Markers of liver fibrosis Predict Adverse Liver Outcomes in a Population of HCV Positive Individuals with and without HIV Coinfection

David Nunes[1,2], Gwynneth Offner[1], Kyle Chambers[1], Carrie Reed[3], Timothy Heeren[3], Sheila Tumilty[2], Sherri Stuver[3], Camilla Graham[4,5], C. Robert Horsburgh[1,2,3], Deborah Cotton[1,2,3].

Boston University School of Medicine, Boston Medical Center, Boston University School of Public Health, Beth Israel Deaconess Medical Center and Harvard Medical School

Abstract

Background: The prognostic value of serological markers of fibrosis in predicting liver disease outcomes was assessed in a prospective cohort study of hepatitis C infected subjects with and without HIV infection. **Methods:** All subjects from the cohort with at least one year of follow-up were included in the analysis . Liver events were defined as new onset ascites, bacterial peritonitis, encephalopathy, variceal bleeding, or liver related mortality. Sera taken at the time of enrollment were analyzed for hyaluronic acid (HA) and YKL-40. These tests were compared to simple prognostic indices Child-Pugh Turcotte (CPT) and MELD scores and to other markers of liver fibrosis: AST/ALT ratio, the AST/Platelet ration index (APRI) and Fib-4. The ability of each marker to predict an adverse clinical event was assessed by the area under the Receiver Operator Characteristic curve (AUROC). Log rank survival analysis was used to assess these data in a time dependent manner. **Results:** 303 subjects were studied of whom 194 (64%) were male. Mean age 44 (+/- 7) years. 207 (68%) were of subjects were co-infected with HIV. Almost all subjects reported injection drug use as their HCV risk factor. Median follow-up was 3.1 years. Adverse liver events occurred in 30 subjects with 28 deaths. There were 26 liver events in HIV infected group versus 5 in the HIV negative group (p=0.08). AUROC for each of the tests are shown in the Table. Comparison of the vvalues showed HA to be superior to MELD (p<0.001) but not significantly different to APRI or CPT score (p>0.05). Survival analysis using the cut-off values showed high predictive value of all of the tests studied (p< 0.001). **Conclusion:** Serum markers of liver fibrosis are highly predictive of adverse liver events in HCV infected individuals with and without HIV coinfection. Use of these markers to monitor disease progression and prognosis warrants further study.

Test	AUROC (95% CI)	Cut-off value	Sens %	Spec %
HA	0.928 (0.885-0.970)	99	90	83
YKL-40	0.764 (0.683-0.846)	327	71	70
APRI	0.897 (0.850-0.944)	1.53	84	85
AST/ALT	0.753 (0.669-0.837)	1.35	71	70
CPT	0.838 (0.742-0.935)	6	54	97
MELD	0.842 (0.771-0.914)	9	81	79

Introduction

Serum markers of hepatic fibrosis have been extensively studied to stage hepatic fibrosis and are gaining increased acceptance as for the noninvasive assessment of liver fibrosis. Most of the available markers are indices which include markers of hepatic function, portal hypertension or are markers of extracellular matrix metabolism (ECM) e.g Hyaluronic acid, YKL-40. Simple indices include the AST/ALT ratio, the AST/platelet ratio index (APRI) and FIB-4. More complex indices include, Fibrotest, Hepascore and Fibrometer. A small number of studies have suggested that some of these markers and indices are useful predictors of liver disease outcome.

Since the stage of hepatic fibrosis is a strong predictor of liver disease outcome we sought to assess the prognostic value of several markers of hepatic fibrosis on short to medium term liver related morbidity and mortality in a cohort of HCV infected individuals, with and without coexisting HIV infection. We also sought to compare the ability of these markers to predict disease outcome to two well established prognostic scores: MELD and Child-Pugh Turcotte (CPT).

Study Design

Prospective cohort study of HIV positive and negative individuals with hepatitis C infection. Patients participated in an ongoing natural history study of HCV infection.

Subjects from the parent cohort were included if the individuals had reached a clinical end-point or had completed at least 12 months of follow-up. Subjects with a single baseline visit for whom no follow-up data were available were excluded from the analysis.

