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Prediction of hepatic fibrosis in HIV/HCV co-infected patients using serum fibrosis markers: The SHASTA index

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***Background/Aims*: To examine if serum fibrosis biomarkers could accurately identify the stage of liver disease amongst hepatitis C (HCV) and HIV co-infected patients.**

***Methods*: One hundred and thirty seven HIV/HCV co-infected persons were randomly selected from the Johns Hopkins HIV Clinic cohort. Ninety five had complete testing for fibrosis markers in sera collected at the time of liver biopsy. Biopsies were scored according to Ishak modified histological activity index (F0 no fibrosis to F6 cirrhosis). Fibrosis was evaluated against alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST to platelet ratio (APRI), albumin, total bilirubin, hyaluronic acid (HA) and YKL-40.**

***Results*: Sixty nine (73%) had no or minimal portal fibrosis (F0-2) and were compared with remaining subjects (F3-6). Fibrosis scores $\geq$F3 were found 27 times more often in persons with HA levels $>$86 ng/ml and 5.5 times more often in persons with HA levels 41–86 ng/ml. Less substantial associations were detected with levels of albumin $<$3.5 g/dl (OR 4.85) and AST $>$60 iu (OR 5.91). All 35 subjects who had favorable results of HA, albumin, and AST had minimal fibrosis (F0-2).**

***Conclusions*: Amongst HIV/HCV co-infected patients, serum testing for HA, albumin, and AST (SHASTA Index) was able to accurately stage mild and advanced fibrosis.**

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***Keywords*: HIV; Hepatitis C; Co-infection; Fibrosis markers**

1. Introduction

Chronic hepatitis C is characterized by slowly progressive hepatic fibrosis. The fibrogenic stimulus transforms the quiescent hepatic stellate cells (HSCs) into myofibroblast-like cells which degrade normal extracellular matrix (ECM) with accumulation of collagen [1]. Collagen accumulation results in architectural distortion with portal fibrosis leading to bridging fibrosis and eventually cirrhosis. The currently accepted gold standard in fibrosis determination is liver biopsy [2,3]. Although widely performed and accepted in diagnosing hepatic fibrosis, liver biopsy has many inherent shortcomings.

Percutaneous liver biopsy is invasive with associated morbidity. Approximately 1–3% of patients require hospital admission following biopsy for an associated complication [4]. Furthermore, even with experienced physicians performing the biopsy and expert pathologists interpreting them, our so called gold standard has up to a 20% error rate in staging disease predominantly related to sampling error [5,6].

Ideally serum markers of fibrosis could either replace the need for liver biopsy or identify those patients with more

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advanced liver disease who may benefit from a liver biopsy. Features essential of such proposed markers include hepatic specificity, ease of determination and ability to discriminate between degrees of fibrosis. Ultimately serum markers may even have the ability to determine response to various therapies and evaluate disease progression [1].

No single marker fulfills all of the proposed criteria to merit routine clinical use. A combination of markers including those that reflect alterations in hepatic synthetic function and markers of extracellular matrix turnover are emerging as useful diagnostic tests for differentiating early from advanced fibrosis [7,8].

Hepatitis C and HIV co-infection is estimated to affect 200,000 people in the US alone [9,10]. Co-infection is associated with more rapid progression of fibrosis, liver failure and hepatocellular carcinoma [11–15]. Consequently, there is an urgent need for reliable markers of fibrosis progression in this patient group. In this study we report the sensitivity, specificity and predictive value of existing putative fibrosis markers in a cohort of HIV/HCV co-infected persons receiving treatment in an urban HIV clinic.

