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Predictors of Hepatotoxicity in Human Immunodeficiency Virus-Infected Patients Receiving Antiretroviral Therapy

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Predictors of Hepatotoxicity in Human Immunodeficiency Virus-Infected Patients Receiving Antiretroviral Therapy

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Clinical Research

by

Debika Bhattacharya

2012

ABSTRACT OF THE THESIS

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Master of Science in Clinical Research

University of California, Los Angeles, 2012

Professor Robert M Elashoff, Chair

ABSTRACT

Hepatotoxicity is a common side effect of antiretroviral therapy with severe hepatotoxicity necessitating a regimen change. This was a retrospective cohort study that investigated the predictors of severe hepatotoxicity in a cohort of HIV-infected patients on antiretroviral (ARV) therapy identified to have clinically significant elevations in transaminases. Severe hepatotoxicity was defined as an alanine aminotransferase level (ALT) greater than 275 IU/ml. The prevalence of severe hepatotoxicity was 23%. In multivariable stepwise regression analysis, only HBsAg, higher median HIV viral load and lower median alanine aminotransferase (ALT) at database entry were predictors of severe hepatotoxicity. In a cohort of HIV and hepatitis B (HBV) coinfected patients, only higher log HIV viral load at database entry showed a trend towards significance as a predictor of severe hepatotoxicity, in multivariable stepwise regression analysis. Neither antiretroviral regimen class nor single agent was associated with severe hepatotoxicity. HBV infection, but not HCV infection, was a significant predictor of severe hepatotoxicity in HIV infected patients on antiretroviral therapy. Conclusion: In a cohort of HIV-infected patients receiving antiretroviral therapy, HBV infection, ALT and log HIV viral load at database entry were significant predictors of severe hepatotoxicity, while antiretroviral regimens were not.

The thesis of Debika Bhattacharya is approved.

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Chapter 1: Background

Brief Background and Rationale: Hepatotoxicity is a common side effect of antiretroviral therapy, occurring in 6-30% of HIV infected subjects[1]. Severe hepatotoxicity occurs in 2-18% of patients and requires discontinuation of therapy and/or therapy modification [2-13]. Given the frequency of low-grade hepatotoxicity, it is important to identify those predictors of severe hepatotoxicity, that which would warrant a regimen change. Although several studies have examined predictors of severe hepatotoxicity, many have only evaluated one agent, a single class of agents, or have had included populations with a low prevalence of hepatitis B and C coinfection (although several studies have examined individuals with hepatitis C infection alone). In this study, we evaluated a cohort of US HIV patients receiving care in the AIDS Healthcare Foundation with ≥ grade 2 hepatotoxicity identified during routine monitoring, and within this cohort, sought to identify predictors of severe hepatotoxicity (≥ grade 3 hepatotoxicity), that which would necessitate regimen change. This cohort is unique given its large sample size (≥ 10,000 patients in clinical care), large variety of antiretroviral agents, and large cohort of patients with viral hepatitis.

Definition of Hepatotoxicity in HIV Infection: Hepatotoxicity, as defined by elevations in plasma transaminase levels, is a common side effect during receipt of antiretroviral therapy in HIV infection. Standard criteria for grading elevations have been developed for use in clinical trials and one such system is the DAIDS ACTG hepatotoxicity criteria[14]. Patients with pretreatment plasma aspartate aminotransferase (AST) and ALT levels within the normal range (AST 35 IU/L and ALT 31 IU/L) will be classified based on changes relative to the upper limit of normal (ULN): grade 0, less than 1.25 x ULN; grade 1, 1.25-2.5 x ULN; grade 2, 2.6-5 x ULN; grade 3, 5.1- 10 x ULN; grade 4, greater than 10 x ULN. Patients with elevated pretreatment plasma AST and ALT levels will be classified based on changes relative to the baseline value

rather than to the ULN: grade 0, less than 1.25 x baseline; grade 1, 1.25-2.5x baseline; grade 2, 2.6- 3.5x baseline; grade 3, 3.6- 5 x baseline; grade 4, greater than 5x baseline. There are small amounts of ALT in the plasma in normal individuals. When there is hepatotoxicity, or liver damage, higher amounts of ALT are released into the blood. Although ALT is primarily found in the liver, it is also found in muscle, pancreas, the kidneys, and the heart.

Incidence of hepatotoxicity in HIV infection: In approximately 6-30% of HIV-infected treated patients, receipt of antiretroviral therapy is associated with significant increases in plasma liver enzymes[2,6,9,10,12,13,15]. Up to 30% of HIV infected patients receiving antiretroviral therapy will only have mild elevations, which do not require changes in therapy. However, symptomatic hepatotoxicity or asymptomatic severe hepatotoxicity (defined as $\geq$ grade 3 ALT elevations) results in a recommendation to discontinue and/or modify antiretroviral therapy[1]. Clinicians are often faced with asymptomatic hepatotoxicity in the grade 2 and above range, yet it is unclear what differentiates those patients who develop severe hepatotoxicity ($\geq$ grade 3) from those with less severe hepatotoxicity ($\leq$ grade 3), which would not require a regimen change. Although several analyses have examined predictors of severe hepatotoxicity, few have included a comparison between less severe and severe hepatotoxicity, that which requires a regimen change. This analysis will examine predictors of severe hepatotoxicity (grade 3 or greater hepatotoxicity), in a cohort of those with grade 2 or greater hepatotoxicity, in a US HIV-infected cohort receiving antiretroviral therapy.

