Biomarkers of Inflammation and Coagulation Are Associated With Mortality and Hepatitis Flares in Persons Coinfected With HIV and Hepatitis Viruses

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Background. Hepatitis C virus (HCV) and/or hepatitis B virus (HBV) coinfection with human immunodeficiency virus (HIV) has a greater risk of mortality than either HCV or HBV infection alone and is frequently associated with hepatitis flares after antiretroviral therapy (ART) initiation.

Methods. We performed a retrospective cohort study of 287 HIV-positive persons coinfected with HBV and/or HCV (70 had HBV coinfection only, 207 had HCV coninfection only, and 10 had HBV and HCV coinfections) who had pre-ART plasma samples evaluated for biomarkers associated with death (within 4 years) and/or hepatitis flare (within 4 months) after ART initiation. A predictive biomarker risk score was calculated.

Results. Forty-eight deaths and 50 hepatitis flares occurred. Nonsurvivors were older, had more prior AIDS-defining events, and had higher pre-ART triglycerides and aspartate transaminase levels. Detectable hyaluronic acid and higher D-dimer, interleukin 6, interleukin 8, and soluble CD14 levels were associated with death in univariate models and with a composite biomarker risk score. The risk of hepatitis flares was higher with HBV coinfection only (24.3%) and with HBV and HCV coinfection (50%) than with HCV coinfection only (13.5%). Higher levels of alanine transaminase and interleukin 10 were also associated with hepatitis flares.

Conclusions. Among HIV-positive patients coinfected with HBV and/or HCV who are initiating ART, biomarkers of inflammation and coagulation are associated with an increased risk of death, whereas HBV coinfection and higher pre-ART interleukin 10 levels are associated with hepatitis flares.

Keywords. hepatic flares; biomarkers; AIDS; hepatitis.

The prognosis for human immunodeficiency virus (HIV)–infected persons receiving antiretroviral therapy (ART) has significantly improved [1–4], but adverse outcomes still commonly occur because of progression of preexisting diseases, new opportunistic infections, immune restoration disease, or medication toxicities [5–8]. Identifying reliable and easily tested biomarkers could enable timely prediction of adverse outcomes.

In the Strategies for the Management of Antiretroviral Therapy (SMART) trial, overall mortality was associated with higher plasma levels of D-dimer, C-reactive protein (CRP), and interleukin 6 (IL-6) [9], and AIDS-defining opportunistic diseases were associated with higher levels of CRP and IL-6 [10]. Moreover, a higher plasma level of soluble CD14 (sCD14), a

marker of monocyte activation, has also been independently associated with death [11]. Notably, most of the participants enrolled into the SMART study were ART experienced. In an ART-naive population, we recently reported that higher levels of CRP, D-dimer, IL-6, and hyaluronic acid were associated with an increased risk for AIDS-defining events, immune reconstitution inflammatory syndrome (IRIS), or death after ART initiation [12]. These findings in both ART-experienced and ART-naive cohorts strongly suggest that biomarkers of inflammation and activation of coagulation could be predictive of undesirable outcomes among HIV-infected persons. Whether these markers can be used in specific subgroups of HIV-infected persons with different coinfections is still unknown.

Coinfections with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) are common among HIV-infected individuals, mainly because of the shared routes of transmission [13, 14]. Interaction between these viruses can lead to accelerated hepatic morbidity and mortality in coinfected individuals [15–17], although ART initiation may delay the progression of liver disease and decrease overall mortality [18–20]. Nevertheless, in such hepatitis virus–coinfected individuals, ART has also been associated with an increased risk of elevated serum liver enzyme levels, and there are rare reports of rapid progression of liver disease or death due to drug toxicity resulting from ART-induced altered pharmacokinetics, ART toxicity, and/or immune restoration disease [21–25]. A substudy of the SMART trial revealed that overall mortality was higher for HIV-positive persons coinfected with HBV and/or HCV and that interruption of ART was particularly unsafe [26], although the cause of death was not always related to hepatic failure or hepatic carcinoma [26, 27].

In this retrospective study, our aim was to identify pre-ART candidate biomarkers associated with death during the first 4 years of ART or hepatitis flares in the first 4 months of ART among HIV-infected persons with HBV and/or HCV coinfections who participated in the Flexible Initial Retrovirus Suppression Therapies (FIRST) open-label randomized clinical trial. Our premise was that persons with an increased risk of hepatitis flares or death could be identified on the basis of biomarkers associated with liver injury and inflammation that differed from those of persons who did not experience these events. Mediators of inflammation, fibrosis, and coagulation were explored, and the choice of candidate biomarkers was based on previous work in other cohorts of HIV-infected individuals with or without hepatitis virus coinfection [10–12, 28]. The hypothesis was that cytokines and chemokines associated with acute inflammation in response to a high antigen load would be associated with flares, whereas biomarkers indicative of chronic inflammation with activation of the coagulation cascade and tissue fibrosis would be associated with death.

