a). When the variables in the earlier HALT-C Trial clinical predictors model were included in a multivariable model for this subset of patients, the hazard ratios were similar to those published previously. When RFSP slope ≥0.2 was included in the model, the slope of progression remained statistically significantly associated with the risk of first outcome (HR = 4.88, P <0.0001), Thus, although the other patient characteristics remain associated significantly with time to a clinical outcome, fast progressors are almost five times more likely to experience an outcome than slow progressors when controlled for other clinical characteristics. When RFSP slope and baseline Ishak score are the only factors in the model fast progressors are more than five times more likely to experience an outcome than slow progressors (Table 4a). Thus, histologic progression is a very strong correlate of clinical outcomes.

We also investigated whether the RFSP slope would contribute significantly to the power of a previously published model for predicting HCC (24). First, we applied an earlier HALT-C Trial HCC prediction model, as reported by Lok et al (24), to the subset of patients in this analysis, and, then, we added the measure of rapid versus slow progression to that model (Table 4b). When the factors used in the earlier HALT-C Trial HCC prediction model were included in a multivariate model for this subset of patients, the hazard ratios were similar to those published previously. When RFSP slope ≥0.2 was included in the model, the hazard ratio was not statistically significant (HR = 2.38, P = 0.12). Thus, the RFSP slope does not add significantly to the model for time to development of HCC.

We explored as well whether the prestudy RFSP slope in the 169 group-B patients was associated with on-study development of cirrhosis during the randomized phase in those with precirrhotic fibrosis (Ishak scores 2–4). Among fast progressors (slope ≥0.2) with precirrhotic fibrosis at screening, cirrhosis developed by month 24 in 4 of 17 (24%), compared to in 8 of 54 (15%) among slow fibrosis progressors (slope <0.2) (P = 0.40). On the other hand, among the rapid progressors who did not have cirrhosis at month 24, cirrhosis developed by month 48 in 7 of 17 (41%), compared to in only 2 of 41 (5%) among slow fibrosis progressors (slope <0.2) (P <0.0005). Thus, in our study population, long-term,

but not short-term, prediction of progression to cirrhosis is possible based on the fibrosis-progression slope.

Finally, an analysis of inflammation in which we controlled for screening fibrosis score and randomization group showed that adjusted mean ± SEM histologic Ishak inflammation score was similar in rapid progressors compared to slow progressors (8.0 ± 0.1 versus 7.9 ± 0.1 Ishak inflammation score). Overall, a significant reduction in inflammation occurred over time (P <0.0001), but inflammation decreased significantly less from baseline (Table 3) in fast progressors (baseline 8.0 ± 1.4, month 24, 7.9 ± 0.3 and month 48, 7.2 ± 0.3 Ishak inflammation score) than in slow progressors (baseline 7.7 ± 1.4, month 24, 6.9 ± 0.2 and month 48, 6.5 ± 0.2 Ishak inflammation score) over time (P <0.0001), during the study (P = 0.02). Thus, inflammatory pressure over time was greater in the rapid progressors.

Discussion

The HALT-C Trial, a large, prospective, well characterized trial in patients with chronic hepatitis C, is unlikely to be duplicated; therefore, investigation of this study population to address issues that are unlikely to be addressed elsewhere commands a high priority. An area of substantial interest currently to the hepatology community is the role of liver biopsy to assess histology and whether the clinical value of the biopsy can be matched by noninvasive indicators. In any consideration of this topical subject, the precision achieved in defining the value of histology is the most important variable, and such characterization of data derived from histological observation is the issue addressed in this paper. We confirmed the gradual accumulation of hepatic fibrosis based on increasing average histologic scores over time (Table 1). The major finding of this study, however, was the strong association in individual patients of RFSP slope with clinical outcomes unrelated to HCC (ascites, hepatic encephalopathy, ≥2-point increase in CTP score, death) (Figure 2a) and with HCC itself (Figure 2b). Furthermore, the RFSP slope predicted histologic progression from precirrhotic fibrosis to cirrhosis. In a previous publication from the HALT-C Trial group, Everhart et al (14) demonstrated for the first time a linear relationship between baseline Ishak fibrosis score and clinical outcomes; more advanced histologic stage at a single point in time was associated with more frequent clinical decompensation. The current study extends those observations by showing the added value of rate of progression of histologic stage in predicting outcomes. Application of a model that includes both prevalent fibrosis score and RFSP slope measured over time reveals that both variables contribute independently (Table 4) to predicting clinical outcomes with a high level of confidence. Thus, these two studies taken together add considerable support to the value of histologic stage as a predictor of clinical severity of chronic liver disease.