2. Materials and methods

2.1. Study subjects

The population for this study derives from HIV/HCV co-infected members of the Johns Hopkins University (JHU) HIV clinic cohort in Baltimore, MD. To obtain an unbiased estimate of the prevalence and severity of liver disease among HIV–HCV co-infected persons treated with anti-retroviral therapy (ART), 137 subjects were randomly selected from a group of 630 HIV–HCV co-infected persons who had received ART for two or more years and had not yet received treatment for HCV infection [16]. Of the 137 sampled, 25 individuals were excluded due to the following reasons: undetectable HCV RNA ($n=5$), medical contraindications ($n=5$), died before biopsy ($n=1$), end-stage liver disease (ESLD) ($n=3$), lost to follow-up ($n=5$) and other ($n=6$). After these exclusions, 112 individuals remained. These patients were not substantially different from other eligible individuals who were not selected with respect to age, gender, liver enzymes, CD4 cell count, and HIV RNA level ($P>0.05$, data not shown). However, patients in the random sample were slightly more likely than the other eligible individuals to be African–American and to have a history of alcohol abuse ($P=0.05$, data not shown). A total of 95 of these 112 had sufficient stored sera to perform complete fibrosis marker testing (Table 1).

Demographic and clinical data was obtained prospectively as previously described [17]. Both the Johns Hopkins University Joint Committee on Clinical Investigation and the Beth Israel Deaconess Human Studies Committee approved the study and written informed consent was obtained for all participants.

2.2. Laboratory testing

Patients had standard laboratory assessments at each visit performed by licensed clinical laboratories including a complete blood cell count with platelets, serum chemistry panels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), CD4 cell count and plasma HIV RNA level (reverse transcriptase polymerase chain reaction). HCV testing was performed using a second- or third-generation enzyme immunoassay (EIA 2.0, Abbott Laboratories, Abbott Park, IL; EIA 3.0, Ortho Diagnostics, Raritan, NJ) and confirmatory HCV RNA testing (COBAS AMPLICOR MONITOR assay, Roche Diagnostic Systems). Individuals also had quantitative PCR testing for HBV DNA.

Table 1
Characteristics of study subjects ($n=95$)

Characteristic	Median (interquartile range [IQR])
Male (%)	60 (63.2)
Age	45 (41–48)
Black race (%)	89 (93.7)
Alcohol use in the past 6 months (drinks/week)	0 (0–0.12)
Injection drug use in past 6 months (%)	8 (8%)
Weight (lbs)	164 (144–190)
CD4+ cells/mm^3	340 (155–523)
HIV RNA (c/ml)	235 (31–19,238)
HCV RNA (IU/ml)	3,740,000 (1,290,000–5,870,000)
HA (ng/ml)	40 (20–86)
YKL-40	155 (69–296)
ALT (IU/l)	41 (24–69)
AST (IU/l)	46 (32–69)
GGT (IU/l) ($n=55$)	134 (65–314)
Total bilirubin (mg/dl)	0.5 (0.3–0.7)
Albumin (g/l)	3.9 (3.7–4.1)
Hemoglobin (g/dl)	13 (12–14)
Platelet count ($\times 10^3$)	206 (156–251)
White blood cell count	4400 (3260–5910)
APRI	0.58 (0.37–1.11)

HIV, human immunodeficiency virus; HCV, hepatitis C virus; HA, hyaluronic acid; AST, aspartate aminotransferase, ALT, alanine aminotransferase; GGT, gama-glutamyl transferase; APRI, AST to platelet ratio index (calculated as AST[/ULN]*100/Platelet count [10^9/l].

2.3. Serum fibrosis markers

Markers of liver fibrosis were assessed in serum collected at the time of the liver biopsy. The levels of hyaluronic acid (HA) and YKL-40 were assessed in serum samples, stored at −80 °C, using commercially available assays. Hyaluronic acid levels (ng/ml) were determined using the enzyme-linked binding protein assay kits supplied by Corgenix Inc. (Colorado, USA). YKL-40 levels (ng/ml) were determined using METRA YKL-40 EIA kits (Quidel Corporation, San Diego, CA).

2.4. Liver histology

Under ultrasound guidance a radiologist performed a transcutaneous liver biopsy with an 18-gauge needle. A single pathologist blinded to all clinical and serological results evaluated all slides. All biopsies were deemed adequate based on specimen size (>10 mm) and number of portal tracts (>5) and scored according to the Ishak modified histological activity index (MHAI) scoring system [18]. The mean biopsy size was 11.8 mm (SD +2.89 mm) with a median of 12 mm. The median number of portal tracts was 8 and all biopsies were deemed adequate for pathological interpretation. Steatosis was classified on a five point scale as follows: 0, none; 1, steatosis involving <5% of hepatocytes; 2, 5–<30%; 3, 30–60%; 4>60%. Distribution of MHAI scores across the study population is shown in Fig. 1.

