Biomarkers of inflammation and coagulation are associated with mortality and hepatitis flares in persons co-infected with HIV and hepatitis viruses

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Abstract

Background: Hepatitis C (HCV) or B virus (HBV) co-infection with HIV increases mortality risk over either infection alone and is frequently associated with hepatitis flares upon antiretroviral therapy (ART) initiation.

Methods: Retrospective cohort study of 287 HIV-positive persons co-infected with HBV or HCV **(**HBV n=70; HCV n=207; HBV+HCV n=10) 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: 48 deaths and 50 hepatitis flares occurred. Non-survivors were older with more prior AIDS events and higher pre-ART triglycerides and AST levels. Detectable hyaluronic acid and higher D-dimer, IL-6, IL-8, and sCD14 levels were associated with death in univariate models and with a composite biomarker risk score. Risk of hepatitis flares was higher with HBV co-infection (24.3%) or both HBV and HCV (50%) displayed than with HCV co-infection only (13.5%). Higher levels of ALT and interleukin (IL)-10 were also associated with hepatitis flares.

Conclusion: Among HIV patients co-infected with HBV and/or HCV initiating ART, biomarkers of inflammation and coagulation are associated with an increased risk of death while HBV co-infection and higher pre-ART IL-10 levels are associated with hepatitis flares.

INTRODUCTION

The prognosis of HIV-infected persons receiving antiretroviral therapy (ART) has significantly improved [1-4], but adverse outcomes still commonly occur due to progression of pre-existing 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 (IL)-6 [9], and AIDS-defining opportunistic diseases were associated with higher levels of CRP and IL-6 [10]. Moreover, higher plasma levels 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-naïve population, we recently reported that higher levels of CRP, D-dimer, IL-6, and hyaluronic acid (HA) were associated with increased risk for AIDS events, immune reconstitution inflammatory syndrome (IRIS), or death after ART initiation [12]. These findings in both ART-experienced and ART-naïve cohorts strongly suggest that biomarkers that infer 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 co-infections is still unknown.

Co-infections with HIV and hepatitis B virus (HBV) and/or hepatitis C virus (HCV) are common, mainly because of the shared routes of transmission [13, 14]. Interaction between these viruses can lead to accelerated hepatic morbidity and mortality in co-infected individuals [15-17], although ART initiation may delay the progression of liver disease and decrease overall

mortality [18-20]. Nevertheless, in such hepatitis co-infected individuals, ART has also been associated with an increased risk of serum liver enzyme elevations 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 sub-study of the SMART trial revealed that overall mortality was higher for persons co-infected with HIV and 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 four years of ART or hepatitis flares in the first four months of ART among HIV-infected persons with HBV and/or HCV co-infections who participated in the Flexible Initial Retrovirus Suppression Therapies (FIRST) open-label randomized clinical trial. Our premise was that persons with increased risk of hepatitis flares or death could be identified by differences in biomarkers associated with liver injury and inflammation when compared with 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 HIV-infected cohorts with and without hepatitis co-infection [10-12, 28]. The hypothesis was that cytokines and chemokines associated with acute inflammation in response to high antigen load would be associated with flares while 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-naïve persons in the USA between 1999 and 2002 to one of three first-line ART strategies using two or three classes of antiretroviral drugs (ClinicalTrials.gov NCT0000092) [6]. The majority of the participants were at an advanced stage of HIV infection with a median CD4 count of 155 cells/μL [6]. All subjects provided written informed consent. Plasma samples were prospectively stored at the study baseline (prior to ART initiation), at months 1 and 4, and every 4 months thereafter.

For this project we initially considered all subjects with HBV and/or HCV co-infection in the FIRST study (N=333). HBV infection was defined as either documented prior seropositivity for hepatitis B surface antigen (HBsAg) at least six months apart or testing seropositive for HBsAg and anti-HBc (either total or IgG) at study entry. HCV infection was defined by HCV antibody seropositivity and detectable plasma HCV RNA. Of HCV antibody seropositive persons (n=263), 46 had plasma HCV RNA levels below the limit of detection and were excluded from the HCV positive group (i.e. reflecting probable prior HCV clearance). Our final analysis cohort included 287 HIV-infected persons with HBV and/or HCV co-infection: 70 persons with HBV infection, 207 with HCV infection, and 10 with both HBV and HCV infection.

