Serum Fibrosis Markers are Associated With Liver Disease Progression in Non-responder Patients With Chronic Hepatitis C

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Serum fibrosis markers are associated with liver disease progression in non-responder patients with chronic hepatitis C

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Section B.

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Abstract

OBJECTIVES—The aim of this study was to explore the association of serum fibrosis marker levels with the risk of clinical and histological disease progression in a large cohort of patients with chronic hepatitis C (CHC).

DESIGN/SETTING—462 prior non-responders to peginterferon and ribavirin enrolled in the randomized phase of the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial had baseline and annual serum samples tested for hyaluronic acid (HA), n-terminal peptide of procollagen type 3, tissue inhibitor of matrix metalloproteinase-1, and YKL-40.

OUTCOME MEASURES—All patients underwent a pretreatment liver biopsy and follow-up biopsies at years 2 and 4. Histological progression was defined as a ≥ 2 point increase in Ishak fibrosis score in non-cirrhotic patients. Clinical outcomes included development of decompensation, hepatocellular cancer, death, or an increase in the CTP score to ≥ 7.

RESULTS—Mean patient age was 49.5 years and 39% had histological cirrhosis at entry. Baseline HA, YKL-40 and TIMP-1 levels combined with other laboratory parameters were all significantly associated with clinical outcomes in the 69 (15%) patients with disease progression (p < 0.0001). The best multivariate model to predict clinical outcomes included baseline bilirubin, albumin, INR, and YKL-40 levels. All of the baseline serum fibrosis marker levels were also significantly associated with histological fibrosis progression that developed in 70 (33%) of the 209 non-cirrhotic patients (p < 0.0001). However, baseline HA and platelet counts were best at predicting histological progression (AUC = 0.663).

CONCLUSION—Pretreatment serum fibrosis marker levels are significantly increased in CHC patients at risk of clinical and histological disease progression. If validated in additional cohorts, measurement of these markers could help identify CHC patients who would benefit from more frequent and intensive monitoring.

Keywords
Cirrhosis; non-invasive markers; liver fibrosis; natural history; viral hepatitis

Introduction

The morbidity and mortality associated with chronic hepatitis C virus (HCV) infection is projected to substantially increase over the next two decades as the proportion of patients with cirrhosis, decompensation, and hepatocellular carcinoma (HCC) increases.[1,2] Management guidelines recommend obtaining a liver biopsy in potential antiviral treatment candidates to grade and stage the severity of liver disease and assist with decision making.
However, due to the risks and sampling variability of liver biopsy, accurate and reliable non-invasive means to assess patients with chronic hepatitis C (CHC) at increased risk of developing worsening hepatic fibrosis are needed. Numerous biochemical indices, serum fibrosis marker algorithms and liver elasticity measurements have been proposed to assess disease severity non-invasively in patients with CHC. However, most of these modalities have not been tested or validated in a large group of patients with CHC who were prospectively followed for histological and/or clinical liver disease progression.

Administration of “maintenance” interferon to patients with CHC who failed to respond to a course of antiviral therapy may slow the rate of liver disease progression. As a result, several large randomized controlled studies were initiated in prior non-responders to determine if maintenance interferon could reduce the rate of clinical decompensation and histological disease progression. In the Hepatitis C Antiviral Long-term Treatment against Cirrhosis Trial (HALT-C), the rate of histological disease progression and clinical outcomes were similar in the 1050 patients with advanced fibrosis that were randomized to receive peginterferon or no additional therapy and followed over 3.5 years. Additional studies were carried out at four HALT-C Trial clinical sites to determine if serum fibrosis marker levels would correlate with initial disease severity and disease progression over time. In our analysis, we detected significant associations between pretreatment serum hyaluronic acid (HA), tissue inhibitor matrix metalloproteinase-1 (TIMP-1), YKL-40, and n-terminal peptide of procollagen type 3 (PIIINP) levels and baseline disease severity. In addition, all of these marker levels significantly declined at week 72 compared to pretreatment baseline in subjects who achieved a sustained virological response following 48 weeks of full dose peginterferon and ribavirin treatment suggesting that these analytes reflect active fibrogenesis and fibrolysis. In the current study, pretreatment baseline serum fibrosis marker levels were tested as potential predictors of clinical and histological disease progression during the randomized phase of the HALT-C Trial. We also hypothesized that the levels of these analytes would increase over time in subjects who experienced clinical or histological disease progression compared to patients who did not progress.

Methods

Patient population

Randomized HALT-C Trial patients had detectable serum HCV RNA and bridging hepatic fibrosis (i.e. Ishak fibrosis score ≥ 3) or cirrhosis on liver biopsy obtained within 12 months of enrollment and had failed to achieve a sustained virologic response to a prior course of peginterferon and ribavirin. Initially, subjects were retreated with peginterferon alfa-2a and ribavirin for 24 weeks in the “lead-in phase” of the study. Subjects who remained viremic at week 20 were eligible for randomization at week 24 to maintenance peginterferon versus no treatment for 3.5 years; subjects with undetectable HCV RNA at week 20, as determined by polymerase chain reaction (PCR) assay (Roche Molecular Systems, COBAS Amplicor v 2.0, sensitivity of 100 IU/ml) continued in the “responder arm” of the study and completed a 48 week course of combination antiviral treatment. Patients who experienced an on-treatment breakthrough or post-treatment virological relapse following the responder arm were also eligible for enrollment. Finally, express patients treated with at least 12 weeks of peginterferon and ribavirin without viral clearance were eligible for randomization. All HALT-C Trial participants entering the randomized phase at the University of Michigan, University of Massachusetts/ University of Connecticut, Massachusetts General Hospital and Virginia Commonwealth University, had serum collected at baseline and study months 12, 24, 36, and 48 following randomization. Serum isolated from whole blood samples was frozen immediately at −80°C and stored at a central repository (SeraCare, Gaithersburg,
Laboratory and clinical assessment during the randomized phase—Lifetime alcohol consumption was estimated with a modification of the Skinner survey.[18] Routine baseline laboratory values (i.e. serum AST, ALT, albumin, bilirubin, platelet count) were obtained at local hospital laboratories. Insulin resistance based on HOMA-IR was calculated as HOMA= [(insulin* glucose)/ 22.5]*0.5551. A baseline liver biopsy was scored for the degree of hepatic fibrosis and inflammation defined by the Ishak scoring system and the degree of hepatic steatosis was estimated as grade 0 to 4.[21,22] Splenomegaly was defined by a spleen length > 13 cm on sonography.