2.5. Statistical analysis

Analyses were designed to differentiate persons with no or 'minimal' liver fibrosis (MHAI 0–2) from those with fibrosis, defined as MHAI scores of 3 or more. Univariate associations between markers and fibrosis were examined using χ^2-tests for categorical variables, Mann–Whitney tests for continuous variables and logistic regression. All markers were examined as continuous and categorical variables. Categories were defined according to the distribution of the marker as well as clinical significance. Commercially available liver enzyme results were examined as a function of the upper limit of 'normal' defined by the laboratory, which are based on averages of testing

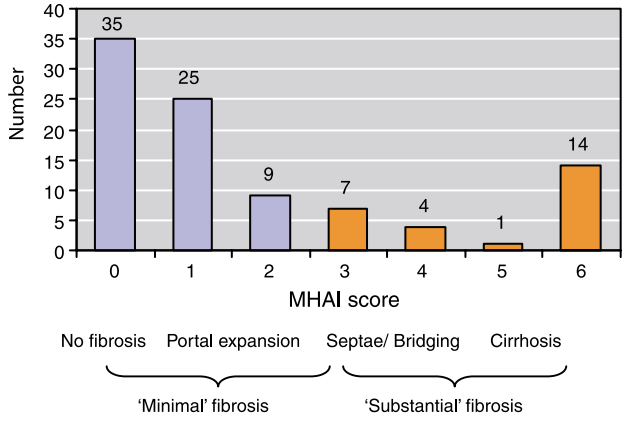


Fig. 1. Distribution of Ishak fibrosis scores in 95 patients with HIV/HCV coinfection. [This figure appears in colour on the web.]

of populations not known to have disease. Research test results (HA and YKL-40) were examined by quartiles in the study population. The AST to platelet ratio index (APRI) was calculated using the formula APRI = AST level(/ULN)*100/platelet count (10^9/l) and categorized according to cutoffs defined by Wai et al. [19]. Variables that were significant in univariate analysis with a P value <0.15 were considered in multivariate analysis after assessment of multicollinearity by variance inflation factors and tolerance. Variables were entered into a multiple logistic regression model in a backwards stepwise fashion. Those that were significant in the multivariate models with a P value <0.05 were retained. A predictive model was constructed by modeling the independent variables (HA, albumin and AST) as categorical variates and their coefficient of regression. The diagnostic value of the model was assessed by calculating the areas under the receiver operating characteristic (ROC) curve. An area under the curve of 1.0 is ideal whereas a score of 0.5 indicates no diagnostic accuracy.

3. Results

Among the 95 subjects, the median age was 45, 63% were male and 94% were African–American (Table 1). The median CD4 cell count was 340 (interquartile range [IQR], 155–523) and the median HIV RNA level was 235 copies/ml (IOR, 31–19,238). The median HCV RNA level was 3,740,000 IU/ml (IQR, 1,290,000–5,870,000). No patients were positive for HBV DNA.

3.1. Fibrosis results

Of the 95 subjects, 35 (37%) had no fibrosis (stage 0) and 34 (36%) had minimal fibrosis (stages 1–2). Twenty six (27%) had bridging fibrosis or cirrhosis (F3 or more) (Fig. 1). The associations of serum markers and APRI with fibrosis are shown in Table 2. Those with ≥F3 fibrosis were significantly more likely to have lower levels of serum albumin (<3.5 g/dl) and lower platelet counts (<150,000) and higher ALT (>37 IU/l) and AST (>60 IU/l) levels ($P<0.01$). Moreover, compared to persons with little or no fibrosis, those with ≥F3 fibrosis had significantly higher serum levels of YKL and HA ($P<0.05$).