We considered two clinical outcomes: death from any cause with 4 years of ART initiation, or hepatitis flare within 4 months of ART initiation. A hepatitis flare was defined as ALT >100 IU/mL at month 1 or 4 with a concomitant ALT increase >50 IU/mL from the pre-ART level. This definition was selected based on the fact that an ALT >100 with an increase from a previous value typically triggers clinical follow up and further testing in hepatitis co-

infected HIV patients who are also receiving ART. In addition, this cut-off value for ALT has been used previously in clinical studies [29, 30]. While primary cause of death was not collected for the FIRST study, up to three 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 (a) AIDS/infectious etiology, (b) liver disease, (c) other, or (d) unknown cause.

Biomarker measurement

Cryopreserved plasma specimens from the baseline visit (pre-ART) were evaluated. Pro-inflammatory (IL-1β, IL-6, TNFα); Th1 (IFNγ, IL-12); Th2 (IL-4, IL-13); Th17 (IL-17), regulatory (IL-10); proliferation/ differentiation cytokines (IL-2, IL-15) and a panel of chemokines including IL-8, CXCL10 (IP-10), CXCL11 (ITAC), CXCL1 (Gro-α), CCL3 (MIP1α), CCL4 (MIP1β), CCL11 (Eotaxin-1), CCL13 (MCP4), CCL17 (TARC), CCL22 (MDC) and CCL26 (Eotaxin-3, MIP-4α) were measured in multiplex ELISA based assays (Meso Scale Discovery, Gaithersburg, MD). ELISA kits were also used to assay plasma CRP levels (CRP; Meso Scale Discovery, Gaithersburg, MD), hyaluronic acid (HA; Corgenix Inc, Westminster, CO), Intestinal Fatty Acid Binding Protein (I-FABP; Hycult Biotechnology BV, Netherlands), sCD14 (R&D Systems, Minneapolis, MN), and free active TGFβ1 (BioLegend, San Diego, CA) according to the manufacturers' protocols. Plasma D-dimer was quantified by Enzyme Linked Fluorescent Assay (ELFA) on a VIDAS instrument (Bio Merieux, Durham, NC).

Plasma HCV and HBV viral load were measured with the Siemen's Versant HBV bDNA 3.0 and Siemen's Versant HCV bDNA 3.0 assays (Siemens Healthcare Diagnostics Inc. Tarrytown, NY), according to the manufacturer's instructions. The HBV assay has a dynamic

range of 2,000–100,000,000 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 with medians presented with interquartile ranges (IQR). For each outcome (death and hepatitis flare) unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (95% CI) were estimated with logistic regression models for each biomarker. Multivariable models were adjusted for baseline covariates: age, gender, history of prior AIDS, triglycerides, HBV infection, HCV infection, ALT, $CD4^+$ T cell count, plasma HIV RNA levels, and use of lamivudine (3TC) in the initial HIV therapeutic regimen. Models were repeated separately for those with HBV infection and for those with HCV infection. Unless otherwise stated, the ORs are per standard deviation of the biomarker after log (natural log scale) transformation. For HA, IL-1β, and IL-4 the OR compares those with detectable levels to those with undetectable levels.

In order to consider the combined effect of the biomarkers that were significantly associated with mortality, an additional exploratory analysis was performed based on a composite mortality risk score. The risk score was calculated as the sum of indicators for the biomarker being above the median value (or detectable for HA). Risk groups were formed based on the total sum: low (sum = 0-1), moderate (sum = 2-3), or high risk (sum = 4-5). An adjusted logistic regression model was performed with the three risk groups as predictors for mortality. All reported p-values were two-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 co-infected with HBV and/or HCV, 50 (17.4%) developed a hepatitis flare within 4 months after ART initiation; 20 occurred during the first month of ART. Hepatitis flares were experienced by 24.3% of those co-infected with HBV only, 13.5%, of HCV only, and 50% of both HBV and HCV co-infected (P=.003). Compared to those HIV positive persons co-infected with HCV only, those persons co-infected with HBV were at 2-fold increased risk of developing a hepatitis flare (unadjusted OR: 2.05; 95% CI: 1.0-4.0; P=.04), while those co-infected with both HCV and HBV had a 6-fold increased risk (unadjusted OR: 6.4; 95% CI: 1.7-23.5; P=.005). Those who did or did not develop a hepatitis flare were similar with respect to most baseline characteristics, lamivudine use in the initial ART regimen, and opportunistic infection (OI) prophylaxis (Supplemental Table 1). There were no differences in baseline $CD4^+$ T cell count between those who did or did not develop a hepatitis flare during follow-up: median (IQR) $CD4^+$ T cell counts were 148 (34, 282) and 155 (36, 343), respectively; P=0.44. There were also no differences in baseline $CD4^+$ T cell count between the individuals who developed a hepatitis flare within 1 month compared to those developing a flare by 4 months (median [IQR], 214 [21-332] vs. 140 [49–269] cells/μL respectively; P=.78). Among the 50 individuals who developed a hepatitis flare, 23 (46%) had ALT levels above 200 IU/L (grade 3) at the time of flare. The distributions of all tested biomarkers prior to ART initiation are provided in Supplemental Table 2 for those who did or did not develop a hepatitis flare.