All patients were seen every 3 months during the randomized phase for laboratory and clinical assessment. In addition, annual liver ultrasounds were obtained to screen for HCC and serum alpha-fetoprotein levels were obtained every 3 months. Clinical endpoints for the study included an increase in CTP score to ≥ 7 on two separate occasions 3 months apart, variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, HCC, or death. For the subgroup of patients with non-cirrhotic fibrosis at baseline, histologic progression was defined as a ≥2 point increase in the Ishak fibrosis score.[18]

There were 624 patients enrolled in the randomized phase of the HALT-C Trial at the four participating sites. The baseline features of the 462 patients with sufficient serum samples for analysis in the current study were similar to the 162 excluded patients including the frequency of cirrhosis and likelihood of disease progression over time. However, the excluded patients were more likely to be Caucasian (86% vs 73%) and less likely to be Black (9% vs 24%), p=0.0001.

Serum fibrosis marker assays

Stored serum samples were tested for TIMP-1 (normal range: 80 to 500 ng/ml) (Quantikine, R & D Systems, Minneapolis, MN), YKL-40 (normal range: 24 to 125 ug/l) (Mitra YKL-40, Quidel Corp, San Diego, CA), and PIIINP (normal range: 2 to 4 ug/l) (UniQ, Orion Diagnostica, Espoo, Finland) using commercially available ELISAs as described previously.[10] Samples that exceeded the upper limit of quantitation were retested using a 1:10 dilution. Serum HA levels were determined by an automated liquid-phase immunoassay using the LiBASys Analyzer (Wako Diagnostics, Richmond, VA) (normal range: 10 to 100 ng/ml) (10). Serum was available for testing in 421 patients at month 12, 404 patients at month 24, 380 patients at month 36, and 364 patients at month 48.

Statistical analyses

Log transformation of non-normally distributed variables was undertaken when needed. When considering all four serum fibrosis markers to predict clinical and histological progression, first, a model adjusting for all non-invasive predictors of clinical progression (variables for which p < 0.20 as per Table 1) as well as race, age, gender and BMI was fit. Then, from this model, only variables for which p < 0.05 were kept and this became the final clinical/lab multivariable model of each serum fibrosis marker. Then, we adjusted for all histologic variables (e.g. cirrhosis, hepatic steatosis) and generated the final model including only significant variables. In addition, a multivariate model that included all of the baseline serum fibrosis marker levels as well as other factors significantly associated with the outcome was constructed to determine the best overall model. For time to first clinical outcome, Cox proportional hazards regression was used and data were censored at the patient’s last follow-up visit or at 1400 days (i.e. 3.8 years) after randomization, whichever occurred first. For time to histological progression, complementary log-log regression
analysis was used. The Akaike’s information criterion (AIC) statistic was used to compare the goodness of fit of individual models to each other and the model with the lowest AIC was considered the best.[23] To demonstrate that the model score is associated with outcome, patients were split into three groups (low, medium and high risk) according to the 75th and 90th percentiles of the distribution of scores from the multivariate model. To assess changes in individual analyte levels over time, random effects models (via SAS’s proc mixed) were used. Specifically, the models included the random effect of time using a repeated statement, as well as the interaction of time with treatment group. All analyses were performed at the data coordinating center (New England Research Institutes, Watertown, MA) with SAS statistical software version 9.2 (SAS Institute, Cary, NC).

Results

Overall study population

The mean age of the 462 HALT-C patients was 49.5 years, 70% were male, 73% were Caucasian, and 49% were randomized to receive low-dose peginterferon (Table 1). Overall, 28% had diabetes mellitus, the mean BMI was 29.9 kg/m², and 39% had histological cirrhosis.

Baseline predictors of clinical outcomes

During a mean follow-up of 51 months, a primary clinical outcome developed in 69 (15%) of the 462 patients, including a CTP score increase (n=40), HCC (n=7), ascites (n=32), encephalopathy (n=15), variceal bleeding (n=8), and death (n=24). Because the rate of outcomes were similar in the peginterferon and untreated- control patients, the two groups were combined for this analysis. As anticipated, patients that developed a primary clinical outcome had baseline features suggestive of more severe liver disease with a higher likelihood of having cirrhosis on biopsy, higher bilirubin and INR levels, and lower albumin and platelet counts (Table 1). In addition, the pretreatment levels of HA, YKL-40, PIIINP, and TIMP-1 were all significantly higher among the subjects who eventually developed a primary clinical outcome compared to those without clinical progression. The overall rate of clinical outcomes was 27.5% in patients with baseline cirrhosis and 6.8% in patients with non-cirrhotic fibrosis.