In multivariate analysis, fibrosis scores ≥F3 were found 27 times more often in persons with HA levels >86 ng/ml (95% CI 5.11, 138.7) and 5.5 times more often in persons with HA levels 41–86 ng/ml (95% CI 1.12–26.8) (Table 3). Distribution of HA scores across each Ishak fibrosis stage are represented in Fig. 2. Less substantial associations were detected with levels of albumin <3.5 g/dl (OR 4.85 95% CI 1.24–19.0) and AST >60 IU/l (OR 5.91, 95% CI 1.62–21.5). Adjustments for age, gender, alcohol use, body weight and ART use did not substantially alter these associations (data not shown). When these three independent markers (HA, AST, and albumin) were considered as categorical variates as predictors of fibrosis (MHAI 0–2 vs 3–6), the regression model was as follows:Risk score = −3.84 + 1.70 (1 if HA 41–85 ng/ml, 0 otherwise) + 3.28 (1 if HA > 85 ng/ml, 0 otherwise) + 1.58 (albumin <3.5 g/dl, 0 otherwise) + 1.78 (1 if AST >60 IU/l, 0 otherwise). The area under a ROC curve was 0.878 (Fig. 3). The area under the ROC curve for APRI was 0.71 and overall the APRI performed less well as a marker for liver fibrosis in this population.

The markers performed best in the extreme categories. For example, a cutoff of 0.8 was associated with a specificity of 100% and a positive predictive value of 100%. So all individuals with scores >0.8 had ≥F3 fibrosis and there were no false positives at this level. However, only four individuals had scores that were in this range. At the other extreme, a cutoff of <0.30 was associated with a sensitivity of >88% and a negative predictive value of >94%. Moreover, all 35 subjects who had favorable results of HA, albumin, and AST had fibrosis scores of 2 or less. It is important to note that cutoffs in between 0.3 and 0.8 performed poorly in terms of sensitivity and specificity. Thus overall 42% of patients could be correctly classified at either extreme but 58% would not be classifiable with scores between 0.3 and 0.8.

We also examined associations between the markers and inflammation and steatosis. In multivariate analysis, albumin <3.5 g/dl (OR, 5.0; 95% CI, 1.41–17.6), YKL 24–69 ng/ml (OR, 5.26; 95% CI, 1.06–26.0) and YKL >69 ng/ml (OR, 6.41; 95% CI, 1.15–35.6) were associated with greater inflammation (MHAI ≥ 5). The area under the ROC curve for these two markers was 0.73. In terms of steatosis, only albumin <3.5 g/dl (OR, 2.83; 95% CI, 0.91–8.80) was marginally associated with steatosis (defined as presence of any fat in the liver).

4. Discussion

Liver biopsy is the predominant diagnostic test upon which determination of prognosis and indeed the need for antiviral therapy is made in patients with chronic hepatitis C although its performance in accurately staging liver disease has recently come into focus. A recent study on virtual liver biopsy has suggested that liver biopsies need to be 25 mm long and non-fragmented to accurately stage disease in 80% of patients [20]. However, that goal is rarely achieved in clinical practice (<25% even in expert liver centers) and

Table 2
Univariate analysis of candidate fibrosis markers in 95 HIV/HCV-infected subjects