The gradual accumulation of hepatic fibrosis is the driving process in chronic liver disease, and, theoretically, clinical outcomes should be related to the individual stage of fibrosis at any given time and rate of fibrosis progression. In this study, we used histology as a marker for prediction of clinical outcomes; however, other clinical data may be as effective as histology as surrogate markers for predicting clinical events. Moreover, deriving fibrosis progression from hepatic fibrosis scores may be confounded by potential limitations. Hepatic fibrosis scores, based on fibrosis patterns, do not correlate well with quantitative measurements of amounts of fibrosis, as shown in a prior report of morphometric imaging of fibrosis in this population (25). Sampling variability is an important source of scoring error on individual biopsies, and a >20-mm biopsy length is preferred (12, 14, 26–31) to minimize this source of error. In this study, however, we selected >10 mm as the threshold for including biopsies in our analyses in order to capture as many study patients as possible. Furthermore, we have already shown in this study cohort that an Ishak score based on a single ≥10-mm biopsy correlated closely with risk of clinical outcomes (14) and

outperformed morphometric analysis of Sirius red staining for quantifying fibrosis in predicting clinical outcomes (25). Although a larger biopsy length might have been preferable, our findings based on shorter-length criteria are impressive. In addition, multiple data points over an adequately extended period of time are likely to have an "averaging" effect, minimizing other sources of error in calculating a rate of change in fibrosis score. Even with a ≥10-mm threshold for biopsy inclusion, the rate of change in Ishak fibrosis score in individual patients as measured in this study correlates with liver disease progression and prediction of clinical outcomes.

Rapid RFSP slope (≥0.2) correlated with baseline screening variables that clustered as markers of more severe chronic liver disease (low albumin and platelet count, high AST/ALT ratio, presence of esophageal varices) and as biochemical markers of more substantial hepatic injury (high AST, ALT, and AFP), markers associated with rapid progression of fibrosis in other studies (16, 17) (Table 3). In fact, we found in multivariable analysis that high AST and AFP and low creatinine were associated independently with rapid progression. The correlation between rapid fibrosis progression and low creatinine may be related to the hyperdynamic circulation in cirrhosis, which, at an early cirrhotic stage, may increase creatinine clearance as a result of an increase in cardiac output and renal blood flow. Alternatively, lower creatinine in early cirrhosis may be a reflection of reduced muscle mass and a catabolic state. Either explanation suggests that lower creatinine is another expression of more advanced liver disease (whereas elevated creatinine is a variable associated in the pretransplantation MELD score with risk of death in decompensated cirrhotics). Unexpectedly, histologic inflammation did not correlate closely with the rate of fibrosis progression and actually decreased over time as fibrosis increased. This observation may be related in part to the effect of treatment during the HALT-C Trial, in which a reduction in hepatic inflammation was observed during the prerandomization lead-in phase in all subjects (32) and during the randomized phase in the maintenance-therapy group (22). Rapid progression did correlate, however, with biochemical markers of more severe injury and liver disease severity.