Multivariate models of clinical outcomes

The utility of individual serum fibrosis marker levels in combination with other baseline parameters were determined using multivariate regression analysis. Although baseline PIIINP levels were significantly associated with clinical outcomes on univariate analysis (Table 1), only baseline bilirubin, INR, and albumin levels remained associated in a multivariate model that included PIIINP (Supplemental Table 1). In contrast, when baseline TIMP-1 levels were combined with other baseline variables, a multivariate model consisting of baseline TIMP-1, bilirubin, INR, and albumin levels was generated. Similarly, when baseline HA levels were combined with other significant variables from Table 1, a multivariate model consisting of HA, bilirubin, INR, and albumin levels was generated. Finally, when baseline YKL-40 levels were combined with other variables, a multivariate model consisting of YKL-40, bilirubin, albumin, and INR levels was generated.

In addition to the above multivariate models, a final model that included all 4 baseline serum fibrosis marker levels entered together with other significant parameters from Table 1 was constructed. This exercise confirmed a final model consisting of baseline total bilirubin, albumin, INR, and YKL-40 levels for predicting clinical outcomes which had the lowest AIC compared to the other multivariate models. Inclusion of diabetes mellitus and baseline Ishak fibrosis and hepatic steatosis scores did not improve the performance of the model.
with the AIC remaining unchanged (data not shown). The equation describing the risk of developing a clinical outcome is \( \text{Clinical Risk score} = 0.883 \times \text{(Total bilirubin)} + 0.809 \times (\text{INR} > 1.0) - 1.63 \times (\text{albumin}) + 0.89 \times (\text{Log YKL-40}) \).

The output of this model can be divided into low (score < −2.5), medium (score between −2.5 and −1.7), and high risk (score > −1.7) for the development of a clinical outcome. Per Figure 1, only 8% of the 324 HALT-C Trial patients with a low risk score would be expected to experience a primary clinical outcome compared to 30% of the 65 patients with an intermediate risk score and 65% of the 44 patients with a high risk score. For example, a patient with a total bilirubin of 0.7 mg/dl, INR of 1.1, albumin of 3.8 g/dl, and a YKL-40 level of 550 ng/ml has a risk score of −2.32 and would be at intermediate risk for the development of a clinical outcome during follow-up.

The model derived from our data set (Model 1) was compared to a model that incorporates serum PIIINP, TIMP-1, and HA levels into an algorithm called the “Enhanced liver fibrosis score” or ELF score. The AIC of Model 1 which reflects its overall goodness of fit for predicting outcomes was 710, while the AIC for the ELF score was 760 indicating that Model 1 explains more of the variation in clinical outcomes than the ELF score.

**Serum fibrosis marker levels over time**

Serial YKL-40 levels in patients who progressed clinically were compared to levels in patients who did not progress using random effects modeling. As seen in figure 2A, the YKL-40 levels increased in both groups of patients over time (p =0.0026) and were significantly higher in the progressors (P< 0.0001). In addition, minimal overlap was observed in the values of progressors compared to those who did not progress, suggesting that assessment of this fibrosis marker over time may prove useful in identifying patients at high risk for disease progression.

The change in baseline PIIINP, TIMP-1, and HA levels over time were also analyzed using random effects modeling. Per Figures 2B–2D, the mean PIIINP, TIMP-1, and HA levels all changed significantly over time both in patients who progressed clinically and in those that did not (P< 0.0001). In addition, the mean values of PIIINP, TIMP-1, and HA were consistently higher in the patients who progressed compared to patients who did not (P < 0.0001). Finally, the changes over time of PIIINP, TIMP-1, and HA were greater in the progressors compared to non-progressors (P< 0.0001).

**Peginterferon therapy and serum fibrosis marker levels**

Serum fibrosis marker levels during the randomized phase of the HALT-C Trial were analyzed by treatment group to determine if the patients receiving low-dose peginterferon had different levels compared to the untreated controls. Both YKL-40 and TIMP-1 levels changed significantly over time in the peginterferon and untreated- control patients (p < 0.0001 for both) but did not differ significantly between the two study groups (YKL-40 p= 0.230, TIMP-1, p=0.34) (Figures 3A, 3B). In contrast, while PIIINP and HA levels changed significantly over time in treated and untreated patients (p < 0.0001), the increase in levels of these two markers was significantly higher in the peginterferon-treated patients (p < 0.0001 for both) (Figure 3C & 3D). In addition, PIIINP levels in treated patients were lower at month 54 compared to month 48 (7.1 vs 7.5 ng/ml) while PIIINP levels were higher in the untreated patients at month 54 vs month 48 (7.6 vs 6.9 ng/ml). In addition, at month 54, HA levels in the treated patients decreased compared to month 48 (1.99 vs 2.18 ng/ml) and HA levels also decreased in the untreated patients (1.93 vs 2.24 ng/ml). Thus, a non-specific increase in both serum PIIINP and HA levels was observed in the peginterferon treated
Baseline predictors of histological progression

Worsening of fibrosis defined as an increase in the Ishak fibrosis score of ≥ 2 points at month 24 or 48 compared to baseline was a primary endpoint in the HALT-C Trial for patients with non-cirrhotic fibrosis.[18] Amongst the 280 patients with a pretreatment Ishak fibrosis score of < 5, 209 had a follow-up biopsy adequate for analysis. These 209 patients had similar pretreatment characteristics to the 71 excluded patients except for older age (50.0 vs 47.6 years) and lower serum AST/ALT ratios (0.84 vs 0.91). During follow-up, 70 (34%) patients had worsening hepatic fibrosis while 139 patients had stable or unchanged Ishak fibrosis scores. In addition, a clinical outcome developed in 3 of the 70 (4.3%) patients with histological progression but in only 2 of the 139 (1.4%) histologically stable patients.

As anticipated, the 70 patients with worsening hepatic fibrosis had baseline laboratory features suggestive of more severe liver disease including significantly lower platelet counts and a trend towards higher bilirubin levels (Table 2). In addition, baseline HA, YKL-40, PIIINP, and TIMP-1 levels were all significantly higher in subjects who histologically progressed compared to those who did not. Furthermore, patients with histological progression had more severe hepatic steatosis at baseline than those who did not progress but they were equally likely to receive peginterferon versus no treatment.