	MHAI 0–2 ($n=69$)	MHAI 3–6 ($n=26$)	P value	OR (95% CI)
Male gender	45 (65.2)	15 (57.7)	0.50	0.73 (0.29–1.83)
Median age (IQR)	36 (33–41)	38 (33–47)	0.32	1.04 (0.98–1.11)
African–American race	64 (92.8)	25 (96.2)	0.54	1.95 (0.22–17.6)
Any alcohol in past 6 months	25 (38.9)	10 (41.7)	0.78	1.17 (0.45–3.03)
Any injection drug use in past 6 months	7 (10.1)	1 (4.0)	0.35	0.37 (0.04–3.16)
Albumin <3.5 g/dl	6 (8.7)	11 (42.3)	<0.0001	7.7 (2.5–24.1)
Platelet <150,000	9 (13.0)	10 (38.5)	<0.01	4.17 (1.45–12.0)
ALT>37 IU/l	34 (49.3)	16 (61.5)	0.12	1.65 (0.66–4.13)
AST>40 IU/l	35 (50.7)	22 (84.6)	<0.01	5.34 (1.67–17.13)
AST>60 IU/l	18 (26.1)	17 (65.4)	<0.0001	5.35 (2.03–14.12)
Bilirubin>1.2 mg/dl	3 (4.4)	3 (11.5)	0.20	2.87 (0.54–15.2)
WBC<4000/uL	28 (40.6)	10 (38.5)	0.85	0.92 (0.36–2.31)
Hemoglobin<13 g/dl	30 (43.5)	16 (61.5)	0.12	2.08 (0.83–5.23)
YKL (ng/ml)				
<69	21 (30.4)	4 (15.4)	0.04	1
69–155	19 (27.5)	6 (23.1)		1.11 (0.24–5.05)
156–296	16 (23.2)	6 (23.1)		1.97 (0.47–8.17)
>296	13 (18.8)	12 (46.2)		4.85 (1.29–18.3)
HA (ng/ml)				
<20	21 (30.4)	1 (3.9)	<0.0001	1
20–40	24 (34.8)	2 (7.7)		1.8 (0.15–20.7)
41–85	15 (21.7)	8 (30.8)		11.2 (1.26–99.3)
>85	9 (13.0)	15 (57.8)		35.0 (4.0–306.4)
APRI				
<0.5	36 (52.2)	6 (23.1)	<0.001	1
0.5–1.5	28 (40.6)	10 (38.5)		2.14 (0.69–6.61)
>1.5	5 (7.3)	10 (38.5)		12 (3.02–47.6)
CD4+/ul				
<200	22 (31.9)	8 (30.8)	0.70	1
200–500	26 (37.7)	12 (46.2)		1.43 (0.52–3.92)
>500	21 (30.4)	6 (23.1)		1.08 (0.35–3.31)
HIV-1 RNA (copies/ml)				
<400	40 (58.0)	13 (50)	0.78	1
400–10,000	9 (13.0)	4 (15.4)		1.39 (0.42–4.61)
>10,000	20 (29.0)	9 (34.6)		1.10 (0.43–2.85)
HCV RNA (IU/ml)				
<500,000	7 (11.7)	1 (5.3)	0.71	1
500,000–2,000,000	13 (21.7)	4 (21.0)		1.33 (0.22–8.10)
>2,000,000	40 (66.8)	14 (73.7)		1.19 (0.23–6.27)

Data is presented as $n(\%)$ unless otherwise indicated; P values are from χ^2 tests for categorical variables and Mann–Whitney tests for continuous variables comparing individuals with MHAI 0–2 vs MHAI 3–6; Odds ratios from univariate logistic regression where outcome is Ishak Fibrosis Score (1 if MHAI 3–6); HIV, human immunodeficiency virus; HCV, hepatitis C virus; OR, odds ratio; CI, confidence interval; IQR, interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; WBC, white blood cell count; HA, hyaluronic acid; APRI, AST to platelet ratio index (calculated as AST[/ULN]*100/Platelet count [10^9/l].

always achieving it may increase the risk of complications. In addition, in another study, laparoscopic biopsies taken at the same time from both lobes of the liver revealed substantial differences even when sampled under direct observation and on the same day [21]. Therefore, there is an obvious need for reproducible and reliable non-invasive markers of fibrosis.

The predominant indication for liver biopsy in HCV is staging to determine the need for anti-viral therapy, one important goal of non-invasive markers is to be able to discriminate treatment need. In co-infected patients the clinical situation is more complex as a liver biopsy may also be necessary for evaluation of drug induced hepatotoxicity or opportunistic infections. However, the main indication

Table 3
Multivariate analysis of candidate fibrosis markers in 95 HIV/HCV co-infected subjects

	OR (95% CI)	*P* value
Albumin <3.5 g/dl	4.8 (1.24–19.0)	0.02
AST>60 IU/l	5.9 (1.62–21.5)	<0.01
HA <41 ng/ml	1	
HA 41–85 ng/ml	5.5 (1.12–26.8)	0.04
HA >85 ng/ml	27 (5.11–138.7)	<0.001

Results are from multiple logistic regression analysis; HIV, human immunodeficiency virus; HCV, hepatitis C virus; AST, aspartate aminotransferase; HA, hyaluronic acid; OR, odds ratio; CI, confidence interval.

