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Validation of Non-invasive Methods for Detecting Hepatic Steatosis in Patients with HIV Infection

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Abstract

Background—Nonalcoholic fatty liver disease (NAFLD) is common among patients with HIV infection; a reliable non-invasive method of detection is needed. We aimed to validate non-invasive means of identifying steatosis in HIV positive patients.

Method—We performed a single-center retrospective study to validate the abilities of liver fat score (LFS) and lipid accumulation product (LAP) to detect hepatic steatosis in HIV positive patients, compared with HIV-negative controls (controls); NAFLD was confirmed by histology. These models were then validated in HIV-positive with NAFLD vs patients co-infected with HIV and hepatitis C virus (HCV) infection without steatosis.

Results—LFS identified steatosis in HIV positive subjects, compared with controls, with an area under the receiver operative curve (AUROC) value of 0.971 ± 0.027 (95% confidence interval [CI], 0.91.8–1.000). At a cut-off value of -0.945 , the LFS identified those with steatosis with 100% sensitivity and 84% specificity. At a cut-off of -0.234 , the LFS differentiated between HIV positive subjects with NAFLD and patients co-infected with HIV and HCV with 100% sensitivity and 74% specificity. LAP scores >38 identified HIV positive patients with steatosis with 89% sensitivity and 83% specificity. LAP scores >42 differentiated between HIV positive subjects with steatosis and patients co-infected with HIV and HCV with 89% specificity and 70% sensitivity.

Conclusion—We validated the accuracy of LFS and LAP in detecting hepatic steatosis in HIV-positive patients.

Keywords

liver disease; diagnosis; prognostic factor; test

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INTRODUCTION

Human immunodeficiency virus (HIV) infection affects 34 million globally. Improvements in highly active antiretroviral therapy (HAART) has shifted the mortality related to AIDS to liver related diseases (1) which have become a leading cause of hospitalization and death in HIV-infected patients (2). Non-alcoholic fatty liver disease (NAFLD) is emerging as an important cause of chronic liver disease in HIV patients (3). NAFLD is closely associated with metabolic syndrome, particularly insulin resistance (IR) (4) and IR associated with HAART can often predispose HIV patients to NAFLD (5). The estimated prevalence of NAFLD in HIV-patients has been reported to be even greater than the general population (3, 6) and a significant proportion of those with NAFLD had non-alcoholic steatohepatitis (NASH) associated with advanced fibrosis highlighting the importance of identifying these individuals early in their disease course (7).

The gold standard for diagnosing the NAFLD is a liver biopsy. Although many noninvasive models to identify fibrosis in those with viral hepatitis have been developed (8), few have focused on NAFLD (9). Non-invasive assessment of steatosis using radiographic imaging is costly and might not be as accurate in individuals with less than 30% steatosis. Liver Fat Score (LFS) and Lipid Accumulation Product (LAP) are non-invasive models that use readily available clinical parameters to predict steatosis, but have not been validated in those with HIV (10, 11). Therefore, the aim of the current study was to validate LFS and LAP histologically in HIV patients with NAFLD.

MATERIALS AND METHODS

This was a single-center retrospective study that validated the use of LFS and LAP in predicting presence of hepatic steatosis in HIV+ patients. The study populations included HIV+ patients who either had steatosis or were coinfectd with HCV. The first cohort consisted of HIV+ patients without HCV with at least 5% steatosis on a liver biopsy (12). The second cohort consisted of HIV-HCV patients. The choice of this cohort as a control group was based primarily on (1) HIV subjects on HAART therapy may develop mild steatosis which can be missed on liver ultrasound and therefore having histological assessment would improve the robustness of the model and (2) patients with HIV may have competing etiologies of elevated liver enzymes (i.e. HCV) and therefore it is important for the model to be able to distinguish between NAFLD and other causes of elevated liver enzymes. The second cohort was further sub-divided into patients with or without concurrent steatosis (>5% liver fat content). Finally, the HIV negative control group consisted of subjects with no known liver disease, normal serum alanine aminotransferase levels (13), and absence of steatosis on ultrasound. Hepatic steatosis was modeled non-invasively using LFS and LAP as described previously (10, 11). We validated these models in a HIV-positive cohort in the following three stages: In the first stage, the receiver-operating curve (ROC) of the LFS and LAP was used to identify optimal cut-off points for predicting steatosis using the Youden Index in the HIV+ cohort with NAFLD compared to HIV-negative controls. Since the models rely on parameters that can be abnormal in patients with any liver disease (i.e. serum aminotransferases), these models were subsequently used in stage 2 to determine if they could be used to differentiate between HIV+ patients with