Predictors of Hepatotoxicity:

Several factors have been associated with hepatotoxicity including coinfection with viral hepatitis, ARV regimen, immune reconstitution, and other medication toxicity (antituberculous therapy).

Viral Hepatitis

Seven studies, two prospective and 5 retrospective[3-5,7,8,16,17], have examined hepatotoxicity during HIV therapy and included viral hepatitis in their analysis. All identified viral hepatitis as a risk for developing hepatotoxicity. Retrospective studies identified HBV as a risk factor with relative risk (RR) ratios clustering from 2-4, with one study of 1047 HIV infected patients initiating protease inhibitors reporting a RR for HBV infection association with severe hepatotoxicity to 6.7[8]. Two prospective studies by Sulkowski emphasize the importance of viral hepatitis in the development of hepatotoxicity during HIV therapy in the U.S. The first study, conducted before non-nucleoside reverse transcriptase inhibitors (NNRTI)s were available, demonstrated that in 298 HIV-infected individuals receiving antiretroviral therapy in Baltimore, 46.6% of patients developed an elevation in their transaminases; 10.4% of whom developed severe hepatotoxicity. Viral hepatitis was associated with a 3.3 fold increase in risk of developing hepatotoxicity[4,5].

A follow-up prospective study by Sulkowski in 2002 examined hepatotoxicity in NNRTI-containing regimens[4]. 15.6% of patients receiving nevirapine and 8% of patients receiving efavirenz developed severe hepatotoxicity. Viral hepatitis infection was associated with a 2.1 fold increase in risk in developing hepatotoxicity. In those patients who developed hepatotoxicity, 69% developed in patients with viral hepatitis; 60% of these patients discontinued antiretroviral therapy[4].

When examining viral hepatitis separately, hepatitis C coinfection itself is associated with a 2.7-5 fold increased risk of severe hepatotoxicity[2,12,13].

HBV infection may carry a higher risk. In one study, Law and colleagues retrospectively examined hepatotoxicity in 692 Thai HIV-positive patients receiving dual nucleoside reverse transcriptase inhibitor (NRTI), protease inhibitor (PI)- based, and NNRTI based antiretroviral

therapies from 1996-2001[17]. The overall combined incidence of severe hepatotoxicity was 5.8%, but this was likely an underestimate as it included patients who were only receiving dual-NRTI therapy, therapy that has been associated with less hepatotoxicity[5] and is no longer the standard of care. Despite this, in multivariate analysis with covariates that included the presence of HBV infection (absent/present), the presence of HCV Infection (absent/present), prior receipt of antiretroviral therapy (yes), and NNRTI containing therapy (yes), the RR of developing hepatotoxicity was 3.20 in patients with HIV/HBV coinfection. In another study of HIV infected individuals, there was a 9.2 hazard risk of grade 4 ALT elevation with HBV infection[12,18]

Although viral hepatitis has been identified as a risk factor for severe hepatotoxicity, less well defined are the predictors of severe hepatotoxicity in those coinfected with HIV and HBV or HCV. In one study of 56 HIV/HCV coinfected subjects, six developed severe hepatotoxicity, on their first regimen. The normalized incidence of severe hepatotoxicity was highest with indinavir. Notably, no cases of severe hepatotoxicity were seen with lopinavir/ritonavir or atazanavir/ritonavir)[18]. However, this study was small and may not have adequately assessed an association with other, less frequently used agents. Additionally, indinavir is no longer a commonly used antiretroviral agent. In another larger study of 105 HIV/HCV coinfected patients, 22% developed $\geq$ grade 3 ALT elevations. In univariate analysis, receipt of lopinavir/ritonavir based regimens and higher baseline ALT were all significantly associated with severe hepatotoxicity[19].

ARV regimens

Hepatotoxicity is a common side effect of all antiretroviral agents. It is difficult to directly compare hepatotoxicity between ARV agents as some studies included, while others excluded, viral hepatitis from their analyses. Individual agents independently associated with hepatotoxicity have included nevirapine, high doses of ritonavir, protease inhibitors, and

prolonged zidovudine or stavudine exposure[4,20,21], maraviroc, and tipranavir. Hepatotoxicity usually occurs by one of four mechanisms: direct drug toxicity and/or drug metabolism, hypersensitivity reactions, mitochondrial toxicity, and immune reconstitution syndrome[1]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are associated with hypersensitivity or direct drug toxicity[22]. Other agents associated with hypersensitivity reactions include the nucleoside reverse transcriptase inhibitor (NRTI) abacavir; and the CCR5 inhibitor maraviroc[23]. Protease inhibitors are associated with direct drug toxicity. All NNRTIs, most NRTIs, and maraviroc are all also associated with direct drug toxicity. The nucleoside reverse transcriptase inhibitors (NRTIs) are associated with mitochondrial toxicity, with stavudine, didanosine, and zidovudine the most commonly implicated. Immune reconstitution in the setting of viral hepatitis can occur with any ARV regimen and is associated primarily with hepatitis B infection.

Other predictors of hepatotoxicity

Other predictors of hepatotoxicity include older age, female sex, low platelets, high HIV RNA levels, and increase in CD4 cell counts of > 50 cells/mm^3, renal insufficiency, high BMI, and non-black ethnicity, concomitant tuberculosis medications, first exposure to antiretroviral therapy, baseline elevated ALT or AST, alcohol use, and preexisting advanced fibrosis[21,24,25].

Given the frequency of hepatotoxicity in HIV infected subjects receiving antiretroviral therapy and the lack of information on predictors differentiating severe from not severe hepatotoxicity, we sought to characterize the predictors of severe hepatotoxicity, as defined by a greater than grade 3 ALT elevation, in comparison to those who developed the more common grade 2 ALT elevation.