There were 48 deaths during the four 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 a higher proportion of prior AIDS

diagnosis than those who survived (Supplemental Table 1). The mortality among persons who experienced hepatitis flares 14% (n=7) and among those without flares 17.3% (n=41) during the 4-year follow up suggested that mortality was more associated with viral hepatitis co-infection than with early flare status after ART initiation. Mortality did not relate to the degree of flare as among the 23 participants with grade 3 hepatitis flares (ALT > 200 IU/L), only 13% (n=3) died within 4 years of follow up, compared to 15% (n=4) among 27 participants with grade 1 or 2 flares. Supplemental Table 3 shows the distributions of all biomarkers prior to ART initiation for those who did or did not die during the first four 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 (n=24), liver-related causes (n=6), other known causes (n=12) and unknown/unidentified cause (n=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 TNFα and CXCL10 levels were not significant in a multivariate model after adjustment for potential confounding variables (Figure 1).

Among the HIV/HBV co-infected 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 the chemokine interferon-inducible T cell alpha

chemoattractant (ITAC, also known as CXCL11), which is produced by human 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 in the patients with HIV/HBV co-infection. In those with HIV/HCV co-infection, 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 five biomarkers (D-dimer, HA, IL-6, IL-8 and sCD14) 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 to 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, footnote), but allowed for identification of persons at high risk for mortality.

Risk of hepatitis flares

The risk of hepatitis flare events within 4 months of ART initiation was increased with the presence of HBV co-infection and higher pre-ART levels of ALT and IL-10 (Figure 3).

Conversely, higher Eotaxin-3 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 was associated with a subsequent hepatitis flare (adjusted OR per $\log_{10}$ 1.30; 95% CI: 1.01-1.65; P=.04).

DISCUSSION

In the present exploratory study, we have identified candidate biomarkers in plasma collected pre-ART that were associated with death and/or hepatitis flares after commencing ART in HIV-infected persons co-infected with HBV and/or HCV. Nearly 30% of North American HIV-infected individuals are co-infected with HCV and 10% are co-infected with HBV [13, 14]. Both HBV and/or HCV co-infections 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 co-infected individuals, ART has been related to an increased risk of liver enzyme elevation and death [22, 24, 25, 35]. Moreover, chronic viral hepatitis has been associated with non-liver, non-opportunistic disease mortality in those HIV-infected individuals who are receiving ART [26]. With regard to HIV/HBV co-infection, current treatment guidelines recommend a tenofovir-based regimen as the treatment of choice, without highlighting particular advantage of HBV combination therapy in this setting [38].

Identification of biomarkers that predict mortality may assist in the clinical management of these patients. We demonstrated in our cohort of HIV-infected individuals co-infected with HBV and/or HCV that detectable hyaluronic acid and higher concentrations of D-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 D-dimer, CRP, IL-6, and hyaluronic acid could be useful in identifying ART-naïve patients at higher risk of AIDS or death after ART initiation [12] and other studies underscoring the importance of these markers as predictors of death in HIV-infected persons [9-11]. While increased D-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 [39]. Indeed, increased expression of tissue factor on activated monocytes may be a cause of increased D-dimer levels [40].