METHODS

Study Participants and Definitions

The FIRST (CPCRA 058) trial randomized 1397 ART-naive persons in the United States between 1999 and 2002 to one of 3 first-line ART strategies that consisted of 2 or 3 classes of antiretroviral drugs (clinical trials registration NCT0000092) [6]. The majority of the participants were at an advanced stage of HIV infection, with a median $CD4^+$ T-cell count of 155 cells/µL [6]. All subjects provided written informed consent. Plasma samples were prospectively obtained and stored at the study baseline visit (ie, prior to ART initiation), at months 1 and 4, and every 4 months thereafter.

For this project, we initially considered all 333 subjects with HBV and/or HCV coinfection in the FIRST study. HBV infection was defined as either documented prior seropositivity for hepatitis B surface antigen (HBsAg) on 2 occasions at least 6 months apart or seropositivity for HBsAg and anti-HBV core antibody (defined on the basis of total immunoglobulin levels or immunoglobulin G levels only) at study entry. HCV infection was defined on the basis of HCV antibody seropositivity and detection of HCV RNA in plasma. Of 263 persons seropositive for HCV antibody, 46 had plasma HCV RNA levels below the limit of detection (which reflects probable prior HCV clearance) and were excluded from the HCV-positive group. Our final analysis cohort included 287 HIV-infected persons with HBV and/or HCV coinfection: 70 persons had HBV infection only, 207 had HCV infection only, and 10 had HBV and HCV infection.

We considered 2 clinical outcomes: death from any cause within 4 years of ART initiation, or hepatitis flare within 4 months after ART initiation. A hepatitis flare was defined as an alanine transaminase (ALT) level of >100 IU/mL at month 1 or 4, with a concomitant increase of >50 IU/mL from the pre-ART level. This definition was selected because an ALT level that increases from a previous value to >100 IU/mL typically triggers clinical follow up and further testing in HIV-positive patients coinfected with hepatitis virus who are receiving ART. In addition, this cutoff value for the ALT level has been used previously in clinical studies [29, 30]. While the primary cause of death was not collected for the FIRST study, up to 3 *International Classification of Diseases, Ninth Revision* (ICD-9) codes for each death were available. Three authors (B. A., D. B., and I. S.) independently reviewed the ICD-9 codes and summarized the causes of death as AIDS/infectious etiology, liver disease, other, or unknown cause.

Biomarker Measurement

Cryopreserved plasma specimens from the baseline visit were evaluated. Proinflammatory cytokines (interleukin 1β [IL-1β], IL-6, and tumor necrosis factor α), T-helper 1 (Th1) cytokines (interferon γ [IFN-γ] and interleukin 12), T-helper 2 (Th2)

cytokines (interleukin 4 [IL-4] and interleukin 13 [IL-13]), a T-helper 17 (Th17) cytokine (interleukin 17), a regulatory cytokine (interleukin 10 [IL-10]), proliferation/differentiation cytokines (interleukin 2 and interleukin 15), and a panel of chemokines, including interleukin 8 [IL-8], CXCL10 (also known as IP-10), CXCL11 (also known as interferon-inducible T-cell α chemoattractant), CXCL1 (also known as Gro-α), CCL3 (also known as macrophage inflammatory protein 1α), CCL4 (also known as macrophage inflammatory protein 1β), CCL11 (also known as eotaxin 1), CCL13 (also known as MCP4), CCL17 (also known as TARC), CCL22 (also known as MDC), and CCL26 (also known as eotaxin 3 and macrophage inflammatory protein 4α), were measured in multiplex enzyme-linked immunosorbent assay (ELISA)–based assays (Meso Scale Discovery, Gaithersburg, MD). ELISA kits were also used to assay plasma CRP levels (Meso Scale Discovery, Gaithersburg, MD), hyaluronic acid (Corgenix, Westminster, CO), intestinal fatty acid binding protein (I-FABP; Hycult Biotechnology, Netherlands), sCD14 (R&D Systems, Minneapolis, MN), and free active transforming growth factor β1 (BioLegend, San Diego, CA) according to the manufacturers' protocols. Plasma D-dimer was quantified by an enzyme-linked fluorescent assay on a VIDAS instrument (BioMerieux, Durham, NC).

Plasma HCV and HBV loads were measured with the Versant HCV bDNA 3.0 and Versant HBV bDNA 3.0 assays (Siemens Healthcare Diagnostics, Tarrytown, NY), respectively, according to the manufacturer's instructions. The HBV assay has a dynamic range of 2000–100 000 000 copies/mL, and the HCV assay has a dynamic range of 615–7 690 000 IU/mL.

Data Analysis

Baseline characteristics for the overall cohort were described using median values presented with interquartile ranges (IQRs). For each outcome (death and hepatitis flare), unadjusted and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were estimated with logistic regression models for each biomarker. Multivariable models were adjusted for the following baseline covariates: age, sex, history of prior AIDS-defining conditions, triglycerides level, HBV infection, HCV infection, ALT level, CD4+ T-cell count, plasma HIV RNA level, and use of lamivudine in the initial therapeutic regimen for HIV infection. Models were repeated separately for individuals with HBV infection and for those with HCV infection. Unless otherwise stated, the ORs are per SD increase in the biomarker level after log (natural log scale) transformation. For hyaluronic acid, IL-1β, and IL-4, the OR compares individuals with detectable levels to those with undetectable levels.