Previously, the HALT-C Trial group described effective models based on routine clinical and laboratory variables to predict clinical and HCC outcomes. When the fibrosis progression slope was added to the previous HALT-C Trial model (25) for the prediction of HCC, little additional predictive power was achieved, i.e., nonhistologic data are sufficient for predicting HCC. In contrast, the fibrosis-progression slope added significantly to the analysis of the risk of non-HCC clinical outcomes in the previously reported HALT-C Trial model (24). In patients with hepatitis C identified as fast progressors, the risk of developing an outcome is approximately 5 times higher than in slow progressors. Thus knowledge of the rate of progression may be an important predictor of subsequent development of clinical outcomes. Pressure is substantial in the clinical community to supplant liver biopsy as the standard for staging and following patients with chronic liver disease. The rationale for adopting surrogate tests for histologic stage notwithstanding, our findings demonstrate that *serial* determination of histologic stage can predict clinical outcomes. Therefore, the fact that noninvasive tests correlate with histology at a single time point may be an inadequate justification for the adoption of such surrogate markers; ideally, the standard for such noninvasive tests should be prediction of clinical outcomes rather than correlation with histology.

We estimated fibrosis progression slope from the data and then used this estimate as an independent variable in the survival analysis for clinical outcomes. This may have resulted in standard errors that are too small and hence the p-value for the slope risk association may be too low. Given that the p-value for the association p of slope risk with clinical outcomes is

<0.0001, we would expect that even if the correct p-value is larger, the association would still be statistically significant.

The RFSP slope we observed in the total group (slope of 0.005–0.124 Ishak Units per year) is similar to that reported in the literature by others (16, 17), although such comparisons are confounded by the different ranges of categorical fibrosis scores (0 to 6-point Ishak scale versus the 0 to 4-point METAVIR scale) and two grades of cirrhosis, 5 and 6, in the Ishak scoring system compared to one in the METAVIR scale (10–13). Furthermore, in a large meta-analysis of studies of histologic progression, Thein et al (18) included reports based on both staging systems converting Ishak to METAVIR scores for the analysis; these investigators concluded that the rate of progression was variable between METAVIR stages. In the current study, the pattern of increase in fibrosis tended to be linear in each of the baseline Ishak 3–6 cohorts (Figure 1) and for individuals (Figure 2); however, extending the group linear regression line back to zero for every Ishak cohort (Figure 1 and Table 2) would yield an estimated duration of disease of >40 years. This estimate of disease duration is unrealistically long in a population whose average age was 52 years, a discrepancy noted previously by Ghany et al (16) in a similar analysis of a different patient cohort at a single clinical site. A more reasonable assumption supported by our data is that fibrosis accumulation is nonlinear (as noted by others) in transition from Ishak stages 0 to 2 but relatively linear between stages 3 and 6.

We demonstrated the importance of a threshold time interval of >4 years for accurate calculation of the individual RFSP slope among serial liver biopsies; the range of progression rates broadened and accuracy decreased as the interval among biopsies was shortened (appendix). Using Ishak scores, both Ryder et al (17) and Ghany et al (16) observed rates of fibrosis progression (0.17 and 0.12 Ishak Units per year, respectively) similar to ours of 0.12 for individual slopes (0.005–0.124 for the group slopes), although the mean time interval for slope calculation was only 44 months (3.67 years) in the Ryder study and only 2.5–3.5 years in 88% of patients in the Ghany study. Many of the individual calculated slopes in these other studies may have been inaccurate (increasing scatter around the mean slope) because of a narrow time interval (<4 years) for slope calculation, while the mean itself remained relatively accurate. Based on our observations, we recommend a minimum of 4 years for accurate individual slope calculation.

In conclusion, we have shown that fibrosis increases slowly over time in patients with chronic hepatitis C, consistent with observations in other studies. The RFSP slope correlated best with laboratory markers of hepatic injury and of the severity of chronic liver disease. The RFSP slope was a powerful predictor of clinical outcomes. Therefore, our observations support caution in rejecting the liver biopsy in favor of noninvasive tests of fibrosis until these surrogate tests are shown to predict clinical outcomes as reliably as the rate of histologic fibrosis progression.