Primary outcome
Liver related mortality or liver related event (variceal bleeding, ascites, hepatic encephalopathy or hepatocellular carcinoma).

Markers of hepatic fibrosis
- AST/ALT
- AST/Platelet ratio index (APRI)
- Hyaluronic acid
- YKL-40
- FIB-4

The ability of each of these markers to predict a liver outcome was compared to Child-Pugh and MELD scores. The predictive value of each test was compared by analysis of receiver operator curves and the independent ability of each marker to predict a liver outcome was assessed by Cox Proportional Hazards analysis. All analyses were performed using either SigmaPlot or Systat version 11.

Results

303 subjects were included in the cohort. Demographic data for the cohort is shown in Table 1 and liver events are shown in Table 2.
- Survival analysis showed that liver related survival:-
  - Was better in females compared to males (p= 0.031)
  - Was not different between HIV infected and uninfected subjects (p=0.11).
- Figure 1 shows survival plots for various markers using the cut-off values in Table 4
- In HIV positive subjects a CD4 count < 200 cells/ μL was associated with reduced survival as compared to those with a CD4 cell count > 200 (O.R. 6.84, 95% CI 2.8-16.6, p< 0.001).

Tables 3 and 4 show the ROC curve analysis for each test.

In summary:
HA showed the best performance characteristics for the prediction of a liver related event.
By comparison of the AUROC HA was significantly better than all other tests except APRI (p=0.14) and the CPT score (p=0.068).
HA performed better than the MELD score the current prognostic model for patients with end-stage liver disease.

Multiple regression analysis demonstrated that HA is an independent predictor of liver disease outcome in this cohort when added to model including both CPT and MELD scores

Table 1

Demographic data

Characteristic	Number (%)
Male	194 (64%)
Female	109 (36%)
Race •White •African American •Hispanic •Other	 92 (30%) 146 (48%) 63 (22%) 3 (1%)
HIV Positive	207 (68%)
Hepatitis BsAg*	5 (2%)
CD4 <200** CD4 200-500 CD4 > 500	50 (17%) 78 (40%) 65 (34%)
Anti-retroviral therapy	138 (66%)
Undetectable HIV RNA	58 (28%)
Liver events	30 (10%)

*Data missing in 24
**Data missing in 14

Table 2

Liver outcomes

Liver event	Number
Liver events	30
Death/liver failure	28
Cholestatic Fibrosing hepatitis C	1
Hepatocellular carcinoma	5
Ascites	14
Spontaneous bacterial peritonitis	2
Gastrointestinal bleeding	3
Encephalopathy	9
Hepatorenal syndrome	1
ART toxicity lactic acidosis with cirrhosis	8

Table 3

ROC curve analysis of predictive ability of each test for adverse liver event

Test	AUROC (95% CI)	P value compared to HA
HA	0.928 (0.885-0.970)	1.0
YKL-40	0.764 (0.683-0.846)	<0.0001
AST	0.835 (0.768-0.902)	0.004
ALT	0.692 (0.606-0.788)	<0.0001
AST/ALT	0.753 (0.669-0.837)	<0.0001
INR	0.837 (0.742-0.931)	0.048
Plats	0.851 (0.781-0.921)	0.04
Albumin	0.828 (0.739-0.917)	0.012
Bili	0.839 (0.756-0.922)	0.037
APRI	0.897 (0.850-0.944)	0.14
FIB-4	0.874 (0.819-0929)	0.017
MELD	0.840 (0.766-0.914)	0.008
CPT	0.838 (0.742-0.935)	0.068