Multivariate models of histological progression

Using complementary log-log regression modeling of individual serum fibrosis marker levels in combination with other laboratory parameters, baseline YKL-40 and TIMP-1 levels did not remain associated with histological progression. However, both baseline PIIINP and HA levels remained significantly associated with histological progression when they were individually combined with other laboratory parameters. When all 4 serum fibrosis marker levels were combined with other baseline laboratory parameters, a final multivariate model consisting of baseline platelet and hyaluronic acid levels was identified (Supplemental Table 1). The risk of fibrosis progression is estimated as Risk = −0.3285 * platelets + 0.8831 * logHA. The area under the receiver operator characteristic curve (AUROC) for the model was 0.663. Inclusion of baseline hepatic steatosis scores did not improve the model performance (data not shown).

Discussion

Serum fibrosis markers have been proposed as a simple and convenient means to estimate the severity of histological fibrosis in patients with liver disease. Although the markers may be effective in differentiating patients with cirrhosis from those with minimal to no fibrosis, the utility of these markers in discriminating between individual stages of fibrosis is limited.[25] Developing accurate and reliable prognostic biomarkers that are linked to clinically important milestones in liver disease progression, such as decompensation and worsening CTP scores, is also an area of active investigation.[24,26]

The HALT-C Trial provided a unique opportunity to test several serum fibrosis markers as individual or combined predictors of the risk of clinical and histological disease progression. Serum PIIINP, HA, TIMP-1, and YKL-40 levels were selected for this study because prior studies had linked them to disease severity and progression in patients with CHC.[27–30] In particular, these serum fibrosis markers were reduced in sustained virological responders compared to relapsers/non-responders suggesting that they are closely linked with hepatic.
fibrogenesis.[20,28,29] In addition, prior longitudinal studies have linked serum and liver tissue expression of YKL-40 with the risk of fibrosis progression.[30]

On univariate analysis, baseline levels of HA, PIIINP, YKL-40 and TIMP-1 were all strongly associated with the risk of a clinical outcome during follow-up (Table 1). Multivariate regression modeling demonstrated that each marker retained significance when combined with other baseline laboratory parameters except for serum PIIINP levels. Interestingly, HA, TIMP-1, and YKL-40 levels are all associated with clinical progression when combined with baseline albumin, bilirubin, and INR levels individually but the model which included YKL-40 performed the best with the lowest AIC. Application of our model to individual patients could provide stratification of the risk of clinical decompensation into low, medium, and high risk and potentially help clinicians determine which patients need closer follow-up and monitoring (Figure 1). Our study findings are consistent with other studies demonstrating the short-term prognostic utility of markers of liver synthetic function (albumin, INR) and excretory function (bilirubin) in patients with CHC and advanced fibrosis.[31–33] However, our data also demonstrates that the addition of baseline HA, TIMP-1, or YKL-40 levels to these routine laboratory parameters provides important incremental ability to predict clinical outcomes in patients with CHC.[34,35]. Furthermore, auxiliary analyses considering time-varying covariates indicate similar results, suggesting that HA, TIMP-1 and YKL-40 levels might not need to be monitored frequently over time.

The identification of baseline YKL-40 levels as an independent predictor of clinical outcomes is a novel and potentially important finding. Baseline YKL-40 levels have been associated with clinical outcomes in patients with alcoholic liver disease.[36] YKL-40 is a growth factor for fibroblasts and endothelial cells and is expressed in areas of active hepatic fibrogenesis.[37,38] In addition, YKL-40 levels strongly correlate with histological markers of stellate cell activation and the risk of rapid fibrosis progression in liver allograft recipients with recurrent hepatitis C.[39] Furthermore, functional polymorphisms in YKL-40 gene expression are associated with the severity of liver fibrosis in CHC patients further strengthening the potential importance of this protein in hepatic fibrogenesis.[40] Although serum YKL-40 levels are increased in patients with various solid organ tumors, the HALT-C Trial excluded patients with known cancer at enrollment and a review of the clinical outcomes indicates only 6 deaths in our cohort were attributed to a non-hepatic malignancy. Therefore, our data add to the growing body of literature supporting the potential utility of YKL-40 as a clinically useful biomarker for hepatic fibrosis.

Serum YKL-40 levels increased over time to a greater extent among patients in whom a clinical outcome developed compared to patients who did not progress clinically (Figure 2). In addition, serum PIIINP and TIMP-1 levels also significantly increased over time and were consistently higher in patients who clinically progressed compared to patients who did not. These data suggest that serial assessment of these markers may prove useful to clinicians following CHC patients. However, our prior study had also demonstrated a non-specific increase in PIIINP and HA levels in patients receiving full dose peginterferon and ribavirin treatment.[20] Therefore, the impact of low-dose peginterferon on serum fibrosis marker levels was explored. Both the YKL-40 and TIMP-1 levels significantly increased over time but no significant difference was noted in these levels between those who did and did not receive peginterferon (Figure 3). In contrast, the serum PIIINP and HA levels also changed significantly over time but were consistently higher in the peginterferon treated patients compared to the untreated patients. These data confirm our prior observations that peginterferon may have systemic effects on other tissues that lead to an increase in these markers over time.[41] In support of this, the PIIINP levels decreased after stopping treatment at month 54 compared to month 48 but remained unchanged or increased in the untreated patients.
Overall, our model explained more of the variation in outcomes than the ELF algorithm with a lower AIC indicative of an improved ability to predict clinical outcomes. In addition, our model performed better than a model derived from the overall HALT-C cohort that did not include serum fibrosis markers (AIC of 710 versus 715). These differences may be due to the fact that our model was derived from this subset of HALT-C Trial patients, our use of commercial assay kits versus an automated platform, and the co-linearity of the serum fibrosis marker levels with each other. The addition of liver histologic features such as baseline fibrosis and steatosis did not improve the model’s performance. However, our model was substantially better at predicting clinical outcomes compared to the baseline Ishak fibrosis score alone (AIC of 710 versus 760). Limited resources precluded us from comparing our serum fibrosis marker panel to that expected with the Fibrotest assays or other proposed algorithms and unfortunately, liver elastography was not available for use when the HALT-C trial was initiated.