To consider the combined effect of the biomarkers that were significantly associated with mortality, an additional exploratory analysis was performed on the basis of a composite mortality risk score. The risk score was calculated as the sum of the biomarkers that were greater than the cohort median value (for D-dimer, IL-6, IL-8, and sCD14 levels) or detectable (for hyaluronic acid), with each risk factor counting as 1 point. Risk groups were formed on the basis of the total points, as follows: low risk (sum = 0–1), moderate risk (sum = 2–3), or high risk (sum = 4–5). An adjusted logistic regression model was performed with the 3 risk groups as predictors for mortality. All reported P values were 2-sided, and there were no adjustments for multiple comparisons.

RESULTS

Cohort Description

Baseline characteristics of the study participants are presented in Table 1. Among the 287 HIV-infected individuals coinfected with HBV and/or HCV, 50 (17.4%) developed a hepatitis flare within 4 months after ART initiation; for 20, hepatic flare

Table 1. Baseline Characteristics of the Study Population

Characteristic	Study Population (n = 287)
Age, y	41 (36–47)
Female sex	47 (16)
Race/ethnicity	
African American	175 (61.0)
White	65 (22.6)
Latino/other	47 (16.4)
History of IDU	143 (49.8)
Prior AIDS-defining event	119 (41.5)
CD4+ T-cell count, cells/µL	155 (34–341)
HIV RNA, $\log_{10}$ copies/mL	5.1 (4.5–5.5)
Hepatitis virus status	
HBV alone	70 (24.4)
HCV alone	207 (72.1)
HBV + HCV	10 (3.5)
AST level, IU/L	49.0 (34.0–75.0)
ALT level, IU/L	47.5 (31.0–72.0)
Cholesterol level, mg/dL	156 (132–178)
Triglycerides level, mg/dL	124 (94–189)
Glucose level, mg/dL	85 (74–95)
Randomization group	
PI	97 (33.8)
NNRTI	99 (34.5)
PI + NNRTI	91 (31.7)
3TC use in initial HIV therapy	239 (83.3)

Data are no. (%) of participants or median (interquartile range).

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug use; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; 3TC, lamivudine.

occurred during the first month of ART. Hepatitis flares were experienced by 24.3% of individuals coinfected with HBV only, 13.5% of those infected with HCV only, and 50% of those infected with both HBV and HCV ($P = .003$). Compared with HIV-positive persons coinfected with HCV only, persons coinfected with HBV had a 2-fold increased risk of developing a hepatitis flare (unadjusted OR, 2.05; 95% CI, 1.0–4.0; $P = .04$), while those coinfected with HCV and HBV had a 6-fold increased risk (unadjusted OR, 6.4; 95% CI, 1.7–23.5; $P = .005$). Individuals who did and those who did not develop a hepatitis flare were similar with respect to most baseline characteristics, lamivudine use in the initial ART regimen, and opportunistic infection prophylaxis (Supplementary Table 1). There was no difference in median baseline CD4$^+$ T-cell counts between persons who did and those who did not develop a hepatitis flare during follow-up (148 cells/µL [IQR, 34–282 cells/µL] and 155 cells/µL [IQR, 36–343 cells/µL], respectively; $P = .44$). There was also no difference in median baseline CD4$^+$ T-cell counts between individuals who developed a hepatitis flare within 1 month and those who developed a flare by 4 months (214 cells/µL [IQR, 21–332 cells/µL] and 140 cells/µL [49–269 cells/µL], respectively; $P = .78$). Among the 50 individuals who developed a hepatitis flare, 23 (46%) had ALT levels of >200 IU/L (grade 3) at the time of the flare. The distributions of all tested biomarkers prior to ART initiation are provided in Supplementary Table 2 for those who did and those who did not develop a hepatitis flare.

There were 48 deaths during the 4 years of follow up (overall 4-year mortality, 16.7%). Prior to ART initiation, the participants who died during the 4 years of follow up were older, had higher plasma levels of AST and triglycerides, and were more likely to have had a past diagnosis of an AIDS-defining event, compared with those who survived (Supplementary Table 1). The mortality among persons who experienced hepatitis flares (14% [7 individuals]) and among those without flares (17.3% [41 individuals]) during the 4-year follow up suggested that mortality was more associated with viral hepatitis coinfection than with early flare status after ART initiation. Mortality did not relate to flare severity, because among the 23 participants with grade 3 hepatitis flares (ALT level, >200 IU/L), only 3 (13%) died within 4 years of follow-up, compared with 4 (15%) of 27 participants with grade 1 or 2 flares. Supplementary Table 3 shows the distributions of all biomarkers prior to ART initiation for individuals who did and those who did not die during the first 4 years of ART.