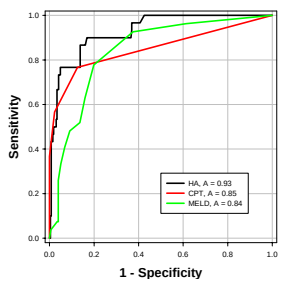
Table 4

Performance of each test at designated cut-off value

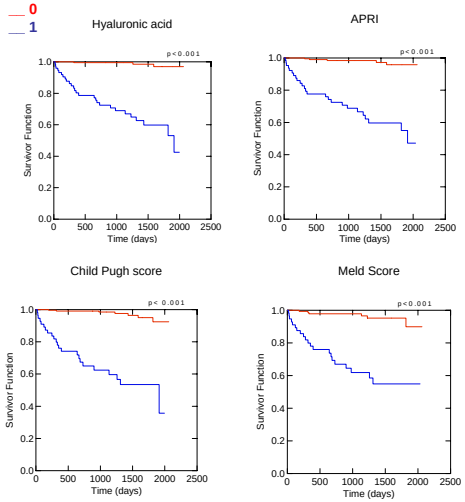
Test	Cut-off value	Odd ratio (95% CI)	Sens	Spec	PPV	NPV
HA	99	42.3 (12.3-145.3)	90	83	92	78
YKL-40	327	7.8 (3.1-19.7)	71	70	85	59
AST/ALT	1.35	5.4 (2.4-12.3)	71	70	84	50
INR	1.1	12.2 (4.4-34.0)	82	74	88	63
Plats	145	24.4 (8.9-67.3)	84	83	92	68
Albumin	3.8	9.3 (3.9-22.0)	72	78	86	59
Bili	0.6	12.4 (4.2-36.7)	77	74	87	57
APRI	1.53	28.2 (10.2-77.8)	84	85	91	70
FIB-4	2.91	25.0 (9.1-68.8)	87	83	92	73
MELD	8	14.1 (5.3-37.6)	78	80	90	61
CPT	6	22.9 (9.1-57.5)	54	97	93	62

Odds ratio n defined by logistic regression analysis: Sens, sensitivity, Spec, specificity, PPV positive predictive Value, NPV negative predictive value.

ROC Curve



Survival curves (using cut-off values from Table 4)



Discussion

Markers of hepatic fibrosis are excellent predictors of liver disease outcome in HCV infected individuals with and without HIV co-infection. Of the assessed markers HA showed the highest predictive value and was found to be an independent predictor of liver disease outcome even when CPT and MELD were included in the model. These data are consistent with the findings of at least one other study that demonstrated that HA was independent of CPT for the prediction of outcome in a cohort of cirrhotic patients.

At the outset of this study we had hypothesized that markers of extracellular matrix metabolism may be predictors of liver disease outcome not only by reflecting the stage of disease, but may also reflect the activity of extracellular matrix metabolism and hence the rate of disease progression. It is also possible that HA showed such excellent predictive value simply because it is a more refined marker of fibrosis stage.

In conclusion this study demonstrates that markers of extracellular matrix metabolism and simple indices of hepatic fibrosis are excellent predictors of liver disease outcome. They perform as well or better than MELD and CPT score, two well established scores of liver disease severity. Further studies to validate these findings and to develop models that incorporate HA into prognostic scores are warranted.

References

Kamal SM, Turner B, He Q, et al. Progression of fibrosis in hepatitis C with and without schistosomiasis: correlation with serum markers of fibrosis. Hepatology 2006;43:771-9.
Ngo Y, Munteanu M, Messous D, et al. A prospective analysis of the prognostic value of biomarkers (FibroTest) in patients with chronic hepatitis C. Clin Chem 2006;52:1887-96.
Nojgaard C, Johansen JS, Christensen E, Skovgaard LT, Price PA, Becker U. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. J Hepatol 2003;39:179-86.
Giannini E, Risso D, Botta F, et al. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. Arch Intern Med 2003;163:218-24.
Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518-26
Korner T, Kropf J, Kosche B, Kristahl H, Jaspersen D, Gressner AM. Improvement of prognostic power of the Child-Pugh classification of liver cirrhosis by hyaluronan. J Hepatol 2003;39:947-53.
Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001;357:1069-75.
Adams LA, Bulsara M, Rossi E, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem 2005;51:1867-73.
Cales P, Oberti F, Michalak S, et al. A novel panel of blood markers to assess the degree of liver fibrosis. Hepatology 2005;42:1373-81.
Guechot J, Serfaty L, Bonnand AM, Chazouillieres O, Poupon RE, Poupon R. Prognostic value of serum hyaluronan in patients with compensated HCV cirrhosis. J Hepatol 2000;32:447-52.
Korner T, Kropf J, Gressner AM. Serum laminin and hyaluronan in liver cirrhosis: markers of progression with high prognostic value. J Hepatol 1996;25:684-8.