Also of interest were some key differences found between the associations of markers for death between HIV/HBV and HIV/HCV co-infected individuals. D-dimer was significantly associated with increased risk of death in the HIV-HBV co-infected cohort, but not in the HIV/HCV co-infected cohort. On the other hand, hyaluronic acid was associated with death in the HIV/HCV co-infected cohort, but not in the HIV-HBV co-infected cohort. IL-6 and sCD14 remained associated with the increased mortality risk in both HIV/HBV and HIV/HCV co-infections. It is unclear why higher levels of I-FABP were associated with decreased risk of death in persons co-infected with HIV and HBV in our study. The inverse relationship between I-FABP and mortality at first glance appears to be in conflict with previous observations suggesting that this marker is increased in patients with HIV and HBV and/or HCV co-infection presenting with elevated markers of inflammation and decreased liver function [41]. 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 out of 48 deaths). I-FABP is produced by intestinal epithelial cells, and plasma detection infers 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 HIV-hepatitis co-infected persons initiating ART. Our results point to differences in the etiology of disease leading to death in ART-naïve persons with HBV or HCV co-infection, although they could be the result of smaller sample size when performing subgroup analyses. Despite these caveats, a risk score for death using a combination of results for D-dimer, hyaluronic acid, IL-6, IL-8, and sCD14 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-naïve persons who were co-infected 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, 42]. 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 [44], and IL-10 has been reported as increased during HBV-related hepatitis flares in HIV-non-infected persons [45]. In chronic HBV infection, HBcAg stimulates IL-10 production by peripheral blood mononuclear cells (PBMCs), blunting Th1 and Th17 responses [46]. Our data suggest two possibilities: (i) that in patients with HIV/HBV co-infection, high HBV antigen load during immune reconstitution triggers stronger immune responses in the liver with production of larger amounts of compensatory IL-10; (ii) 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 (e.g. monocytes/macrophages) as evidenced by sCD14, from antigen presenting cell signaling (e.g.

IL-6, IL-8). . Although HBV-related immune restoration disease can be severe with acute hepatic failure and death on rare occasions [25, 43], overall, patients have a higher chance of clearing the infection suggesting a robust immune response following ART [42]. In our study we were unable to assess potential clearance of either HBsAg or HBeAg of patients with flares.

We finally found an inverse association with plasma levels of eotaxin-3 and hepatitis flares. Eotaxin-3 (CXCL26) is a chemokine induced by IL-4 and IL-13 that has a chemotactic effect on eosinophils by binding to CCR3. Eotaxin-3 also has an antagonistic effect on CCR2, inhibiting monocyte activity and migration [47]. The potential protective effect of eotaxin-3 may therefore be down-regulation of inflammatory responses in the liver. Similar protective association of eotaxin-3 has also been observed in cryptococcal paradoxical IRIS [48].

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, 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 pro-coagulant status, fibrosis, and systemic inflammation may enable risk stratification to predict death after ART initiation in HIV-infected persons with HBV and/or HCV co-infection. 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.

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Table 1. Baseline characteristics of the study population

Characteristic	Study population (N=287)
Age, median years (IQR)	41 (36 - 47)
Female gender	47 (16)
Race/ethnicity	
African American	175 (61.0)
White	65 (22.6)
Latino/other	47 (16.4)
History of intravenous drug use	143 (49.8)
Prior AIDS event	119 (41.5)
$CD4^+$ T cells/μL, median (IQR)	155 (34 – 341)
HIV RNA, median $\log_{10}$ copies/mL plasma (IQR)	5.1 (4.5 – 5.5)
Hepatitis status	
Hepatitis B	70 (24.4)
Hepatitis C	207 (72.1)
Hepatitis B and C	10 (3.5)
AST, median IU/L (IQR)	49.0 (34.0-75.0)
ALT, median IU/L (IQR)	47.5 (31.0 – 72.0)
Cholesterol, median mg/dL (IQR)	156 (132 – 178)
Triglycerides, median mg/dL (IQR)	124 (94 – 189)
Glucose, median mg/dL (IQR)	85 (74 – 95)
Randomization Group	
PI	97 (33.8)
NNRTI	99 (34.5)
PI+NNRTI	91 (31.7)
Lamivudine (3TC) use in initial HIV therapy	239 (83.3)

NOTE. Data represent no. (%) of participants unless otherwise specified. IQR, interquartile range; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

Table 2. A composite biomarker risk score for prediction of death.