Chapter 2: Predictors of Hepatotoxicity in Human Immunodeficiency Virus-Infected Patients Receiving Antiretroviral Therapy

ABSTRACT

Hepatotoxicity is a common side effect of antiretroviral therapy with severe hepatotoxicity necessitating a regimen change. This was a retrospective cohort study that investigated the predictors of severe hepatotoxicity in a cohort of HIV-infected patients on antiretroviral therapy identified to have clinically significant elevations in transaminases. Severe hepatotoxicity was defined as an alanine aminotransferase level (ALT) greater than 275 IU/ml. The prevalence of severe hepatotoxicity was 23%. In multivariable stepwise regression analysis, only HBsAg, higher median HIV viral load and lower median ALT at database entry were predictors of severe hepatotoxicity. In a cohort of HIV and hepatitis B (HBV) coinfected patients, only higher log HIV viral load at database entry showed a trend towards significance as a predictor of severe hepatotoxicity, in multivariable stepwise regression analysis. Neither antiretroviral regimen class nor single agent was associated with severe hepatotoxicity. HBV infection, but not HCV infection, was a significant predictor of severe hepatotoxicity in HIV infected patients on antiretroviral therapy. Conclusion: In a cohort of HIV-infected patients receiving antiretroviral therapy, HBV infection, ALT and log HIV viral load at database entry were significant predictors of severe hepatotoxicity, while antiretroviral regimens were not.

Key Words: hepatitis, HIV, antiretroviral treatment

Introduction:

Hepatotoxicity is a common side effect of antiretroviral therapy, occurring in 6-30% of HIV infected subjects[1]. Severe hepatotoxicity occurs in 2-18% of patients and requires discontinuation of therapy and/or therapy modification[24] . Clinicians are often faced with patients who develop hepatotoxicity, but most cases of mild hepatotoxicity will resolve even with the continuation of antiretroviral agents [3,26]. Therefore, within a cohort of those with hepatotoxicity, it is critical to identify the risk factors associated with severe hepatotoxicity, or that hepatotoxicity that would necessitate a regimen change. There is little published data on the predictors of severe hepatotoxicity, when compared to less severe hepatotoxicity, in a cohort of individuals identified to have hepatotoxicity. Additionally, although several studies have examined predictors of severe hepatotoxicity, many have only evaluated one agent, a single class of agents, or have had limited viral hepatitis enrollment. In this study, we evaluated a cohort of US HIV patients receiving care in the AIDS Healthcare Foundation (AHF) with ≥ grade 2 hepatotoxicity, and within this cohort, sought to identify predictors of severe hepatotoxicity (≥ grade 3 hepatotoxicity), that which would necessitate regimen change. We elected to exclude examination of a cohort with < grade 2 hepatotoxicity as this is common and would not necessitate regimen change and as this was a preliminary analysis and we were unsure of the nature of the characterization of the database.

Patients and Methods: This study included HIV infected adults receiving care at the AIDS Healthcare Foundation (AHF) outpatient clinics in California and Florida who received antiretroviral therapy from 2003 to 2011. The cohort included both antiretroviral-naïve and –experienced patients. Patients routinely visited the outpatient clinics every 12 weeks.

Information on demographic factors and laboratory data were all obtained from the AHF patient database. This study received institutional review board approval at the University of California, Los Angeles.

Data collected from the patients' medical records included date of birth, sex, antiretroviral therapy regimen, HIV risk factor, history of ETOH abuse, and BMI. Severe hepatotoxicity was defined as a $\geq$ grade 3 elevation in ALT ($\geq$ 3.1 x the upper limit of normal (ULN)). The ULN was 55 for ALT in our cohort. The first hepatotoxic event in the database was chosen for analysis. Information on patient adherence and concomitant medications or tuberculosis diagnoses was not captured.

Laboratory data, such as plasma HIV RNA levels, CD4 cell counts, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, and hepatitis B virus (HBV) and hepatitis C virus (HCV) serologies, were retrieved electronically from the AHF database. Plasma HIV RNA levels, CD4 cell counts, and AST and ALT levels were obtained at database entry. The FIB-4 score, a non-invasive marker of liver fibrosis[27], was calculated at database entry.

Definitions of chronic viral hepatitis. Patients were considered to have chronic HBV infection when HBV surface antigen (HBsAg) could be detected in the plasma. Patients were considered to have chronic HCV infection when antibodies against HCV (anti-HCV) were present.

Definition of Endpoint: We selected grade 3 or greater hepatotoxicity, as defined by an absolute ALT value of $\geq$ 275 IU/ml, as this is the grade of hepatotoxicity that would warrant regimen change. Individuals with elevated ALT at database entry were included; we did not assess hepatotoxicity as measured by change from database entry ALT.

Statistical analysis: All statistical analyses were performed using JMP v.10 software.

Baseline characteristics of patients with and without severe hepatotoxicity were tabulated: state in which therapy was given (California or Florida), age, sex, risk group for HIV transmission (men who have sex with men status and injection drug use), white vs non-white ethnicity, database entry CD4 cell count, database entry plasma HIV RNA levels, database entry AST and ALT levels, database entry FIB-4 score, database entry BMI, history of alcohol abuse, injection drug use, and methamphetamine use, and infection with HBV or HCV. Antiretroviral regimens were also tabulated. Group comparisons were made with the Wilcoxon rank sum test or Student's t-test, and with the x^2 (Chi square) or Fisher's exact test for categorical data. The level of significance was set at 5% throughout the analyses. All reported p values are 2-sided.