Among those who died, the median time to death was 26 months (95% CI, 11–34 months). The cause of death was retrospectively classified as related to AIDS and/or infection (for 24 individuals), liver-related causes (for 6), other known causes (for 12), and unknown/unidentified cause (for 6).

Risk of Death

The overall risk of death within 4 years of ART initiation was associated with detectable hyaluronic acid and higher levels of D-dimer, IL-6, IL-8, and sCD14 at baseline (Figure 1). The unadjusted univariate associations between increased TNF-α and CXCL10 levels were not significant in a multivariate model after adjustment for potential confounding variables (Figure 1).

Among the HIV/HBV-coinfected cohort, the associations of higher levels of D-dimer, IL-6, and sCD14 with increased risk of death were maintained in the multivariable models, while higher levels of IL-8 and detectable hyaluronic acid were no longer significantly associated with death (Figure 2A). Moreover, higher levels of CXCL11, which is produced by neutrophils in response to IFN-γ in combination with either TNF-α or bacterial liposaccharides [31], was also associated with death during 4 years of ART. Higher levels of I-FABP, a biochemical marker of intestinal cell damage [32, 33], were related to a decreased risk of death among patients with HIV/HBV coinfection. In those with HIV/HCV coinfection, detectable hyaluronic acid and increased levels of IL-6 and sCD14 remained significantly associated with death (Figure 2B).

Combination of Biomarkers to Predict Mortality

Next, we developed a simple composite biomarker score predictive of mortality. Table 2 presents the results from modeling the joint effects of 5 biomarkers (D-dimer level, hyaluronic acid detectability, IL-6 level, IL-8 level, and sCD14 level) on mortality. While the mortality risk groups were fairly equal in size, when scored into low-, moderate-, and high-risk groups, the percentage dying within 4 years of ART initiation was 4.6%, 16.8%, and 28.3%, respectively. Compared with the low-risk group as reference, the OR (95% CI) for death within 4 years of ART initiation was 3.8 (95% CI, 1.2–12.1) for those in the moderate-risk group and 7.7 (95% CI, 2.4–24.6) for those in the high-risk group. The goodness of fit from the model with the composite biomarker score was similar to a model with biomarkers on a continuous scale (Table 2) but allowed for identification of persons at high risk for mortality.

Risk of Hepatitis Flares

The risk of hepatitis flare events within 4 months after ART initiation was increased with the presence of HBV coinfection and higher pre-ART levels of ALT and IL-10 (Figure 3). Conversely, a higher CCL26 level at baseline was associated with decreased risk of hepatitis flares (Figure 3). Among those who were HBV seropositive, higher plasma HBV DNA was associated with the development of a hepatitis flare (adjusted OR, 1.36; 95% CI, 1.11–1.66; $P = .003$). Similarly, among those who were HCV seropositive, higher plasma HCV RNA load
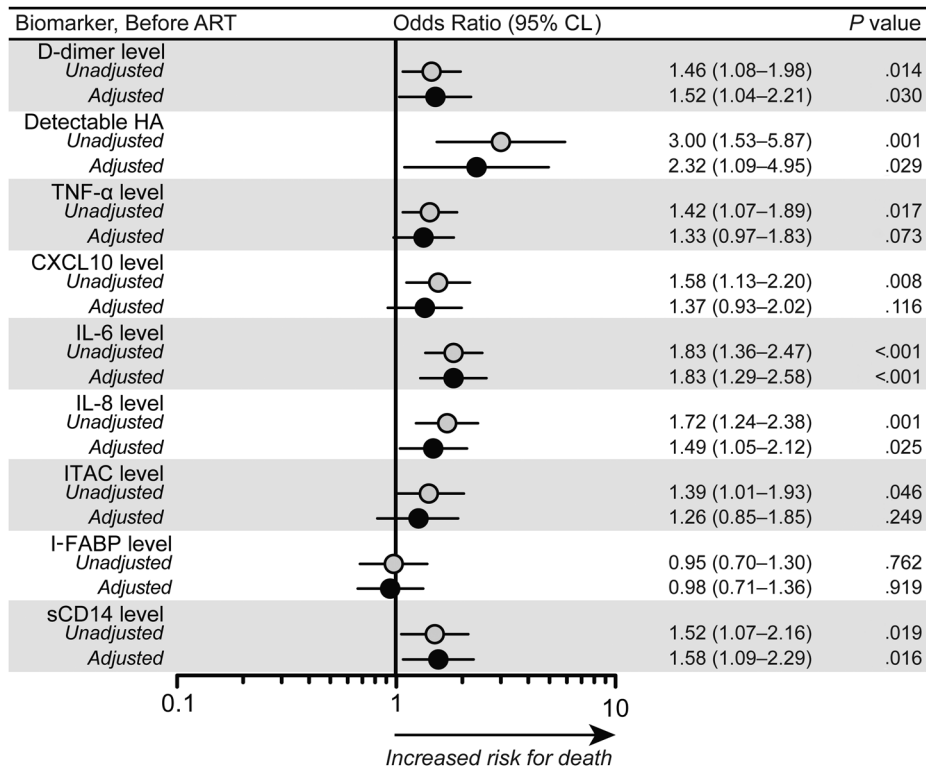
Figure 1. Baseline (ie, pre–antiretroviral therapy [ART]) biomarkers associated with subsequent all-cause mortality within 4 years after initiation of therapy for human immunodeficiency virus (HIV) infection are shown. Odds ratios (ORs) are per SD increase after log transformation. ORs for hyaluronic acid (HA) are for individuals with detectable HA, compared with those with undetectable HA. ORs were adjusted for age; sex; history of prior AIDS-defining events; triglycerides level; hepatitis B virus infection; hepatitis C virus infection; baseline alanine transaminase level, CD4⁺ T-cell count, and plasma HIV RNA level; and use of lamivudine in the initial therapeutic regimen. Abbreviations: CI, confidence interval; I-FABP, intestinal fatty acid binding protein; IL-6, interleukin 6; IL-8, interleukin 8; sCD14, soluble CD14; TNF-α, tumor necrosis factor α.