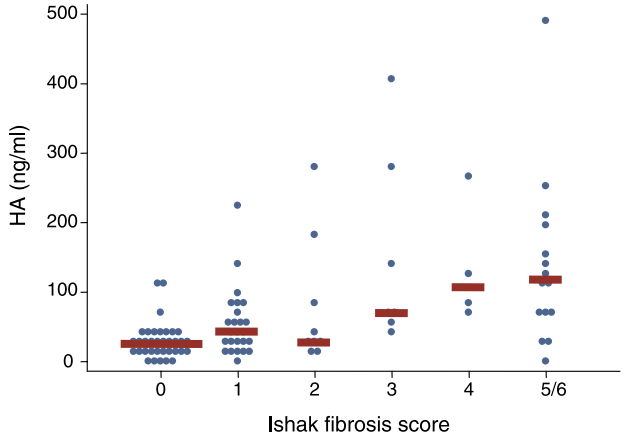
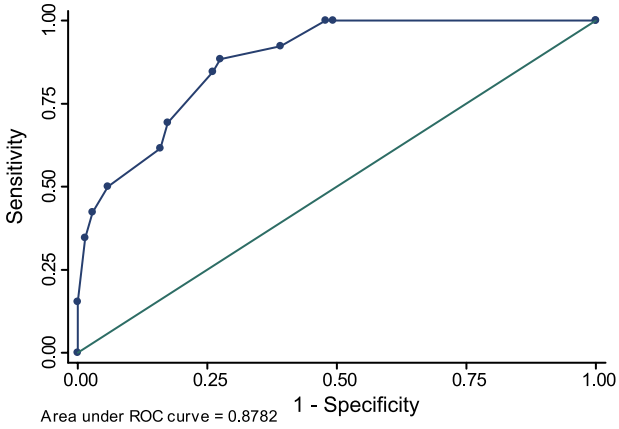


Fig. 2. Distribution of hyaluronic acid by Ishak fibrosis stage. Median score represented by solid bar. [This figure appears in colour on the web.]

still remains disease staging and accordingly, in this study, we sought to use serum markers to differentiate persons across the fibrosis gradient traditionally used for therapy (MHAI Stage >3).

In our study, seven biochemical tests, each with biological plausibility and supporting literature were considered, most were correlated to some degree with liver fibrosis in our study. The strongest associations were found with levels of HA. Increased serum HA may be a result of increased hepatic stellate cell production as well as a decrease in removal by hepatic sinusoidal endothelial cells. Our data confirm several other studies of HCV infected persons without HIV that detected an association of HA with hepatic fibrosis [22,23]. HA appears to be especially reliable as a predictor of the absence of advanced fibrosis (cirrhosis). In one study of chronic hepatitis C patients a serum HA less than 60 ug/l had a 99% accuracy in predicting the absence of cirrhosis on liver histology (negative predictive value), however, conversely its accuracy in diagnosing cirrhosis was low (30% positive predictive value) [23].

In our own cohort, HA levels were even more discriminating for low amounts of fibrosis when combined with albumin and AST results, a fibrosis index that we suggest be named the SHASTA index (Serum HA, AST, Albumin). All 35 subjects with favorable results for the SHASTA index had Ishak fibrosis scores <3, and these represented half of those in that low fibrosis group and one third of those in the study. If confirmed, these results suggest that liver biopsies could have been avoided in one third of all subjects by use of these serological tests. The relative simplicity of adding a quantitative ELISA for HA to AST



Cutoff†	Positive†	Negative†	Sensitivity	Specificity	PPV	NPV
0.1	59	36	100	52	44	100
0.2	42	53	88	72	55	94
0.3	42	53	88	72	55	94
0.4	27	68	62	84	59	85
0.5	17	78	50	94	76	83
0.6	17	78	50	94	76	83
0.7	17	78	50	94	76	83
0.8	4	91	15	100	100	76
0.9	4	91	15	100	100	76

*ROC, receiver operating characteristic; HA, hyaluronic acid; AST, aspartate aminotransferase; PPV, positive predictive value; NPV, negative predictive value; Cutoff reflects the fibrosis score from the final regression model including HA, albumin and AST above which individuals would be considered to have significant liver disease (MHAI 3–6, positive) and below which would be considered not to have significant liver disease (MHAI 0–2, negative).