NAFLD and HIV-HCV patients without steatosis. The ROC and Youden Index were used to re-define the optimal cut-off. Finally, in stage 3 we determined if these models could be used to identify presence of steatosis in a larger cohort of HIV-HCV patients.

RESULTS

The four cohorts consisted of HIV-negative controls (N=19), HIV+ patients with NAFLD (N=9), HIV-HCV patients with no steatosis (N=53), and HIV-HCV patients with at least 5% hepatic steatosis on a liver biopsy (N=18). The clinical characteristics of the cohorts are summarized in table 1. Genotype 3 was more common in HIV-HCV patients with steatosis than without steatosis (13% vs. 2%; $p<0.001$).

Validation of the LFS in HIV Patients with NAFLD

In stage 1, the ROC was 0.971 ± 0.027 (95% CI: 91.8–1.000) for LFS in predicting >5% steatosis (Figure 1A). The optimal cut-off point was determined to be -0.945 . A value of greater than -0.945 predicted underlying steatosis in HIV+ patients with 100% sensitivity and 84% specificity. The cut-off of -0.945 was then applied to see how well the LFS could predict NAFLD in HIV+ compared to HIV-HCV patients without steatosis. Although the sensitivity remained 100%, the specificity dropped from 84% to 53%.

In order to improve the accuracy of the LFS in identifying HIV+ with NAFLD compared to HIV-HCV patients, the Youden index was reapplied to determine the optimal cut-off between HIV+ subjects with underlying steatosis and those with HIV-HCV but no histological evidence of steatosis (stage 2). The ROC was 0.923 ± 0.038 (95% CI: 0.848–0.998) and the optimal cut-off was determined to be -0.234 (Figure 1B). The LFS of greater than -0.234 was 100% sensitivity and 74% specific in predicting NAFLD in HIV patients compared to HIV-HCV patients. Re-applying the new cut-off of -0.234 , the LFS was 100% sensitive and 84% specific in identifying HIV patients with NAFLD compared to controls. Therefore, the LFS of -0.234 appears to be the ideal cut-off for screening HIV patients with abnormal aminotransferases and suspected NAFLD.

Validation of the LAP in HIV patients with NAFLD

The LAP score was first validated in HIV patients with NAFLD using the control group similar to the validation of LFS. The ROC was 0.907 ± 0.058 (95% CI: 0.794–1.000) for LAP in predicting NAFLD in HIV patients (stage 1). The optimal LAP cut-off was determined to be 38 and values greater than 38 predicted presence of steatosis with 89% sensitivity and 83% specificity. When this cut-off was used to differentiate HIV+ with NAFLD from HIV-HCV patients with no steatosis, the specificity remained at 89% while the sensitivity dropped to 70%. By increasing the cut-off to 42, the sensitivity improved to 74% while the specificity remained at 89%. Re-applying the cut-off of 42 to HIV-positive patients with NAFLD, the LAP was 89% sensitive and 83% specific in identifying HIV patients with NAFLD compared to controls (stage 2). Therefore, the optimal cut-off for LAP was 42 in predicting NAFLD in the HIV+ patients.

Using LFS and LAP to Predict Hepatic Steatosis In Co-Infected HIV-HCV patients

To determine if the LFS and LAP could be used in predicting presence of >5% steatosis in HIV-HCV patients, the entire HIV-HCV cohort was sub-divided further based on presence or absence >5% steatosis (stage 3). New cut-offs specific for this cohort derived above were used, since previously established cut-off were sub-optimal; LFS > -0.234 was 67% sensitive and 74% specific while LAP > 42 was 53% sensitive and 74% specific. In co-infected patients, the optimal cut-offs were identified to be -0.413 and 23 for LFS and LAP respectively. A LFS of greater than -0.413 predicted presence of steatosis in HIV-HCV patients with 80% sensitivity and 71.4% specificity while a LAP of greater than 23 predicted steatosis with 73% sensitivity and 40% specificity.