To identify independent risk factors for the development of antiretroviral therapy–associated grade 3 or greater hepatotoxicity, we constructed a multiple variable regression model using forward stepwise regression. All baseline variables were included.

In a separate analysis, we evaluated predictors of severe hepatotoxicity in an HIV/HBV coinfected cohort. Baseline characteristics of patients with and without severe hepatotoxicity were tabulated and included all of the baseline characteristics examined in the larger cohort. Additionally, log HBV viral load was also examined as a predictor. Finally, instead of examining individual antiretroviral agents, because of the smaller sample size, we examined class of antiretroviral agent, rather than individual drugs. Statistical analyses were conducted as above.

Results:

In total, 2180 patients with unique hepatotoxic events that were greater than grade 2 were identified. Of these, 1591 patients had events while on antiretroviral therapy. Three hundred sixty (360) patients had events that met the definition for severe, or greater than grade 3

hepatotoxicity. The baseline demographic and clinical characteristics of these patients are listed in Table 1. 87% of patients were identified in California. 32% of patients were of white ethnicity while 92% were male. HBsAg and HCV prevalence were 9% and 4%, respectively. Median CD4 was 340 while median HIV viral load was 3.6 $\log_{10}$ copies.

Table 2 summarizes the antiretroviral regimens. Protease inhibitors accounted for 47% of ARV regimens, with boosted protease inhibitors comprising 36% of regimens. Approximately one quarter of patients were receiving non-nucleoside reverse transcriptase inhibitors. If fewer than 5 subjects received an antiretroviral agent, this agent was excluded. These agents were tipranavir, rilpivirine, delavirdine, combination nelfinavir/ritonavir, and combination tipranavir/ritonavir.

Predictors of Grade 3 Hepatotoxicity.

Univariate Analyses: In univariate analyses, when comparing to those with grade 2 hepatotoxicity, patients with grade 3 or severe hepatotoxicity were more likely to be of white ethnicity (37% vs. 30%; p 0.02). Patients with grade 3 hepatotoxicity were also more likely to be infected with hepatitis B or hepatitis C than those with grade 2 (12.5% vs. 8.5%, p=0.01 and 5.6% vs. 3.0% p=0.03). HIV viral load was also higher in the severe hepatotoxicity group. Median CD4 was lower in the severe hepatotoxicity group (281 vs. 302 cells/mm^3), but this trend did not reach statistical significance.

With regards to antiretroviral regimens, nevirapine was represented more in the severe hepatotoxicity group (15.3% vs. 12.6%) but this trend did not reach statistical significance.

Multivariate Analysis: A forward stepwise regression analysis was performed to assess which factors, when combined, were significantly associated with the occurrence of severe

hepatotoxicity. Factors tested in the stepwise regression evaluation included those that were considered to be potentially clinically relevant and included all baseline predictor variables.

In the forward stepwise regression model, only HBsAg, median HIV viral load, median ALT at database entry were significant. Antiretroviral regimens were not significant in multivariate analysis. Table 3 lists the risk factors significantly associated with severe hepatotoxicity in multivariate analysis.

We then separately assessed a cohort of HIV/HBV coinfection, to examine the predictors of severe hepatotoxicity. In this cohort, although CD4 and log HIV viral load at database entry were numerically lesser and greater in the severe hepatotoxicity group (184 vs. 232 cells/mm^3 and 4.4 and 3.6 log10, respectively), these did not reach statistical significance (See Appendix). Using a forward stepwise regression model (see methods for covariates selected), there were no predictors that achieved statistical significance. However, a higher log HIV viral load at database entry trended towards significance (p=0.07)

Discussion: In a large cohort of HIV infected patients receiving antiretroviral therapy, HBsAg status, lower median ALT, and higher median HIV viral load, were predictors of severe hepatotoxicity in univariate analyses. Individual HIV regimens or classes were not associated with severe hepatotoxicity. In a cohort of HIV/HBV coinfected patients receiving antiretroviral therapy, a higher log HIV viral load at database entry was predictive of severe hepatotoxicity.

The fact that ARV regimens did not predict for severe hepatotoxicity was surprising. Our particular study design may explain the lack of association between antiretroviral regimens and severe hepatotoxicity. We chose patients who already had hepatotoxicity and wanted to evaluate what distinguished severe from less severe hepatotoxicity. Within this background of

hepatotoxicity, we were then able to isolate key predictors, including HBsAg status. One other study examining severe hepatotoxicity, as defined as grade 4 liver enzyme elevations, also found a minimal effect of antiretroviral regimen, citing only nevirapine and high dose ritonavir as significant ARV risk factors, even when examining several different antiretroviral regimens. Our study had few patients with each of these agents and thus may have been limited to detect these effect sizes.

Hepatitis B coinfection has been a predictor of hepatotoxicity in several studies. In a Thai HIV study, the relative risk of developing hepatotoxicity was 3.20 in patients with HIV/HBV coinfection[17]. In another study of HIV infected individuals, there was a 9.2 hazard risk of grade 4 ALT elevation with HBV infection[12]. We have also shown that hepatitis B infection is associated with more hepatotoxicity than HCV infection. In our study, HCV infection did not retain statistical significance in the multivariable model. The fact that HBV was a more significant predictor than HCV is similar to a finding in another study, where the hazard ratio for hepatotoxicity due to HBV was 9.2 compared to 5.0 for HCV[12]. Hepatitis B infection may be more associated with immune reconstitution with a larger inflammatory response leading to greater hepatotoxicity. In a study of 36 patients with HIV/HBV coinfection, 22.2% developed immune reconstitution inflammatory syndrome (HBV-IRIS)[28] while in a study of HIV/HCV coinfected patients, only 2% of patients who started antiretroviral therapy developed HCV associated IRIS[29].