The HALT-C Trial also provided a unique opportunity to explore the role of serum fibrosis marker levels in predicting the likelihood of histological disease progression over time. Baseline levels of YKL-40, PIIINP, TIMP-1 and HA were all associated with the risk of fibrosis progression on univariate analysis (Table 2). However, a multivariate model of baseline HA and platelet counts was most strongly associated with histological progression. Low platelet counts are well known to be associated with more severe hepatic fibrosis, a reflection of portal hypertension and hypersplenism as well as reduced thrombopoietin production. Similarly, baseline and serial platelet levels were reported to be associated with a higher likelihood of developing varices and death in patients with alcoholic cirrhosis. The increase in serum HA levels associated with worsening hepatic fibrosis has been attributed to reduced HA clearance by hepatic sinusoids and increased HA production by hepatic stellate cells. However, the clinical utility of this model may be limited due to the anticipated frequency of both false positive and false negative results expected with the observed AUROC. In addition, only CHC patients with advanced fibrosis were enrolled into HALT-C which may limit the generalizability of our findings.

Strengths of our study include the large number of well-characterized patients who underwent serial testing for the analytes of interest. In addition, the patients were treated in the setting of a controlled clinical trial and clinical disease progression was defined by prospectively identified, objective endpoints that were reviewed and confirmed by an independent expert committee. Lastly, histological progression was defined by a 2 point increase in Ishak fibrosis score rather than 1 fibrosis stage as used in other studies. However, the potential for sampling error and understaging of fibrosis remains possible. The generalizability of our findings to other patients with CHC and prior non-response to peginterferon and ribavirin may be limited due to the strict inclusion criteria of the HALT-C Trial. Finally, logistical constraints precluded us from obtaining direct portal pressure measurements in enrolled patients to compare with our serum fibrosis marker data. Still, this study represents the largest and longest prospective assessment of serum fibrosis markers in association with liver disease progression in patients with CHC reported to date.

In summary, pretreatment levels of TIMP-1, YKL-40, and HA in combination with baseline albumin, bilirubin, and INR enhance the ability to identify patients with CHC who are at increased risk of disease progression. In our final model that includes pretreatment serum YKL-40 levels, the risk of disease progression could be stratified into low, medium, and high risk groups. The observed increase in YKL-40 levels and TIMP-1 levels in patients who progress clinically compared to patients who do not further demonstrates the potential utility of these fibrosis markers in tracking patients with CHC at risk for disease progression. However, validation of our exploratory models in independent patient cohorts that are longitudinally followed is needed as well as in a group of CHC patients with a broader...
distribution of fibrosis severity. If validated, measurement of serum fibrosis marker levels in conjunction with standard laboratory parameters may help clinicians identify which patients with CHC need closer follow-up and monitoring.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**Abbreviations**

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<th>Abbreviation</th>
<th>Description</th>
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</thead>
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<tr>
<td>AIC</td>
<td>Akaike’s information criterion</td>
</tr>
<tr>
<td>AUROC</td>
<td>Area under the receiver operator curve</td>
</tr>
<tr>
<td>CHC</td>
<td>Chronic hepatitis C</td>
</tr>
<tr>
<td>CTP</td>
<td>Child-Turcotte-Pugh</td>
</tr>
</tbody>
</table>

_Gut._ Author manuscript; available in PMC 2013 August 10.
ELF  Enhanced liver fibrosis score
HA   Hyaluronic acid
HALT-C Hepatitis C Antiviral Long-term Treatment against cirrhosis
HCC  Hepatocellular carcinoma
HCV  Hepatitis C virus
PIIINP n-terminal peptide of procollagen type III
TIMP-1 Tissue inhibitor of matrix metalloproteinase

References


Summary Box

What is already known about this subject.
- Progression of hepatic fibrosis is a key determinant of the natural history of CHC which is a leading cause of liver-related morbidity and mortality worldwide.
- Serum fibrosis markers have been proposed to non-invasively assess disease severity in CHC patients but have not been validated against clinical and/or histological disease progression in a large group of well-characterized CHC patients prospectively followed.

What are the new findings:
- An algorithm consisting of baseline YKL-40 and other routine laboratory parameters was strongly associated with the likelihood of clinical disease progression in 462 CHC patients with advanced fibrosis and prior non-response to antiviral therapy that were followed over 3.5 years.
- Consistent increases in serum YKL-40 and TIMP-1 levels over time in patients with clinical disease progression compared to non-progressors demonstrates the potential utility of these markers to non-invasively monitor CHC patients.
- Baseline hyaluronic acid levels combined with platelet counts were associated with the risk of histological fibrosis progression.