was associated with a subsequent hepatitis flare (adjusted OR per $\log_{10}$ increase, 1.30; 95% CI, 1.01–1.65; $P = .04$).

DISCUSSION

In the present exploratory study, we have identified candidate biomarkers in plasma collected before ART initiation that were associated with death and/or hepatitis flares after commencing ART in HIV-infected persons coinfected with HBV and/or HCV. Nearly 30% of North American HIV-infected individuals are coinfected with HCV, and 10% are coinfected with HBV [13, 14]. HBV and/or HCV coinfections have been linked to accelerated liver fibrosis progression rates and shorter times to development of cirrhosis [32–34]. In HIV/HBV- and/or HIV/HCV-coinfected individuals, ART has been related to an increased risk of elevated liver enzyme levels and death [22, 24, 25, 35]. Moreover, chronic viral hepatitis has been associated with non-liver, non–opportunistic disease mortality in HIV-infected individuals who are receiving ART [26]. With regard to HIV/HBV coinfection, current treatment guidelines recommend a tenofovir-based regimen as

the treatment of choice, without highlighting the particular advantage of HBV combination therapy in this setting [36].

Identification of biomarkers that predict mortality may assist in the clinical management of these patients. We demonstrated in our cohort of HIV-infected individuals coinfected with HBV and/or HCV that detectable hyaluronic acid and higher concentrations of ᴅ-dimer, IL-6, IL-8, and sCD14 were associated with death within 4 years of ART initiation. These results are in agreement with our previous study suggesting that ᴅ-dimer, CRP, IL-6, and hyaluronic acid could be useful in identifying ART-naive patients at higher risk of AIDS or death after ART initiation [12] and with other studies underscoring the importance of these markers as predictors of death in HIV-infected persons [9–11]. While increased ᴅ-dimer levels suggest activation of the