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Abbreviations

CTP	Child-Turcotte-Pugh
HALT-C Trial	Hepatitis C Antiviral Long Term Treatment Against Cirrhosis Trial
HCC	hepatocellular carcinoma
HR	hazard ratio
RFSP	rate of hepatic fibrosis score progression

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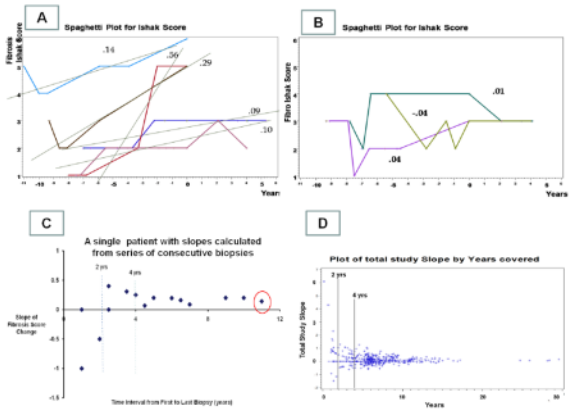
Appendix

Selection of criteria for slope calculation in individual subjects

Liver biopsy scores are categorical and, therefore, sensitive to the time interval over which the slope (rate of fibrosis score change) is calculated. To determine an adequate interval for the slope calculation that would yield a rate of progression representative of the individual patient, we reviewed the slope versus time for the eight patients with the largest number of biopsies (5–8 prestudy and on-study biopsies with a minimum of 10 years between first and last liver biopsy) and calculated the overall slope as an indication of the rate of progression. In five patients (Panel A), the rate of fibrosis increased over time, with slopes of 0.09 to 0.56 Ishak Units/year (progression to cirrhosis from 60 to 11 years), and, in three (Panel B), scores fluctuated between Ishak 2 and 4, with no significant change over time (slope −0.04 to +0.04). In these 8 patients with slopes calculated from multiple values over 10 years, we considered a slope <1.0 as a reasonable upper limit of RFSP (progression to cirrhosis in 6 years).



Then, we examined the distribution of slopes calculated from every combination of consecutive biopsies in each of these patients to determine the minimum interval between biopsies that yielded slopes similar to the slope calculated based on inclusion of all values. For example, this would yield 14 sets of RFSP slopes from 6 liver biopsies over 11 years in one of the patients in Panel A with interval versus RFSP slope in Panel C; the last value is the slope calculated based on all biopsies included. In this example, as well as in each of the eight patients, the RFSP slope was comparable to that obtained from all biopsies only when the interval between biopsies is at least 2 years and is closest to the actual slope if the interval between biopsy pairs is ≥4 years.

We then compared the distribution of individual RFSP slopes for all 457 patients with >10-mm biopsy length to the interval between first and last biopsy (Figure D) (Figure 1: 455 patients with 575 prestudy biopsies) without any interval restriction (RFSP calculated from intervals of 2 months to 24 years). The distribution of RFSP slope was similar in range to those in individual patients with biopsy intervals ≥4 years; both positive and negative slopes fall into nonrepresentative ranges when RFSP slopes are calculated from intervals of <2 years, and, therefore, we concluded these calculations were inaccurate. Thus, we used patients with a ≥4-year interval for calculatgion of RFSP slope. As a test of the impact of scatter in calculating the slope without the slope interval criterion (slopes displayed in panel D), we evaluated slope < or ≥0.2 in the 457 patients for clinical outcomes and the histological and temporal factors in Table 2. No significant correlations were found.