We found that lower ALT at database entry was associated with severe hepatotoxicity. Other studies have also identified ALT as a risk factor for hepatotoxicity [7,12], including in cohorts of HIV/HBV coinfection[28]; however, these studies identified elevated ALT as a risk factor. ALT, alanine aminotransferase is a marker for hepatic inflammation and baseline elevated inflammation has been implicated in the development of hepatotoxicity with other potentially hepatotoxic agents. Our finding of lower ALT at database entry being predictive of severe

hepatotoxicity was surprising. Lower ALT values may be associated with immune dysfunction in patients coinfected with viral hepatitis; in one study, 25% of patients with normal ALT had moderate to severe liver fibrosis. These patients were more likely to have lower CD4 counts (below 500 cells/mm^3). Thus, in our cohort, these lower ALT values may have been a marker of low CD4 counts.

We found that a higher median HIV viral load at database entry was associated with severe hepatotoxicity. Baseline HIV viral load is a marker for immune deficiency and several other studies have identified immune deficiency, identified as low CD4 counts, as a risk factor for hepatotoxicity. Baseline immunodeficiency may be a marker of those most at risk for developing immune reconstitution syndrome (IRIS) from viral hepatitis, particularly hepatitis B infection. This is supported by the finding that HIV viral load at database entry was the only factor significantly associated with hepatotoxicity in our HIV/HBV coinfected cohort.

Our study had several limitations. First, by identifying those already with hepatotoxicity, we may have inadvertently cancelled out the effect of antiretroviral therapy, as most hepatotoxicity with antiretroviral therapy is less than grade 2 hepatotoxicity. This may explain why we could not identify ARV regimens as predictors. Conversely, this allowed us to single out the most important predictors of severe hepatotoxicity. Secondly, we could not distinguish between those who were initiating their first antiretroviral regimen or who were on their second or later regimens. This is important, as first regimen initiation has been independently associated with severe hepatotoxicity[12]. Also, the effect of prior antiretroviral regimens with the potential for hepatotoxicity could not be studied. One additional important limitation is that this analysis is a cross-sectional study; therefore, we may have missed those who would have, over time, developed more severe hepatotoxicity. Also, we could not assess time to hepatotoxicity, which may have helped delineate types of hepatotoxicity. Finally, in the HIV/HBV coinfection cohort, 40 patients had missing data with regards to HBV viral load. This may have explained why HBV

viral load was not predictive of hepatotoxicity in our study, but has been associated with severe hepatotoxicity in other studies.

In summary, HBsAg status, higher median log HIV viral load and lower median ALT at database entry were predictors of grade 3 or greater hepatotoxicity. HBsAg status, along with baseline immune and hepatic inflammation status should be considered risk factors for the development of severe hepatotoxicity, regardless of ARV regimen. In those with HIV/HBV coinfection, baseline HIV viral load was associated with hepatotoxicity.

Table 1: Baseline and Clinical Demographics

	All N=1591, (%)	Not Severe Hepatotoxicity N=1231	Severe Hepatotoxicity N=360	P value
Age Median (IQR)[1]	 41 (35-47)	 41 (35-47)	 41 (35-47)	 0.89
ALT at first hepatotoxic event[2,3] Median (IQR)[1]	 187 (158-257)	 170 (154-202)	 400 (327-601)	
Sex F M Trans[4]	 102 (6.4%) 1456 (92%) 29 (1.8%)	 76 (6.2%) 1124 (92%) 27 (2.2%)	 26 (7.2%) 332 (92%) 2 (0.6%)	 0.10
Race				**0.02**
White	507 (31.9%)	374 (30.1%)	133 (36.9%)	
Non-White	1084 (68.1%)	857 (69.6%)	227 (63.1%)	
IDU[5] ETOH[6] Meth[7]	29(1.8%) 110 (6.9%) 176 (11%)	21 (1.7%) 83 (6.7%) 136 (11.1%)	8(2.2%) 27 (7.5%) 40(11.1%)	0.52 0.62 0.97
MSM[8]	971 (61%)	750(60.9%)	221 (61%)	0.87
State CA FL	 1389 (87%) 202 (13%)	 1089(88.4%) 142 (11.5%)	 300 (83.3%) 60 (16.7%)	 **0.01**
HBsAg	150 (9%)	105 (8.5%)	45 (12.5%)	**0.01**
HCV Ab	57 (4%)	37 3.0%	20 5.6%	**0.03**
CD4 Median (IQR)	 299 (133-482)	 302 (137-487)	 281 (108-468)	0.11
HIV VL (log) Median (IQR)	 3.6 (2.1-4.9)	 3.5 (2.1-4.8)	 4.1 (2.1-4.9)	 **0.04**
ALT Median (IQR)	 47 (27-90)	 49 (28-91)	 41 (25-86)	0.11
AST[9] Median (IQR)	 38 (26-67)	 39 (26-69)	 35 (25-64)	0.11
BMI[10] Median (IQR)	 25.1 (22.7-27.7)	 25.2 (22.7-27.8)	 24.8 (22.8-27.2)	0.79
FIB4[11] Median (IQR)	 1.1 (0.78-1.74)	 1.1 (0.78-1.77)	 1.0 (0.71-1.62)	0.82

1. IQR: Interquartile range 2. ALT: Alanine aminotransferase 3. ALT- ALT at first hepatotoxic event 4. Trans: Transgender 5. IDU: Injection Drug Use 6. ETOH: Alcohol use 7. Meth: Methamphetamine use 8. MSM: Men who have sex with men 9. AST: Aspartate aminotransferase 10. BMI: Body mass index 11.FIB4: Fibrosis 4 score. Bolded variables indicate a statistical significance of ≤ 0.05 in univariate analysis. The following variables had missing data; sex n=4, HIV viral load n=24, CD4 n=6, BMI n=686, FIB-4 n=103.