DISCUSSION

Mirroring the NAFLD pandemic in the general population, NAFLD is becoming an increasingly important cause of chronic liver disease in HIV infected patients (3, 6, 7). With improvements in therapies for HIV and viral hepatitis, the morbidity and mortality associated with NAFLD in HIV infected patients is expected to increase further. Given the projected increase and long-term morbidity associated with undiagnosed NAFLD, there is a need to evaluate HIV patients for presence of steatosis that is cost-effective, easy to administer and accurate. In the present study, the LFS and LAP performed well in predicting presence of steatosis in patients with HIV compared to both controls and HIV-HCV. The LFS was more sensitive and specific than LAP. Additionally, LFS was able to predict steatosis in HIV-HCV patients with reasonable accuracy. The ability to predict presence of underlying steatosis using routinely available clinical information, makes these models an attractive tool for both population based research and clinical screening for steatosis.

Strengths of the current study were that these models were evaluated in a well-characterized control group and histologically defined HIV patients and were evaluated HIV+ patients with two common causes of liver disease, HCV and NAFLD. The models were also fairly accurate in differentiating presence of hepatic steatosis in HIV-HCV patients. The accuracy of the two models decreased when the model was validated in HIV-HCV with and without steatosis. This is primarily due to an increase serum aminotransferases with concurrent HCV.

Limitations of our analysis included potential selection bias of those undergoing liver biopsy, the high proportion of African-Americans in our HIV-HCV population, and the cross-sectional nature of our data collection. Unfortunately, due to a small cohort of patients with HIV and NAFLD, we were unable to determine if these models could also be used to predict underlying steatohepatitis or bridging fibrosis. Our study also did not include a control group consisting of HIV+ patients with no evidence of hepatic steatosis.

In conclusion, we provide, for the first time, novel data on the application of two non-invasive models to predict hepatic steatosis in patients living with HIV. These models, first validated in a defined cohort of HIV patients with NAFLD and then applied to subjects with HIV-HCV, were effective in identifying HIV patients with underlying steatosis. Larger studies will need to be done to determine if these models can now be combined with other

simple models of hepatic fibrosis (such as FIB-4, APRI) that use routine clinical data, in the hopes of reducing the need for liver biopsy.

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Figure 1a

Figure 1b**Figure 1.**

Figure 1A: Receiver operating curve (ROC) for HIV and hepatic steatosis (HIV vs controls) for Liver Fat Score (LFS)

Figure 1B: The ROC for the LFS for presence of hepatic steatosis in HIV infected patients vs those with HIV-HCV co-infection

Table

Baseline clinical characteristics in the 4 cohorts

	Controls	HIV with Steatosis	HIV-HCV	HIV-HCV with Steatosis
N	19	9	53	18
Age (years)	33.1±12.6	46.8±9.5	48.7±8.5	47±8.5
BMI	25.6±4.0	31.1±8.7	24.7±7.0	28.0±3.5
Gender (% Male)	58%	77%	76%	78%
Ethnicity (%cohort)				
White	58	67	9.4	39
Blacks	5	33	91	61
Other	37	--	--	--
ALT (IU/L)	18±13	109±51	49±19	75±22
AST (IU/L)	20±12	87±49	52±24	77±32
HCV Genotype (%cohort)				
1	--	--	92	88
2	--	--	6	0
3	--	--	2	13
HIV Viral Load (% cohort)				
Less than 400	--	100%	78%	80%
More than 400	--	0	22%	20%
CD4	--	605±408	460±337	621±379
CD4%	--	26.3±14.4	24.7±10.9	30.5±13.5
CD8	--	815±164	812±431	783±503
CD8%	--	50±24.6	44.5±14.6	40.1±16.7