coagulation pathway, increased plasma levels of hyaluronic acid, IL-6, IL-8, and sCD14 might indicate systemic inflammation and tissue fibrosis in response to injury, resulting from activation of cells of the innate immune system, particularly monocytes [37]. Indeed, increased expression of tissue factor on activated monocytes may be a cause of increased ᴅ-dimer levels [38].
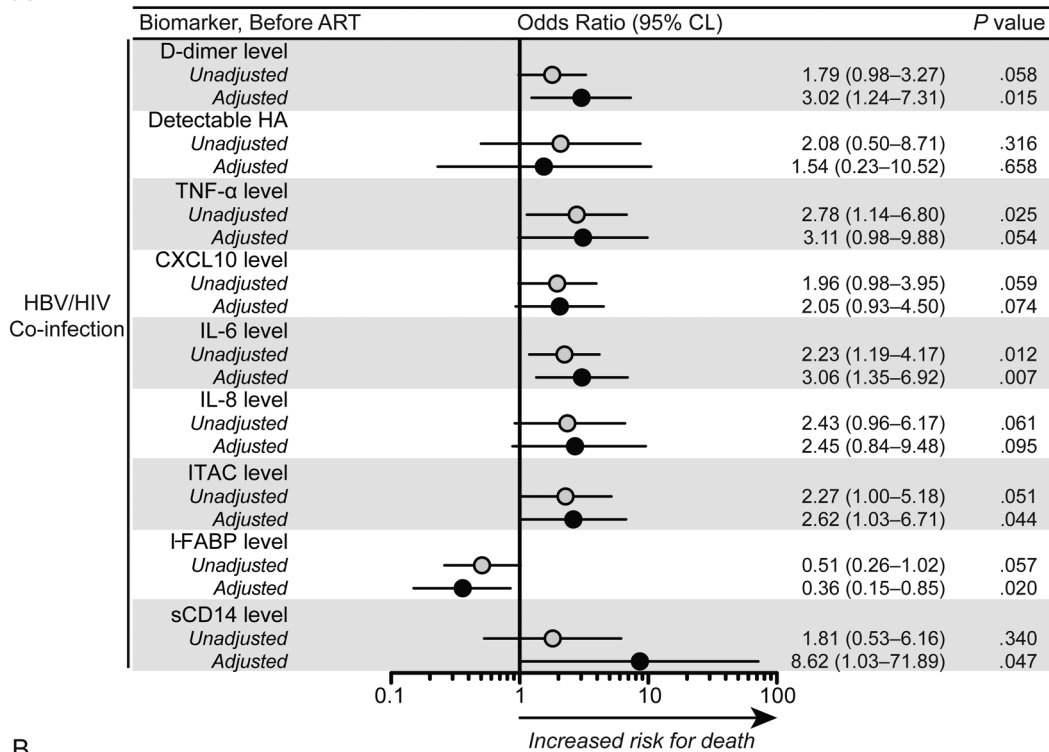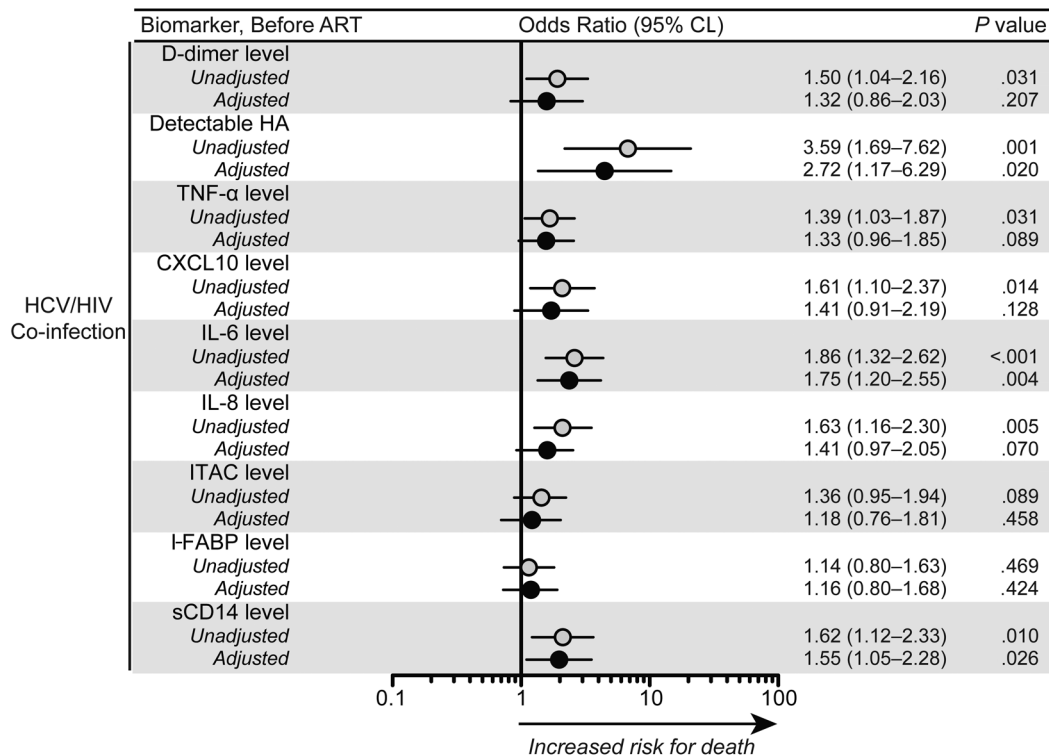
Figure 2. Baseline (ie, pre–antiretroviral therapy [ART]) biomarkers associated with subsequent death during ART, stratified by hepatitis B virus (HBV) coinfection (*A*) and hepatitis C virus (HCV) coinfection (*B*). Odds ratios (ORs) for hyaluronic acid (HA) are for individuals with detectable HA, compared with those with undetectable HA; other ORs are per SD increase after log transformation. ORs were adjusted for age; sex; history of prior AIDS-defining events; triglycerides level; HBV infection; HCV infection; baseline alanine transaminase level, CD4+ T-cell count, and plasma HIV RNA level; and use of lamivudine in the initial therapeutic regimen. Abbreviations: CI, confidence interval; I-FABP, intestinal fatty acid binding protein; IL-6, interleukin 6; IL-8, interleukin 8; sCD14, soluble CD14; TNF-α, tumor necrosis factor α.

Table 2. Composite Biomarker Risk Score for Prediction of Death

Risk Group	Risk Factor(s),[a] No.	People, No.	Deaths, No. (%)	Adjusted OR[b](95% CI)	P
Low	0–1	88	4 (4.6)	…	
Moderate	2–3	107	18 (16.8)	3.8 (1.2, 12.1)	.02
High	4–5	92	26 (28.3)	7.7 (2.4, 24.6)	<.001

Model fit statistics for the risk score model include an AIC of 245.2, a rescaled R^2 of 0.17, and a C statistic of 0.74. For comparison, an adjusted model with an indicator for detectable hyaluronic acid and continuous values (on the log scale) for D-dimer, IL-6, IL-8, and SCD14 has an AIC of 246.3 (lower is better), a rescaled R^2 of 0.19, and a C statistic of 0.76 (higher is better).