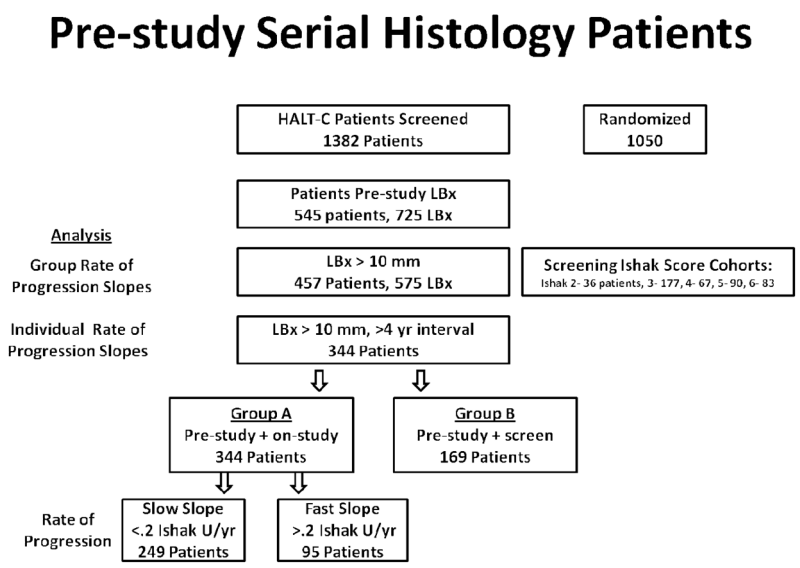
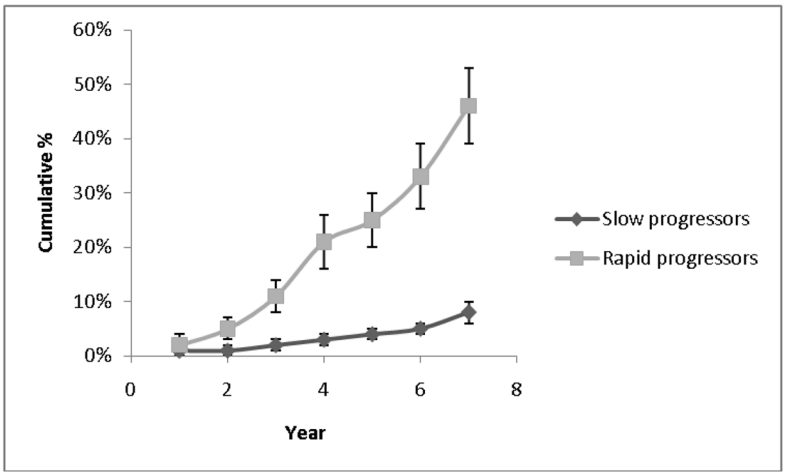


Figure 1.

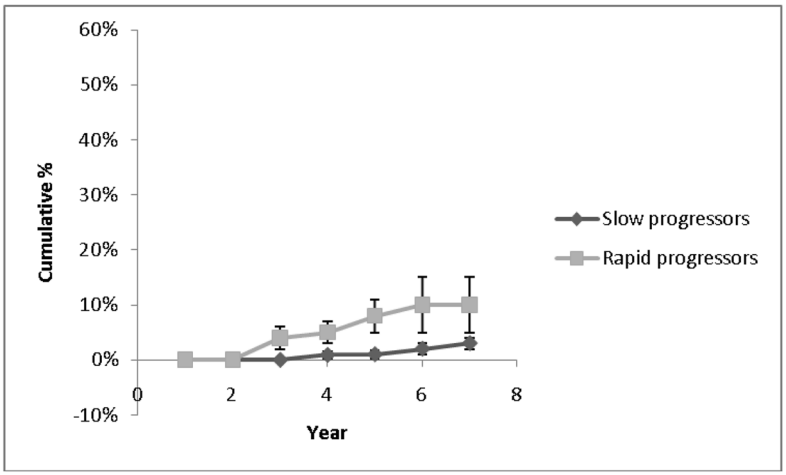
Flow diagram of prestudy serial histology patients. Of 1,050 patients enrolled, 545 had a pre-study biopsy, but in only 457 of these patients was a biopsy available longer than >10 mm, and in only 344 was the interval between the first and the HALT-C Trial screening liver biopsy (LBx) at least 4 years. The analysis performed in each group of biopsies is listed on the left side of the figure and described in the text..

Figure 2a



Log rank test of equality of distributions P <0.0001.

Figure 2b



Log rank test of equality of distributions P = 0.04.

Figure 2.

Figure 2a. The rate of clinical outcomes excluding HCC (± SEM) by rate of Ishak score progression.

Log rank test of equality of distributions, P <0.0001.

Figure 2b. Distribution of cumulative HCC incidence rates (± SEM) by rate of Ishak score progression.

Log rank test of equality of distributions P = 0.04.