Table 2: Comparison of Antiretroviral Regimen and Hepatotoxicity

	All N=1591	Not Severe N=1231	Severe N=360	pvalue
ARV[1] regimen during event				
Protease inhibitor	755 (47.4%)	588(47.8%)	167(46.4%)	0.64
Atazanavir	264 (16.6%)	211(17.1%)	53 (14.7%)	0.28
Darunavir	81 (5.1%)	69 (5.6%)	12 (3.3%)	0.08
Saquinavir	57 (3.6%)	41 (3.3%)	16 (4.4%)	0.32
Amprenavir	59 (3.7%)	47 (3.8%)	12 (3.3%)	0.67
Fosamprenavir				
Nelfinavir	63 (4.0%)	12 (3.3%)	51(4.1%)	0.49
Tipranavir	5 (0.3%)	2 (0.2%)	3 (0.8%)	*
Ritonavir Alone	21 (1.3%)	14 (1.1%)	7(1.9%)	0.24
Boosted protease inhibitor	574(36.1%)	450 (36.6%)	124 (34.4%)	0.46
a. Lopinavir/rit[2]	218 (13.7%)	165 (13.4%)	53 (14.7%)	0.52
b. Atazanavir/rit	202 (12.7%)	165 (13.4%)	37 (10.3%)	0.12
c. Darunavir/rit	61 (3.8%)	52 (4.2%)	9 (2.5%)	0.13
d. Saquinavir/rit	30 (1.9%)	23 (1.9%)	7 (1.9%)	0.93
e. Amprenavir/rit	45(2.8%)	36 (2.9%)	9 (2.5%)	0.67
f. Fosamprenavir/rit	26 (1.6%)	21 (1.7%)	5 (1.4%)	§0.82
g. Nelfinavir/rit	2 (0.13%)	2 (0.16%)	0	*
h. Tipranavir/rit	3 (0.19%)	1 (.08%)	2 (0.6%)	*
Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)	119(24.3%)	371(30.1%)	119 (33.1%)	0.29
a. Efavirenz	97 (6.1%)	73 (5.9%)	24 (6.7%)	0.61
b. Nevirapine	210 (13.2%)	155 (12.6%)	55 (15.3%)	0.19
c. Etravirine	13 (0.8%)	9 (0.7%)	4 (1.1%)	§0.48
d. Rilpivirine	2 (0.13%)	1 (.08%)	1 (0.3%)	*
e. Delavirdine	3 (0.19%)	3 (0.2%)	0	*
Stavudine (D4T)	147 (9.2%)	114 (9.3%)	33(9.2%)	0.96
Didanosine (DDI)	114 (7.2%)	92 (7.5%)	22 (6.1%)	0.38
DDI or D4T	236 (14.8%)	188 (15.2%)	48 (13.3%)	0.37
DDI and D4T	25 (1.6%)	18 (1.5%)	7 (1.9%)	0.52
Abacavir	304 (19.1%)	240 (19.5%)	64 (17.8%)	0.47
Zidovudine (AZT)	212 (13.3%)	160 (13%)	52 (14.4%)	0.48
Raltegravir	43 (2.7%)	35 (2.8%)	8 (2.2%)	0.53
Maraviroc	1	1	0	*

1. ARV: Antiretroviral 2. Rit: Ritonavir * P values are not included for regimens for which there were 5 or fewer subjects. § P values are calculated with the Fisher's exact test.

Table 3: Predictors of Severe Hepatotoxicity: Results of Multivariable Logistic Regression Analysis with Forward Stepwise Regression

	Not Severe	Severe Hepatotoxicity	OR, MV	95% CI	P value
HBsAg	105 (8.5%)	45 (12.5%)	1.53	(1.04-2.22)	0.04
HIV VL (log)					
Median	3.5	4.1	0.88	(0.81-0.96)	0.05
ALT[1]					
Median	49	41	0.996	(0.995-0.998)	0.003

1. ALT: Alanine aminotransferase Predictors that were significant in forward stepwise regression were placed into a multivariable logistic regression model.

Appendix:

Design Methodology

Approach to Study Design

The overarching intent of this preliminary analysis was to examine the AIDS Healthcare Foundation (AHF) patient database and identify further opportunities for research in viral hepatitis coinfection.

Our study design is a retrospective cohort analysis, examining predictors of severe hepatotoxicity in HIV infected patients on antiretroviral therapy, in a cohort of those identified to have hepatotoxicity.

Why did we choose this approach?