Abbreviations: AIC, Akaike information criterion; CI, confidence interval; IL-6, interleukin 6; IL-8, interleukin 8; OR, odds ratio; sCD14, soluble CD14.

[a] Defined as D-dimer, IL-6, IL-8, and sCD14 levels above the cohort median level and detectable hyaluronic acid, each counting as 1 point.

[b] Adjusted for age, sex, prior AIDS-defining conditions, triglycerides level, and hepatitis C status.

Also of interest were some key differences between the associations of markers for death between HIV/HBV- and HIV/HCV-coinfected individuals. D-dimer levels were significantly associated with an increased risk of death in the HIV/HBV-coinfected cohort but not in the HIV/HCV-coinfected cohort. On the other hand, hyaluronic acid was associated with death in the HIV/HCV-coinfected cohort but not in the HIV/HBV-coinfected cohort. IL-6 and sCD14 levels remained associated with the increased mortality risk in both the HIV/HBV- and HIV/HCV-coinfected cohorts. It is unclear why higher levels of I-FABP were associated with a decreased risk of death among persons coinfected with HIV and HBV in our study. The inverse relationship between I-FABP level and mortality appears at first glance to conflict with previous observations suggesting that this marker is increased in HIV-infected patients with HBV and/or HCV coinfection presenting with elevated markers of inflammation and decreased liver function [39]. Although this discrepancy may reflect distinct patient populations, another likely explanation is that in the present study, death caused by liver-related issues was relatively infrequent (only 6 of 48 deaths). I-FABP is produced by intestinal epithelial cells, and plasma detection implies intestinal damage due to enterocyte necrosis. Yet, there is no described role of I-FABP for differential induction of inflammatory responses, and its biology may be more complex. Further studies are necessary to verify the association between I-FABP levels and outcomes in the context of person who are coinfected with HIV and hepatitis virus and initiating ART. Our results point to differences in the etiology of disease leading to death in ART-naive persons with HBV or HCV coinfection, although the differences could be the result of the smaller sample size when performing subgroup analyses. Despite these caveats, a risk score for death, calculated using a combination of results for D-dimer level, hyaluronic acid detection, IL-6 level, IL-8 level, and sCD14 level, was significantly associated with mortality within 4 years of ART initiation.

In this study, the risk of hepatitis flares after ART initiation was evaluated and found to be elevated in ART-naive persons who were coinfected with HBV. Furthermore, higher plasma HBV DNA or HCV RNA levels were associated with hepatitis flares. These findings indicate that the pathogen load may be a critical trigger of liver inflammation driving the flares and is consistent with other reports [28, 40]. Despite the fact that our definition of hepatitis flare was more conservative, we also found that increased baseline ALT and IL-10 levels were highly associated with hepatitis flares within 4 months of ART initiation. Polymorphisms in the IL-10 gene promoter linked to higher systemic concentrations of IL-10 are directly associated with accelerated progression of chronic HBV infection [41], and IL-10 has been reported to be increased during HBV-related hepatitis flares in persons without HIV infection [42]. In chronic HBV infection, HBV core antigen stimulates IL-10 production by peripheral blood mononuclear cells (PBMCs), blunting Th1 and Th17 responses [43]. Our data suggest 2 possibilities: (1) in patients with HIV/HBV coinfection, high HBV antigen load during immune reconstitution triggers stronger immune responses in the liver, with production of larger amounts of compensatory IL-10; and (2) higher levels of IL-10 suppress effective Th1 and Th17 responses, leading to higher HBV viremia. In the latter possibility, inflammation could be driven by innate immune responses (eg, monocytes/macrophages) as evidenced by sCD14, from antigen-presenting cell signaling (eg, IL-6 and IL-8). Although HBV-related immune restoration disease can be severe, with acute hepatic failure and death on rare occasions [25, 44], overall, patients have a higher chance of clearing the infection, suggesting a robust immune response following ART [40]. In our study, we were unable to assess potential clearance of either HBsAg or HBV e antigen in patients with flares.

We finally found an inverse association with plasma levels of CXCL26 and hepatitis flares. CXCL26 is a chemokine induced by IL-4 and IL-13 that has a chemotactic effect on eosinophils by binding to CCR3. CXCL26 also has an antagonistic effect on CCR2, inhibiting monocyte activity and migration [45]. The potential protective effect of CXCL26 may therefore be downregulation of inflammatory responses in the liver. Similar protective association of CXCL26 has also been observed in cryptococcal paradoxical IRIS [46].