How might these findings impact clinical practice in the foreseeable future:
- Measurement of serum fibrosis marker levels in combination with routine laboratory parameters may help clinicians identify which CHC patients need closer follow-up and monitoring.
Figure 1.
Risk of developing a primary clinical outcome during follow-up. The 324 HALT-C Trial patients with a risk score of under −2.5 have a low risk of a clinical outcome (8%) while the 44 patients with a risk score > −1.7 are at the highest risk of a clinical outcome (65%) and would warrant the most intensive monitoring.
The graph shows the mean log YKL-40 (µg/l) over time (months) for two groups: those with no primary outcome and those with a primary outcome. The data points are as follows:

- No Primary Outcome:
  - 0 months: 383
  - 12 months: 348
  - 24 months: 346
  - 36 months: 333
  - 48 months: 313

- Have Primary Outcome:
  - 0 months: 68
  - 12 months: 61
  - 24 months: 55
  - 36 months: 43
  - 48 months: 29

The graph indicates a generally increasing trend in YKL-40 levels over time for both groups, with slightly higher levels in the Have Primary Outcome group compared to the No Primary Outcome group.
Gut. Author manuscript; available in PMC 2013 August 10.
Figure 2.
Serum fibrosis marker levels in HALT-C patients with and without clinical progression. 2A). Serum YKL-40 levels significantly changed over time in the 69 patients with disease progression and in the 393 without disease progression (p= 0.0026). YKL-40 levels also differed significantly in the progressors compared to the non-progressors (p< 0.0001), but the changes over time did not differ by outcome group (p= 0.21). 2B). Serum TIMP-1 levels also changed significantly over time (p< 0.0001), were significantly different in progressors compared to non-progressors (p < 0.0001), and the changes over time did differ by outcome group (p=0.0023). 2C). Serum PIIINP levels changed significantly over time (p < 0.001), were significantly different in the two outcome groups (p < 0.0001), and the changes over time did differ by outcome group (p < 0.0001). 2D). Serum HA levels significantly changed over time (p< 0.0001), were significantly different in the two outcome groups (p< 0.0001) and also differed over time by outcome group (p = 0.0111).
Figure 3.
Serum fibrosis marker levels over time by treatment group. 3A) Serum YKL-40 levels changed significantly over time in both groups of patients (p< 0.0001) but the changes did not differ by treatment group (p=0.23). 3B) Serum TIMP-1 levels changed significantly over time (p< 0.0001), but the changes did not differ by treatment group (p= 0.34). 3C). Serum PIIINP levels increased significantly over time in the two treatment groups (p< 0.0001). The levels were higher in the peginterferon-treated patients (p= 0.0015) and the changes over time were greater in the peginterferon treated patients (p< 0.0001). 3D). Serum HA levels also changed significantly over time in the two patient groups (p< 0.0001), were higher in the peginterferon treated patients (p= 0.0007), and the changes over time were greatest in the peginterferon group (p< 0.0001).
Table 1