Table 1

No. of Patients with valid prestudy liver biopsies

# Biopsies per patient	# Patients	# patients with biopsies ≥10 mm
1	426	370
2	83	67
3	22	12
4	6	5
5	5	3
6	3	0
Total Patients	545	457
Total Biopsies	725	575

Table 2

Mean Ishak fibrosis scores of prestudy (intervals of > −3 years and −3 to 0 years), screening, month 24, and month 48 biopsies and group slope of fibrosis progression for each cohort of Ishak score at screening.

Ishak Score at Screening	Time	N *	Years ± screening	Ishak score	Group Slope
2 **	>3 yr pre	21	−6.4	2.6	
	0 –3 yr pre	19	−2.0	2.1	
	Screening	36	0	2.0	.005
	Month 24	24	2.4	2.5	
	Month 48	27	4.3	2.6	
3	>3 yr pre	95	−5.8	2.6	
	0 –3 yr pre	101	−1.9	2.9	
	Screening	177	0	3.0	.062
	Month 24	133	2.3	3.0	
	Month 48	115	4.3	3.3	
4	>3 yr pre	31	−5.1	3.1	
	0 –3 yr pre	40	−1.9	3.7	
	Screening	67	0	4.0	.090
	Month 24	49	2.3	3.9	
	Month 48	45	4.3	4.0	
5	>3 yr pre	45	−6.8	3.7	
	0 –3 yr pre	50	−1.9	4.4	
	Screening	90	0	5.0	.076
	Month 24	62	2.3	4.5	
	Month 48	48	4.2	4.5	
6	>3 yr pre	45	−5.9	4.1	
	0 –3 yr pre	44	−1.7	4.9	
	Screening	83	0	6.0	.124
	Month 24	56	2.3	5.3	
	Month 48	42	4.3	5.3	

* Note that not all patients had biopsies in each period; the largest category was screening, because a screening biopsy was required for inclusion in the study.

** 36 patients were down staged after consensus group assessment from the local reading of Ishak 3 required for inclusion in the study.

Table 3

Screening characteristics of Group-A participants by slow or fast fibrosis progression rate.

Participant characteristics	Ishak Fibrosis Score Rate of Progression		P-value
	Slow (slope ≤0.2) N=249	Fast (slope >0.2) N=95	
	Mean (s.d)	*Mean (s.d)*	
Age (yr)	50.7 (6.9)	50.2 (6.6)	0.56[1]
Duration of Infection (years)	28.6 (8.7)	27.7 (7.6)	0.36[1]
Total lifetime alcohol (drinks)	17,276 (26,916)	15,850 (30,674)	0.67[1]
Alk Phos ratio (ULN)	0.77 (0.30)	0.93 (0.41)	**0.0002**[1]
ALT (U/L)	99.7 (65.2)	129.2 (97.6)	**0.001**[1]
AST (U/L)	74.4 (40.3)	107.4 (73.9)	**<0.001**[1]
AST/ALT	0.82 (0.25)	0.90 (0.28)	**0.01**[1]
Serum Ferritin (ng/mL)	313.6 (286.7)	447.8 (509.1)	**0.002**[1]
AFP (ng/mL)	9.8 (14.2)	23.0 (38.7)	**<0.001**[1]
Albumin (g/dL)	3.98 (0.36)	3.80 (0.42)	**<0.001**[1]
Creatinine (mg/dL)	0.87 (0.16)	0.82 (0.16)	**0.009**[1]
Iron saturation	0.34 (0.13)	0.38 (0.18)	**0.04**[1]
Platelets (1000/mm^3)	188.5 (61.8)	164.9 (70.8)	**0.003**[1]
INR	1.01 (0.08)	1.05 (0.11)	**0.002**[1]
WBC (1000/mm^3)	5.88 (1.94)	5.93 (2.33)	0.84[1]
Total Bilirubin	0.74 (0.32)	0.83 (0.41)	**0.04**[1]
Log (HCV RNA)	6.5 (0.5)	6.4 (0.5)	0.27[1]
Ishak Inflammation Score	7.7 (1.4)	8.0 (1.4)	0.10[1]
Ishak Fibrosis Score			
First Prestudy Biopsy	3.1 (1.1)	2.8 (0.9)	0.06[1]
Time of initial biopsy (years)	−4.8 (3.9)	−4.7 (2.7)	0.77[1]
Screening biopsy	3.6 (1.2)	4.4 (1.2)	**<0.0001**[1]
	N (%)	*N (%)*	
Gender			0.75[2]
Men	180 (72)	67 (71)	
Women	69 (28)	28 (29)	
Race/ethnicity			0.20[2]
White, non-Hispanic	188 (76)	64 (67)	
Black	43 (17)	19 (20)	
Hispanic or other	18 (7)	12 (13)	