Risk Group	Number of Risk Factors[1]	Number of People	Deaths N (%)	Adjusted OR (95% CI)	P-value
Low	0-1	88	4 (4.6%)	-	
Moderate	2-3	107	18 (16.8%)	3.8 (1.2, 12.1)	0.02
High	4-5	92	26 (28.3%)	7.7 (2.4, 24.6)	<0.001

[1] Risk Factors are: D-dimer, IL-6, IL-8 and sCD14 above the cohort median and detectable hyaluronic acid (HA), each counting as one point. The adjusted Odds Ratio (OR) is adjusted for age, gender, prior AIDS, triglycerides and hepatitis C status.

Model fit statistics for the risk score model include Akaike's Information Criterion (AIC) = 245.2, rescaled R^2 = 0.17, and C-statistic = 0.74. For comparison, an adjusted model with an indicator for detectable HA and continuous values (on the log scale) for D-dimer, IL-6, IL-8 and SCD14 has AIC = 246.3 (lower is better), re-scaled R^2 = 0.19 and C-statistic = 0.76 (higher is better).

FIGURE LEGENDS

Figure 1. Biomarkers associated with subsequent death on ART.

Baseline (pre-ART) biomarkers associated with subsequent all-cause mortality within 4-years of HIV therapy initiation are shown. Odds ratios (OR) are per standard deviation increase after log transformation. For hyaluronic acid (HA), the odds ratios are for detectable versus undetectable values. Adjusted OR models were adjusted for age, gender, history of prior AIDS events, triglycerides, HBV infection, HCV infection, pre-ART values of ALT, $CD4^+$ T cell count and plasma HIV RNA levels, and use of 3TC in the initial therapeutic regimen.

95% CI, 95% confidence interval.

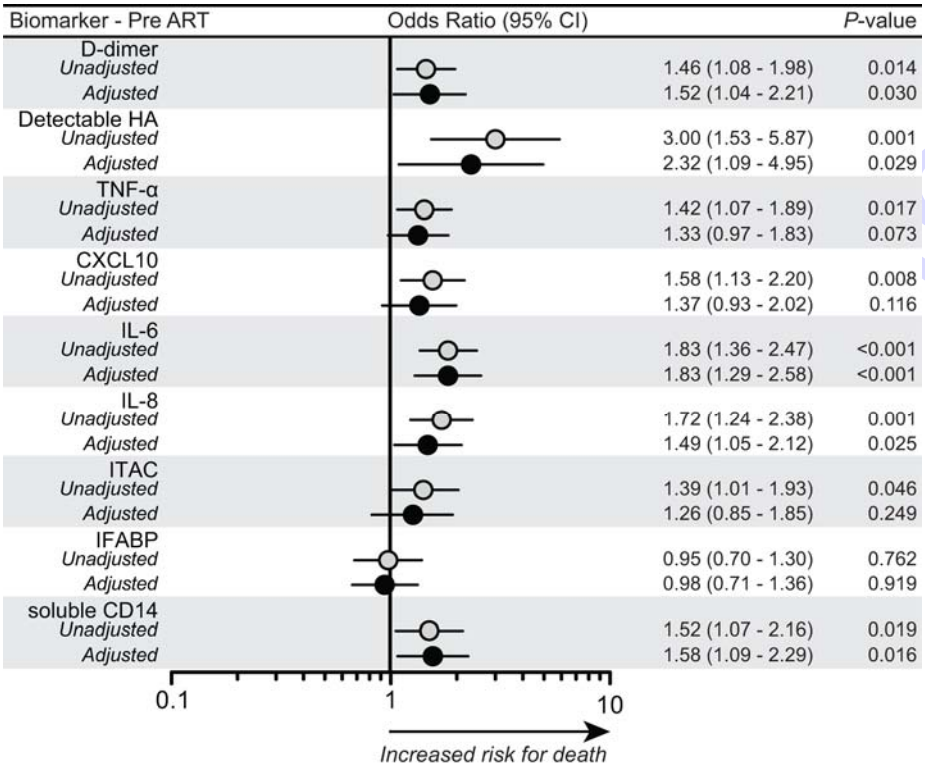
Figure 2. Pre-ART biomarkers associated with subsequent death on ART, stratified by hepatitis co-infection.

Panel A is among those with HBV co-infection; Panel B is among those with HCV infection. Odds ratios (OR) for hyaluronic acid (HA) are for those with detectable values compared to those with undetectable values; other OR are per standard deviation increase after log transformation. OR were adjusted for age, gender, history of prior AIDS events, triglycerides, HBV infection, HCV infection, pre-ART values of ALT, $CD4^+$ T cell count and plasma HIV RNA levels, and use of 3TC in the initial therapeutic regimen. 95% CI, 95% confidence interval.