Pretreatment baseline characteristics of the 462 HALT-C Trial patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients N=462</th>
<th>With Clinical progression n=69</th>
<th>Without clinical progression n=393</th>
<th>p-value *</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>49.5 (6.95)</td>
<td>50.0 (6.58)</td>
<td>49.5 (7.02)</td>
<td>0.78</td>
<td>1.05 (0.75, 1.47)</td>
</tr>
<tr>
<td>% Male</td>
<td>70.3%</td>
<td>72.5%</td>
<td>70%</td>
<td>0.74</td>
<td>1.09 (0.65, 1.86)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Hispanic</td>
<td>3.7%</td>
<td>1.4%</td>
<td>4.1%</td>
<td>0.40</td>
<td>0.60 (0.28, 0.87)</td>
</tr>
<tr>
<td>% Black</td>
<td>23.8%</td>
<td>23.2%</td>
<td>23.9%</td>
<td>0.90</td>
<td>0.52 (0.24, 1.58)</td>
</tr>
<tr>
<td>% Caucasian</td>
<td>72.5%</td>
<td>75.4%</td>
<td>72%</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Diabetes mellitus</td>
<td>27.9%</td>
<td>42%</td>
<td>25.4%</td>
<td>0.0042</td>
<td>2.01 (1.25, 3.24)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>29.9 (5.55)</td>
<td>29.9 (5.48)</td>
<td>30 (5.57)</td>
<td>0.97</td>
<td>1.00 (0.80, 1.25)</td>
</tr>
<tr>
<td>% Truncal obesity</td>
<td>51.1%</td>
<td>48.5%</td>
<td>51.6%</td>
<td>0.64</td>
<td>0.89 (0.55, 1.43)</td>
</tr>
<tr>
<td>% Current smoker</td>
<td>31.1%</td>
<td>36.2%</td>
<td>30.2%</td>
<td>0.19</td>
<td>1.39 (0.85, 2.27)</td>
</tr>
<tr>
<td>Total lifetime drinks</td>
<td>18,514 (28,053)</td>
<td>19,097 (23,846)</td>
<td>18,412 (28,757)</td>
<td>0.66</td>
<td>1.02 (0.93, 1.12)</td>
</tr>
<tr>
<td><strong>HCV parameters</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Prior HALT-C status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.114</td>
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<tr>
<td>% Non-responder</td>
<td>62.3%</td>
<td>66.7%</td>
<td>61.6%</td>
<td>0.90</td>
<td>0.53 (1.23, 1.53)</td>
</tr>
<tr>
<td>% Breakthru/relapse</td>
<td>13.6%</td>
<td>5.8%</td>
<td>15%</td>
<td>0.32</td>
<td>0.11 (0.37, 0.95)</td>
</tr>
<tr>
<td>% Express</td>
<td>24%</td>
<td>27.5%</td>
<td>23.4%</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Log HCV RNA (IU/ml)</td>
<td>6.46 (0.51)</td>
<td>6.24 (0.45)</td>
<td>6.5 (0.51)</td>
<td>&lt;0.0001</td>
<td>0.46 (0.32, 0.66)</td>
</tr>
<tr>
<td>% HCV genotype 1</td>
<td>93.9%</td>
<td>95.7%</td>
<td>93.6%</td>
<td>0.74</td>
<td>1.21 (0.38, 3.86)</td>
</tr>
<tr>
<td>% Peginterferon arm</td>
<td>49.4%</td>
<td>52.2%</td>
<td>48.9%</td>
<td>0.62</td>
<td>1.13 (0.70, 1.81)</td>
</tr>
<tr>
<td><strong>Laboratory parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum AST/ALT</td>
<td>0.90 (0.28)</td>
<td>1.09 (0.33)</td>
<td>0.86 (0.26)</td>
<td>&lt;0.0001</td>
<td>6.54 (3.63, 11.77)</td>
</tr>
<tr>
<td>Alk phos ratio (ULN)</td>
<td>0.91 (0.46)</td>
<td>1.14 (0.55)</td>
<td>0.87 (0.43)</td>
<td>&lt;0.0001</td>
<td>2.14 (1.55, 2.96)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.74 (0.37)</td>
<td>0.90 (0.48)</td>
<td>0.71 (0.35)</td>
<td>&lt;0.0001</td>
<td>2.68 (1.64, 4.38)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>All patients N=462</td>
<td>With Clinical progression n=69</td>
<td>Without clinical progression n=393</td>
<td>p-value *</td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------------------</td>
<td>-------------------------------</td>
<td>-----------------------------------</td>
<td>-----------</td>
<td>----------------------</td>
</tr>
<tr>
<td>INR (% &gt; 1)</td>
<td>33.1%</td>
<td>63.8%</td>
<td>27.7%</td>
<td>&lt;0.0001</td>
<td>0.24 (0.15, 0.40)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.83 (0.37)</td>
<td>3.53 (0.41)</td>
<td>3.88 (0.33)</td>
<td>&lt;0.0001</td>
<td>0.091 (0.05, 0.16)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.84 (0.16)</td>
<td>0.84 (0.18)</td>
<td>0.84 (0.16)</td>
<td>0.91</td>
<td>0.92 (0.20, 4.16)</td>
</tr>
<tr>
<td>Platelets (10^3/ml)</td>
<td>170.6 (67.5)</td>
<td>131.1 (70)</td>
<td>177.6 (64.7)</td>
<td>&lt;0.0001</td>
<td>0.98 (0.98, 0.99)</td>
</tr>
<tr>
<td>Log YKL-40 (ug/l)</td>
<td>2.45 (0.41)</td>
<td>2.69 (0.38)</td>
<td>2.40 (0.40)</td>
<td>&lt;0.0001</td>
<td>4.82 (2.69, 8.63)</td>
</tr>
<tr>
<td>PIIINP (ug/l)</td>
<td>6.19 (2.64)</td>
<td>7.36 (2.94)</td>
<td>5.99 (2.53)</td>
<td>&lt;0.0001</td>
<td>1.16 (1.08, 1.24)</td>
</tr>
<tr>
<td>TIMP-1 (ng/ml)</td>
<td>237.34 (65.7)</td>
<td>267.28 (79.4)</td>
<td>232.01 (61.6)</td>
<td>&lt;0.0001</td>
<td>1.08 (1.04, 1.11)</td>
</tr>
<tr>
<td>Log HA (ng/ml)</td>
<td>1.96 (0.43)</td>
<td>2.23 (0.46)</td>
<td>1.91 (0.41)</td>
<td>&lt;0.0001</td>
<td>4.79 (2.82, 8.13)</td>
</tr>
<tr>
<td>% Cirrhosis (Ishak 5/6)</td>
<td>39.4%</td>
<td>72.5%</td>
<td>33.6%</td>
<td>&lt;0.0001</td>
<td>4.48 (2.64, 7.61)</td>
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<tr>
<td>% Hepatic steatosis ≥ 2</td>
<td>44.2%</td>
<td>46.4%</td>
<td>43.8%</td>
<td>0.65</td>
<td>1.12 (0.70, 1.79)</td>
</tr>
</tbody>
</table>

* Hazard ratio for lifetime drinks is for a change of 10,000 units.