We evaluated two other approaches to data extraction. Initially, we considered evaluating all HIV infected patients initiating antiretroviral therapy within the AHF database with the intent to identify predictors of severe and less severe hepatotoxicity, compared to those who did not experience hepatotoxicity. However, with over 15,000 patients in the database, we felt that the volume and manipulation of data would be beyond the scope of a master's thesis. Additionally, upon review of the database, we found that one key variable was incompletely characterized, whether patients were antiretroviral therapy naïve or not. Based on these two factors, we decided that it was more appropriate to evaluate a smaller dataset, acknowledging the limitations of selecting a cohort without hepatotoxicity. Secondly, we considered evaluating predictors of hepatotoxicity in those with just viral hepatitis coinfection. This would have been a smaller dataset, however, the incompletely characterized exposure to therapy variable (naïve vs experienced) made us hesitate to pursue this approach. Thus, we chose to identify all greater than grade 2 hepatotoxic events, a common problem, and identify predictors of grade 3 or

greater hepatotoxicity. The next step in this study is to perform a case-control study, identifying those patients in the AHF database who did not develop grade 3 or greater hepatotoxicity, and identify predictors of grade 3 or greater hepatotoxicity.

Selection of Primary Endpoint

We selected grade 3 or greater hepatotoxicity, as this is the grade of hepatotoxicity that would warrant regimen change.

We considered performing a time-to-event analysis, i.e. time to first hepatotoxic event after initiating an antiretroviral regimen, as a primary endpoint. However, when we assessed the duration on therapy, we found that 10% had less than 1 day of antiretroviral therapy, suggesting that the antiretroviral regimen may have been the regimen that patients were switched to after an hepatotoxic event, or that the dataset was insufficiently characterized. See Figure 1 below.

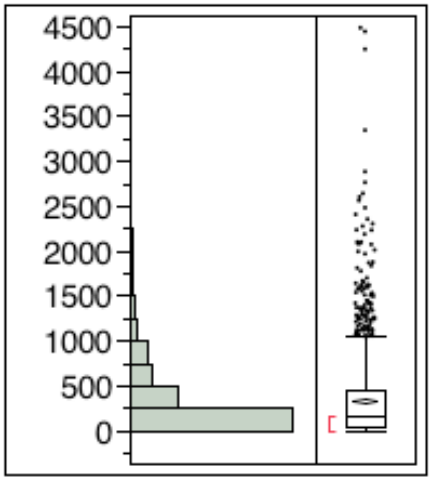

Figure 1: Distribution of Time From Regimen Initiation to First Hepatotoxic Event, in Days

Quantiles

100.0%	maximum	4474.46
99.5%		2596.02
97.5%		1592.49
90.0%		890.656
75.0%	quartile	441.89
50.0%	median	152.592
25.0%	quartile	40.4458
10.0%		0.64667
2.5%		0.45833
0.5%		0.36628
0.0%	minimum	0

Summary Statistics

Mean	331.67745
Std Dev	466.50155
Std Err Mean	11.695479
Upper 95% Mean	354.61763
Lower 95% Mean	308.73727
N	1591

Selection of Covariates (Predictors)

The covariates selected included age, sex, ethnicity, injection drug user status, history of alcoholism, history of methamphetamine use, HIV risk factor (MSM vs not), state, hepatitis B and C, CD4 and HIV viral load at database entry, ALT and AST at database entry, body mass index at database entry, and fibrosis score at database entry. Other important covariates included the antiretroviral agents. If fewer than 5 subjects received the antiretroviral agent, this agent was excluded. These agents were tipranavir, rilpivirine, delavirdine, combination nelfinavir/ritonavir, and combination tipranavir/ritonavir. We chose these covariates as they have all, with the exception of state, MSM status, and methamphetamine use, been associated with hepatotoxicity in previous studies. We chose to include geography as it was important to evaluate whether major differences in hepatotoxicity rates could be seen between regions, suggestive of provider preference for, or availability of, certain antiretroviral therapy regimens. Additionally, the prevalence of viral hepatitis could have been different between states. Although more patients in Florida experienced severe hepatotoxicity (statistically significant in univariate analyses), this did not retain significance in the stepwise regression model. We chose to include MSM status and methamphetamine use, as both have been associated with acute hepatitis C acquisition and hepatotoxicity.

Model Selection

We initially chose a multivariable logistic regression model to analyze the data. However, the model was unstable; this was likely related to the large number of covariates. We then chose a

forward stepwise regression model, as this model is thought to be more suitable for exploratory analyses.

Primary Endpoint Analysis

Univariate analyses:

The t-test was used to evaluate normally distributed continuous variables. The Wilcoxon sign-rank test was chosen when variables were not normally distributed. Chi-squared tests were used to examine dichotomous variables. All statistical tests were two-sided with a significance level cut-off of of 0.05.

Multivariate Regression Analysis:

Table 4. Forward Stepwise Regression for Entire HIV-infected Cohort:

Step History								
Step	Parameter	Action	L-R ChiSquare	Sig Prob	RSquare	p	AICc	BIC
1	**ALTAtDbEntry**	**Entered**	**8.88952**	**0.0029**	**0.0097**	**2**	**914.469**	**923.986**
2	**HBSAG**	**Entered**	**4.382379**	**0.0363**	**0.0144**	**3**	**912.101**	**926.368**
3	**Log HIV Viral Load**	**Entered**	**3.809832**	**0.051**	**0.0186**	**4**	**910.31**	**929.323**
4	CD4AtDbEntry	Entered	3.548423	0.0596	0.0224	5	908.785	932.54
5	EFV	Entered	2.690924	0.1009	0.0254	6	908.122	936.614
6	DAR	Entered	2.591657	0.1074	0.0282	7	907.563	940.788
7	HCV	Entered	1.711844	0.1907	0.03	8	907.888	945.841
8	BMIAtDbEntry	Entered	1.319594	0.2507	0.0315	9	908.611	951.286
9	Sex	Entered	1.825356	0.1767	0.0335	10	908.832	956.226
10	ETOHismEver	Entered	1.303589	0.2536	0.0349	11	909.581	961.687

The above table demonstrates the results of the forward stepwise regression. Variables whose p values were $\leq$ 0.25 were selected for entry in the stepwise regression model. Bolded variables represent predictors that were significant in forward stepwise regression. These were then tested in a logistic regression model and odds ratios were calculated. Only HBsAg status,

database entry HIV viral load, and database entry ALT were significant. Odds ratios are presented in the manuscript in the multivariate logistic regression table (Table 3: Predictors of Severe Hepatotoxicity: Results of Multivariable Logistic Regression Analysis with Forward Stepwise Regression).