Figure 3. Baseline biomarkers associated with hepatitis flare on HIV antiretroviral therapy (ART).

Pre-ART baseline biomarkers associated with subsequent hepatitis flare within 4-months of ART initiation. Odds ratios (OR) for Hepatitis B positive are for those with detectable HBsAg values compared to those with undetectable values; OR for HA are for detectable values compared to undetectable values; other OR are per standard deviation increase after log transformation. OR were adjusted for age, gender, history of prior AIDS events, triglycerides, HBV infection, HCV

infection, baseline values of ALT (except when association with ALT and hepatitis flares is tested), $CD4^+$ T cell count and plasma HIV RNA levels, and use of 3TC in the initial therapeutic regimen. CI, confidence interval.



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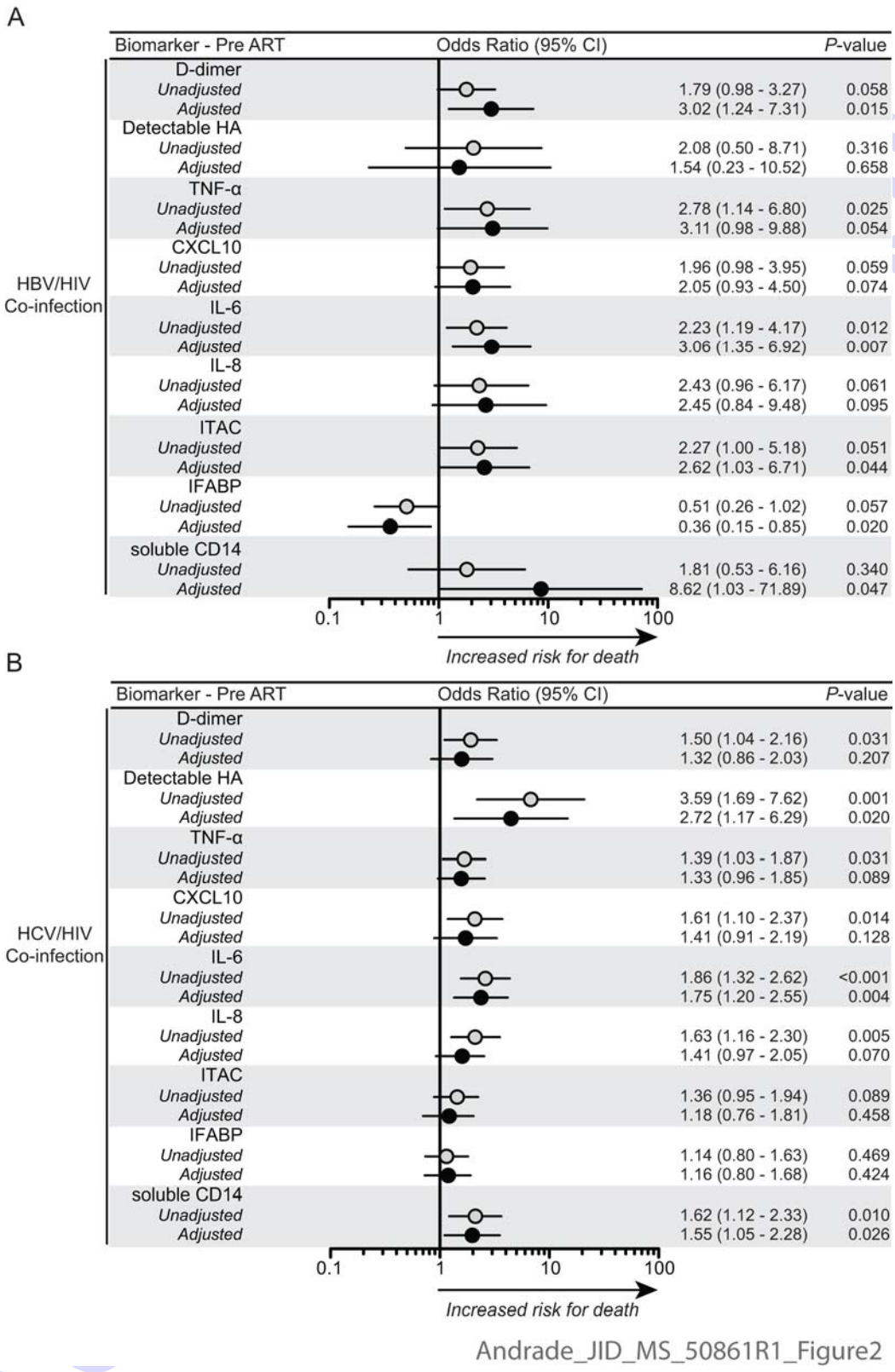