** Hazard ratio for age is per 10 years

^ Hazard ratio for BMI per 5 kg/m^2

# Hazard ratio for TIMP-1 is for every 10 ng/ml Data reported as mean ± std dev (in parentheses) or %

* comparing clinical progressors vs non-progressors
Table 2

Pretreatment characteristics of the 209 HALT-C Trial patients included in the histological progression analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients N=209</th>
<th>With Histological progression n=70</th>
<th>Without Histological progression n=139</th>
<th>p-value</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>50.0 (6.45)</td>
<td>49.4 (5.4)</td>
<td>50.3 (6.9)</td>
<td>0.34</td>
<td>0.98 (0.95, 1.02)</td>
</tr>
<tr>
<td>% Male</td>
<td>72.2%</td>
<td>72.9%</td>
<td>71.9%</td>
<td>0.82</td>
<td>1.06 (0.54, 1.59)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Hispanic</td>
<td>2.4%</td>
<td>2.9%</td>
<td>2.2%</td>
<td>0.19</td>
<td>1.19 (0.24, 2.14)</td>
</tr>
<tr>
<td>% Black</td>
<td>23.9%</td>
<td>17.1%</td>
<td>27.3%</td>
<td>0.72</td>
<td>0.72 (0.11, 1.32)</td>
</tr>
<tr>
<td>% Caucasian</td>
<td>73.7%</td>
<td>80%</td>
<td>70.5%</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Diabetes mellitus</td>
<td>23.4%</td>
<td>27.1%</td>
<td>21.6%</td>
<td>0.39</td>
<td>1.26 (0.73, 1.79)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.9 (5.5)</td>
<td>30.1 (5.7)</td>
<td>29.8 (5.4)</td>
<td>0.82</td>
<td>1.00 (0.96, 1.05)</td>
</tr>
<tr>
<td>% Truncal obesity</td>
<td>52.5%</td>
<td>52.9%</td>
<td>52.2%</td>
<td>0.91</td>
<td>0.97 (0.50, 1.44)</td>
</tr>
<tr>
<td>% Current smoker</td>
<td>27.3%</td>
<td>27.1%</td>
<td>27.3%</td>
<td>0.92</td>
<td>1.03 (0.50, 1.55)</td>
</tr>
<tr>
<td>Total lifetime drinks</td>
<td>15840 (21074)</td>
<td>12951 (17143)</td>
<td>17295 (22717.73)</td>
<td>0.19</td>
<td>0.91 (0.78, 1.05)</td>
</tr>
<tr>
<td><strong>HCV parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior HALT-C status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>% Non-responder</td>
<td>57.4%</td>
<td>54.3%</td>
<td>59%</td>
<td>0.93</td>
<td>(0.60, 1.25)</td>
</tr>
<tr>
<td>% Breakthru/relapse</td>
<td>17.7%</td>
<td>15.7%</td>
<td>18.7%</td>
<td>0.85</td>
<td>(0.41, 1.28)</td>
</tr>
<tr>
<td>% Express</td>
<td>24.9%</td>
<td>30%</td>
<td>22.3%</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Log HCV RNA (IU/ml)</td>
<td>6.58(0.46)</td>
<td>6.52(0.50)</td>
<td>6.61(0.45)</td>
<td>0.1832</td>
<td>0.71 (0.21, 1.21)</td>
</tr>
<tr>
<td>% HCV genotype 1</td>
<td>95.2%</td>
<td>92.8%</td>
<td>96.4%</td>
<td>0.25</td>
<td>0.58 (0.33, 1.50)</td>
</tr>
<tr>
<td>% Peginterferon arm</td>
<td>47.4%</td>
<td>44.3%</td>
<td>48.9%</td>
<td>0.66</td>
<td>0.90 (0.43, 1.37)</td>
</tr>
<tr>
<td><strong>Laboratory parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum AST/ALT</td>
<td>0.84(0.23)</td>
<td>0.87(0.26)</td>
<td>0.82(0.21)</td>
<td>0.0968</td>
<td>2.19 (1.27, 3.12)</td>
</tr>
<tr>
<td>Alk phos ratio (ULN)</td>
<td>0.84(0.45)</td>
<td>0.93(0.57)</td>
<td>0.80(0.37)</td>
<td>0.0661</td>
<td>1.47 (1.06, 1.89)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.71(0.37)</td>
<td>0.77(0.45)</td>
<td>0.68(0.32)</td>
<td>0.1711</td>
<td>1.66 (1.06, 2.27)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>All patients N=209</td>
<td>With Histological progression n=70</td>
<td>Without Histological progression n=139</td>
<td>p-value *</td>
<td>Hazard Ratio (95% CI)</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>--------------------</td>
<td>-----------------------------------</td>
<td>----------------------------------------</td>
<td>-----------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>INR (% &gt; 1)</td>
<td>20.1%</td>
<td>21.4%</td>
<td>18.4%</td>
<td>0.73</td>
<td>1.11 (0.54, 1.68)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.93 (0.31)</td>
<td>3.91 (0.35)</td>
<td>3.94 (0.29)</td>
<td>0.69</td>
<td>0.86 (0.09, 1.63)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.84 (0.16)</td>
<td>0.83 (0.14)</td>
<td>0.85 (0.17)</td>
<td>0.38</td>
<td>0.52 (0.12, 2.24)</td>
</tr>
<tr>
<td>Platelets (10^3/ml)</td>
<td>190 (60)</td>
<td>172 (65)</td>
<td>200 (54)</td>
<td>0.0008</td>
<td>0.68 (0.46, 0.91)</td>
</tr>
<tr>
<td>Log YKL-40 (ug/l)</td>
<td>2.38 (0.38)</td>
<td>2.46 (0.37)</td>
<td>2.33 (0.38)</td>
<td>0.00204</td>
<td>2.01 (1.42, 2.60)</td>
</tr>
<tr>
<td>PIIINP (ug/l)</td>
<td>5.65 (2.43)</td>
<td>6.44 (2.61)</td>
<td>5.24 (2.24)</td>
<td>0.0013</td>
<td>1.12 (1.05, 1.19)</td>
</tr>
<tr>
<td>TIMP-1 (ng/ml)</td>
<td>222.7 (53)</td>
<td>234.6 (49.6)</td>
<td>216.5 (53.8)</td>
<td>0.0186</td>
<td>1.06 (1.01, 1.1)</td>
</tr>
<tr>
<td>Log HA (ng/ml)</td>
<td>1.82 (0.38)</td>
<td>1.95 (0.41)</td>
<td>1.75 (0.35)</td>
<td>0.0008</td>
<td>2.88 (2.30, 3.46)</td>
</tr>
<tr>
<td>% Ishak fibrosis score of 4</td>
<td>29.7%</td>
<td>35.7%</td>
<td>26.6%</td>
<td>0.22</td>
<td>1.36 (0.83, 2.22)</td>
</tr>
<tr>
<td>% Hepatic steatosis ≥ 2</td>
<td>40%</td>
<td>54.3%</td>
<td>32.4%</td>
<td>0.0022</td>
<td>2.09 (1.62, 2.56)</td>
</tr>
<tr>
<td>Liver biopsy length (cm)</td>
<td>1.95 (0.86)</td>
<td>1.86 (0.81)</td>
<td>2.01 (0.88)</td>
<td>0.20</td>
<td>0.82 (0.49, 1.16)</td>
</tr>
</tbody>
</table>

**Hazard ratio for lifetime drinks is for a change of 10,000 units.**

^Hazard ratio for age is per 10 years

^^Hazard ratio for BMI is per 5 kg/m^2

#Hazard ratio for TIMP-1 is for every 10 ng/ml Data reported as mean + std dev (in parentheses) or %

*comparing patients with histologic progression vs patients without histologic progression.