Fig. 3. ROC plot of HA, AST, and albumin (SHASTA Index) according to liver fibrosis (MHAI 0–2 vs 3–6). [This figure appears in colour on the web.]

and serum albumin is attractive for both a cross-sectional diagnostic test and for disease monitoring. In addition, this is only the second cohort study that has focused on evaluating fibrosis markers in patients co-infected with HIV.

Currently, there are multiple methodologies proposed to evaluate liver fibrosis in HCV and all appear to perform reasonably well and with a similar diagnostic accuracy. The FibroTest (FibroSure, LabCorp) is one of the most commonly utilized tests and probably the best validated test, even in HIV/HCV patients [24]. The optimal AUC for FibroTest is approximately 0.856 and comparable to the 0.878 with SHASTA index. A recent study from Rosenberg and the European Liver Fibrosis Group used HA, procollagen peptide III and TIMP-1 to stage fibrosis in a variety of liver diseases and had again a comparable AUC of 0.804 overall [25]. All of these indices appear to be non-specific and are more fibrosis indices rather than disease specific indices. The SHASTA index in HIV/HCV has similar accuracy to FibroTest and in this study performed significantly better than the APRI test. Validation of the use of these tests in clinical practice algorithms deserves further study.

Studies of fibrosis markers are important in persons co-infected with HIV and HCV. In the United States and Europe, about one quarter of HIV infected persons also have HCV infection [26]. Co-infected patients have more rapid progression of cirrhosis, liver failure and hepatocellular carcinoma[11–14,27–30]. Furthermore, many of those co-infected with both HCV and HIV due to intravenous drug use often have poor access and compliance with available healthcare. Thus, fibrosis marker research needs to be conducted in this setting not only because the pathogenesis of disease appears to differ (or at least accelerated), but also because biopsies are more difficult to obtain.

In this and other studies, the correlation of fibrosis markers and liver histology is not perfect. Not surprisingly, the best correlation is found at the extreme spectra of fibrosis, i.e. minimal fibrosis and cirrhosis. The inability to always identify patients with moderate disease, Ishak stage 2–4 is not surprising since fibrosis is a complex, multidimensional process. Since liver biopsy is only 80% accurate in staging disease, biomarkers can only be accurate to the same extent. Recently, Poynard and colleagues reported that an inadequate biopsy rather than inaccuracy of biomarkers was more commonly the cause for divergent results between FibroTest and biopsy [31]. This relative inadequacy of biopsy specimens has resulted in some authorities suggesting that biomarkers may even be more accurate than biopsy in staging disease [32].

There are several important limitations to our study. First, the cohort is chiefly composed of African—American males of relatively low body weight and who are chiefly infected with genotype 1 HCV infection acquired by injection drug use. Further validation of this fibrosis algorithm should be undertaken in a more diverse HIV/HCV population and also expanded into the HCV monoinfected patients. However, this population reflects a homogeneous cohort of HIV co-infected males that frequently are resistant to liver biopsy and thus represent an appropriate cohort for evaluation of non-invasive markers. Second, ART affects blood levels of liver enzymes [33] and consequently could affect the degree to which biochemical tests predict liver disease. There is no evidence that ART will effect HA or albumin levels but it could impact the AST level. In this study, all patients were on ART and thus it is highly unlikely to have affected the analysis. Utilization of this algorithm in different HIV populations should control for ART therapy.

In reality biomarkers are probably complementary to biopsy and may in fact be additive in helping to correctly classify the degree of fibrosis. We would suggest that the fibrosis markers could be performed at the initial evaluation and that patients whose score was below 0.3 could be offered treatment at the discretion of the physician and patient or followed clinically. Patients with indeterminant scores could have a biopsy and serial fibrosis markers utilized to follow patients. In fact, the optimal role for markers may be to monitor disease progression or therapy. Research in biomarkers needs to focus on their utilization in longitudinal cohort studies and evaluation of their role as markers of prognosis and disease progression.

In conclusion, in this HIV/HCV co-infected clinic based cohort, 50% of patients with mild hepatic fibrosis could be identified by the SHASTA index. If these results are confirmed, serum fibrosis markers may be used to identify persons with a low stage of liver disease who could be followed conservatively allowing resources to be focused on those with greatest need.

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