The present study was retrospective in nature. The FIRST study did not capture data related to some potential confounding factors, such as smoking, alcohol use, or other biochemical markers of liver function at study entry. In addition,
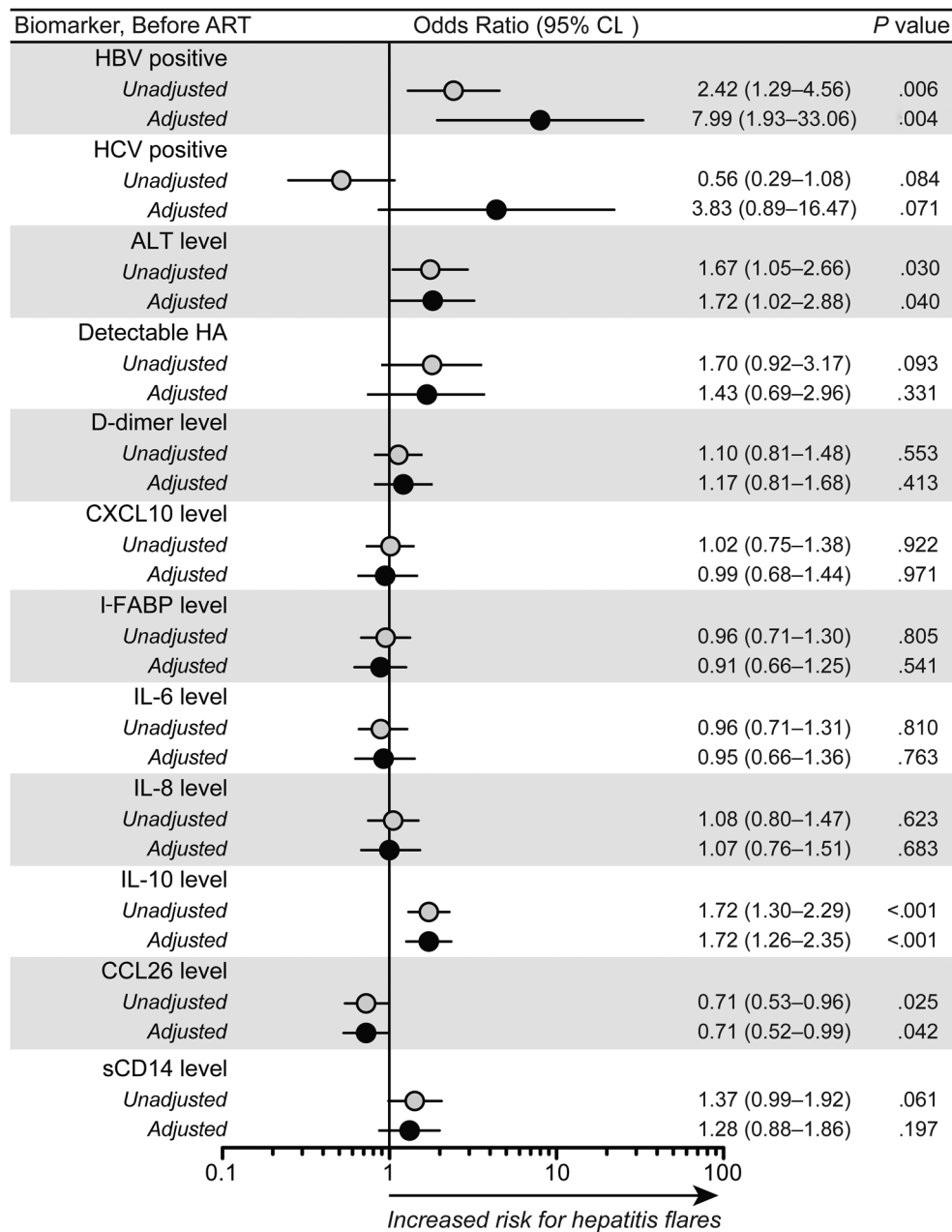
Figure 3. Baseline (ie, pre–antiretroviral therapy [ART]) biomarkers associated with subsequent hepatitis flare within 4-months of initiating ART for human immunodeficiency virus (HIV) infection. Odds ratios (ORs) for hepatitis B virus (HBV) positivity are for individuals with detectable HBV surface antigen (HBsAg), compared with those with undetectable HBsAg; ORs for hyaluronic acid (HA) are for individuals with detectable HA, compared with undetectable HA; other ORs are per SD increase after log transformation. ORs were adjusted for age; sex; history of prior AIDS-defining events; triglycerides level; HBV infection; hepatitis C virus (HCV) infection; baseline alanine transaminase (ALT) level (except when the association between ALT level and hepatitis flares was tested), CD4+ T-cell count, and plasma HIV RNA level; and use of lamivudine in the initial therapeutic regimen. Abbreviations: CI, confidence interval; I-FABP, intestinal fatty acid binding protein; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; sCD14, soluble CD14.

longitudinal data on HCV viremia or even HBsAg status after randomization were unavailable. It is also possible that there was referral bias that led to fewer patients with severe liver disease being enrolled in the main clinical trial. Despite these limitations, the results were consistent with similar biomarker studies.

In summary, we have shown that plasma biomarkers of procoagulant status, fibrosis, and systemic inflammation may enable risk stratification to predict death after ART initiation in HIV-infected persons with HBV and/or HCV coinfection. Pre-ART high pathogen load(s) and plasma biomarkers associated with inflammation may be useful to estimate the risk of

hepatitis flares soon after ART initiation. The early identification of individuals at high risk for hepatitis flares and, in particular, death may help optimize individualized patient care.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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