Secondary Analysis

We also wanted to examine predictors of hepatotoxicity in a HIV/HBV coinfected cohort. Table 5 demonstrates the baseline and clinical factors, including antiretroviral therapy regimen. Because of the smaller sample size, we chose to examine antiretroviral regimens grouped by drug class, rather than explore individual antiretroviral agents. In addition, we added log HBV viral load as a predictor, given its importance in predicting hepatotoxicity in another study[30]. The t-test was used to evaluate normally distributed continuous variables. The Wilcoxon sign-rank test was chosen when variables were not normally distributed. Chi-squared tests were used to examine dichotomous variables. All statistical tests were two-sided with a p value of 0.05.

Table 5: HIV/HBV Coinfection
Baseline and Clinical factors, including Antiretroviral Therapy Regimen

Total: n=150		**Not severe N=105**	**Severe N=45**	**pvalue**
Age	Median(IQR[1])	39 (35-45)	42 (32-46)	0.99
State				
CA		99(66.0%)	43(28.67%)	0.75
FL		6(4.00%)	2(1.33%)	
Sex				§0.67
F		1(0.67%)	0	
M		102(68.46%)	44(29.53%)	
Trans[2]		1(0.67%)	1(0.67%)	
Race				
White		43 (41.0%)	11(24.4%)	0.05
HCV		0	1	*
CD4	Median(IQR)	232 (117-415)	184 (90-360)	0.33
ALT[3]	Median(IQR)	58 (36-95)	54 (28-82)	0.47
AST[4]	Median(IQR)	44 (30-72)	43 (29-66)	0.62
BMI[5]	Median(IQR)	24.64 (21.7-27.2)	24.57	0.97
ETOH[6]		2 (1.33%)	2 (1.33%)	*
IDU[7]		2(1.33%)	0	*
Meth[8]		18 (12.00%)	6 (4.00%)	0.56
MSM[9]		83(55.33%)	35(23.33%)	0.86
Log HIV VL	Median(IQR)	3.58 (2.0-4.9)	4.43 (2.63-5.0)	0.14
Log HBV VL	Median(IQR)	4.45 (2.0-7.0)	3.37 (2.0-7.0)	0.60
FIB4[10]	Median(IQR)	1.19 (0.80-2.0)	1.09 (0.82-1.66)	0.49
NNRTI[11]		23 (15.33%)	10 (6.67%)	0.97
PI[12]		52 (34.67%)	20 (13.33%)	0.57
Boosted PI		42(28.00%)	16(10.67%)	0.61

1. IQR: Interquartile range 2. Trans: Transgender 3. ALT: Alanine aminotransferase 4. AST: Aspartate aminotransferase 5. BMI: Body mass index 6. ETOH: Alcohol use 7. IDU: Injection Drug Use 8. Meth: Methamphetamine use 9. MSM: Men who have sex with men 10.FIB4: Fibrosis 4 score. 11. NNRTI: Non-nucleoside reverse transcriptase inhibitor 12. Protease inhibitor. The following variables had missing data; sex n=1, BMI n=64, FIB-4 n=10, HBV viral load n=42. * P values are not included for predictors for which there were 5 or fewer subjects. § P values are calculated with the Fisher's exact test.

In a forward stepwise regression model, all of the above variables were examined and none demonstrated a statistical significance of $p<0.05$. Variables whose p values were ≤ 0.25 were selected for entry in the stepwise regression model. However, log HIV viral load trended towards significance (p=0.07). See Table 6. The bolded variable represents a predictor that trended towards significance in forward stepwise regression.

Table 6: Forward Stepwise Regression for HIV/HBV Infected Cohort:

Step History

Step	Parameter	Action	L-R ChiSquare	Sig Prob	RSquare	p	AICc	BIC
1	MSMEver	Entered	2.987827	0.0839	0.0324	2	93.3104	97.6593
2	AgeAtFirstALTGr143	Entered	2.284787	0.1306	0.0572	3	93.2073	99.6372
3	**logHIVVL**	**Entered**	**3.25866**	**0.071**	**0.0926**	**4**	**92.1965**	**100.641**
4	ALTAtDbEntry	Entered	2.945333	0.0861	0.1246	5	91.5682	101.959
5	White	Entered	1.6977	0.1926	0.143	6	92.2599	104.524

In summary, we performed a retrospective cohort analysis using a large database in order to identify predictors of severe hepatotoxicity in a cohort of HIV infected receiving antiretroviral therapy. A stepwise forward regression model was employed. In subanalyses, we evaluated predictors of severe hepatotoxicity in an HIV/HBV coinfected cohort, again using a forward stepwise regression model. Future work will include designing a case control study to evaluate predictors of all grade hepatotoxicity and performing time to event analyses for hepatotoxicity.

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