Participant characteristics	**Ishak Fibrosis Score Rate of Progression**		**P-value**
	Slow (slope ≤0.2) N=249	**Fast (slope >0.2) N=95**	
	Mean (s.d)	***Mean (s.d)***	
Smoking status			0.64[2]
Never smoked	58 (23)	27 (29)	
Former smoker	124 (50)	43 (46)	
Current smoker (<1 pk/day)	32 (13)	9 (10)	
Current smoker (≥1 pk/day)	35 (14)	14 (15)	
Randomization stratum			0.31[2]
Not randomized	24 (10)	12 (13)	
Lead-in	144 (58)	60 (63)	
Breakthrough/relapse	46 (18)	10 (11)	
Express	35 (14)	13 (14)	
Randomization group			
Treatment	108 (48)	35 (42)	0.36[2]
Control	117 (52)	48 (58)	
Esophageal Varices			**0.003**[2]
Yes	36 (16)	26 (32)	
No	186 (84)	56 (68)	
Decline in HCV RNA during lead-in			**0.03**[2]
<1 Log_{10}	59 (31)	34 (49)	
1 to < 2 Log_{10}	35 (18)	12 (17)	
2+ Log_{10}	96 (51)	24 (34)	

[1] P-value for test of equality of means using analysis of variance

[2] P-value for test of homogeneity of distributions using Chi-square.

Table 4

Multivariable survival analysis of clincial outcomes and HCC: adding Ishak progression to previously published models (24,25).

a. Clinical outcome (excluding HCC and death not liver related) (24);

	Published Ghany Model (24) N = 1,050			Ghany model in current data N = 308			Ghany model + slope risk N = 308			Screening Ishak Score + slope risk N = 308		
	HR	95%CI	P-value	HR	95%CI	P-value	HR	95%CI	P-value	HR	95% CI	P-value
Log(AST/ALT)	3.06	1.69,5.55	0.0002	4.41	0.38,51.8	0.24	4.02	0.37,44.2	0.25			
Albumin	0.21	0.13,0.33	<0.0001	0.33	0.14,0.78	0.01	0.34	0.13,0.88	0.03			
Platelet count	0.66	0.53,0.81	<0.0001	0.91	0.68,1.24	0.56	0.91	0.671.22	0.52			
Bilirubin	1.92	1.44,2.55	<0.0001	2.14	1.06,4.34	0.03	2.29	1.14,4.59	0.02			
Ishak Score	1.31	1.12,1.53	0.001	1.47	1.11,1.95	0.008	1.32	0.99,1.75	0.06	1.62	1.25,2.09	0.0002
Slope risk							4.88	2.58,9.23	<0.0001	5.54	2.92,10.5	<0.0001

b. HCC (25);

	Published Lok Model (25) N = 1,050		Lok model in current data N = 304			Lok model + slope risk N = 304			Ishak Score + slope risk N = 308		
	HR	P-value	HR	95%CI	P-value	HR	95%CI	P-value	HR	95% CI	P-value
Age	1.04	0.03	1.05	0.99,1.12	0.11	1.05	0.99,1.13	0.09			
Alkaline Phosphatase	1.003	0.001	1.79	0.57,5.59	0.32	1.58	0.47,5.30	0.46			
Platelet count	0.98	0.002	0.98	0.97,0.99	0.003	0.98	0.97,0.995	0.006			
Esophageal Varices	1.97	0.03	1.49	0.49,4.54	0.56	1.14	0.35,3.75	0.83			
Ishak Score									1.41	0.93,2.15	0.11
Slope risk						2.38	0.81,7.05	0.12	3.06	1.07,8.73	0.04