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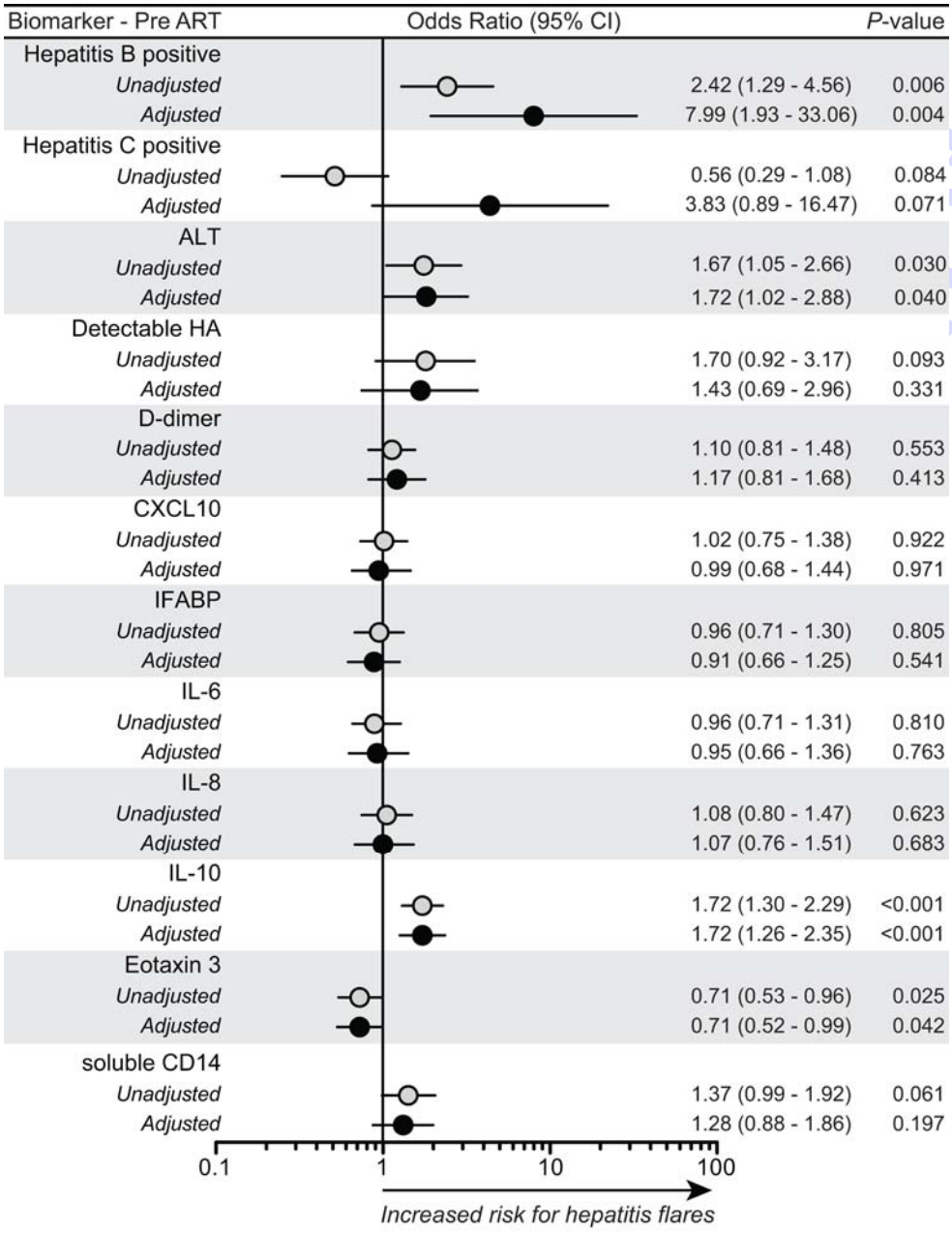

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