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Bilirubin Is Inversely Associated With Cardiovascular Disease Among HIV-Positive and HIV-Negative Individuals in VACS (Veterans Aging Cohort Study)

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Background—Bilirubin may protect against cardiovascular disease (CVD) by reducing oxidative stress. Whether elevated bilirubin reduces the risk of CVD events among HIV+ individuals and if this differs from uninfected individuals remain unclear. We assessed whether bilirubin independently predicted the risk of CVD events among HIV+ and uninfected participants in VACS (Veterans Aging Cohort Study).

Methods and Results—We conducted a prospective cohort study using VACS participants free of baseline CVD. Total bilirubin was categorized by quartiles. CVD as well as acute myocardial infarction, heart failure, and ischemic stroke events were assessed. Cox regression was used to evaluate hazard ratios of outcomes associated with quartiles of total bilirubin in HIV+ and uninfected people after adjusting for multiple risk factors. There were 96 381 participants (30 427 HIV+); mean age was 48 years, 48% were black, and 97% were men. There were 6603 total incident CVD events over a mean of 5.7 years. In adjusted models, increasing quartiles of baseline total bilirubin were associated with decreased hazards of all outcomes (hazard ratio, 0.86; 95% confidence interval, 0.80–0.91). Among HIV+ participants, results persisted for heart failure, ischemic stroke, and total CVD, but nonsignificant associations were observed for acute myocardial infarction.

Conclusions—VACS participants (regardless of HIV status) with elevated bilirubin levels had a lower risk of incident total CVD, acute myocardial infarction, heart failure, and ischemic stroke events after adjusting for known risk factors. Future studies should investigate how this apparently protective effect of elevated bilirubin could be harnessed to reduce CVD risk or improve risk estimation among HIV+ individuals. (J Am Heart Assoc. 2018;7:e007792. DOI: 10.1161/JAHA.117.007792.)

Key Words: cardiovascular disease • bilirubin • HIV • stroke • myocardial infarction • heart failure

After achieving HIV virologic suppression, cardiovascular disease (CVD) emerges as one of the principal causes of morbidity and mortality for individuals living with HIV.1 HIV infection has been shown to increase the risk of CVD, independent of established risk factors, by 50% to 100%2–4. The mechanisms underlying this increased risk may be mediated by inflammation, immune activation, and oxidative stress.5 Thus, the search for better methods of identifying those PLWH at highest risk and discovering novel ways to address the cause of CVD has been of crucial importance for the past several years.6
Bilirubin is known to have antiatherogenic properties, mediated in part by reducing oxidative stress. Individuals with Gilbert syndrome have elevated levels of unconjugated bilirubin because of a defect in uridine diphosphate glucuronosyltransferase 1A1 enzyme. Longitudinal studies of this syndrome have demonstrated reductions in coronary artery disease, stroke, and other inflammatory conditions.

Certain medications can also increase bilirubin levels. For example, the antiretroviral drug atazanavir can competitively inhibit uridine diphosphate glucuronosyltransferase 1A1, leading to an increase in unconjugated bilirubinemia. Studies have shown atazanavir use can improve endothelial function and reduce lipid levels and blood pressure in HIV-uninfected diabetic patients. Among HIV-positive individuals, atazanavir decreased markers of inflammation and slowed progression of carotid intimal-medial thickness when compared with other antiretroviral treatment (ART), an effect that appears to be partially mediated by bilirubin.

We sought to determine whether increased total bilirubin levels were associated with reduced CVD events in VACS (Veterans Aging Cohort Study) and to determine if those associations differed by HIV status, atazanavir use, and liver disease.

Methods

Study Design and Data Source

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedures. VACS is a prospective longitudinal cohort of HIV-positive veterans matched 1:2 with uninfected veterans on age, sex, race/ethnicity, and clinical site; they were enrolled in the same calendar year. We included all VACS participants enrolled on or after April 1, 2003, in whom the baseline was the first clinic visit on or after this date. Participants were followed from baseline until development of CVD, date of death, or December 31, 2011. Participants with known CVD (myocardial infarction, coronary heart disease, stroke, heart failure (HF), or cardiomyopathy) before the baseline visit were excluded. Prevalent CVD was determined using International Classification of Diseases, Ninth Revision (ICD-9) codes from Medicaid, Medicare, and Veterans Affairs (VA) data. In addition, veterans who seroconverted to HIV-positive status during follow-up were excluded. The institutional review boards at Vanderbilt University, Yale University, and West Haven VA Medical Center approved this study. VACS has a waiver of informed consent.

Exposure, Outcome, and Covariates

The primary outcome of interest was total incident CVD, defined as the composite of the following incident events: acute myocardial infarction (AMI), ischemic stroke (IS), and HF. ICD-9 codes for AMI included 1 inpatient or 2 outpatient 410.xx codes; for HF, 1 inpatient or 2 outpatient 402.xx, 404.xx, and 428.xx codes; and for IS, 1 inpatient 433.x1, 434.x1 (excluding 434.x0), or 436 code or 2 outpatient 438.x codes. Secondary outcomes were the individual incident events that made up the composite CVD outcome.

The primary exposure was total bilirubin level closest to baseline (up to 180 days after baseline), which was pulled from laboratory data for each participant. All measurements of bilirubin were performed in a Clinical Laboratory Improvement Amendments–certified and compliant clinical laboratory.
Various clinical laboratories across the VA Healthcare System may differ in testing platforms over time, but this would serve to increase standard error and likely bias results toward the null. The variation in total bilirubin results between testing platforms from laboratories that have received Clinical Laboratory Improvement Amendments approval would be within 20%. Age, sex, and race/ethnicity were gathered from VA administrative data, whereas systolic blood pressure, diabetes mellitus status, and lipid levels were collected from clinical outpatient and laboratory data closest to the baseline date. Body mass index was taken from VA vital sign data. ART receipt was based on pharmacy data, and smoking status was ascertained from the health factors data that are collected on a standard VA form. Systolic blood pressure was the mean of 3 routine outpatient measurements closest to baseline. Diabetes mellitus status was determined on the basis of at least 1 inpatient or 2 outpatient codes for this diagnosis and a validated algorithm that incorporates glucose levels and use of antidiabetic agents. Body mass index was dichotomized using a cut point of at least 30 kg/m². Smoking status was categorized as current, former, or never. Alcohol and cocaine abuse or dependence was determined using ICD-9 codes. ICD-9 codes or the presence of a positive hepatitis C virus antibody test result was used to determine hepatitis C virus status. As a measure of liver fibrosis, we used FIB-4, which is calculated from age, alanine aminotransferase, aspartate aminotransferase, and platelet count. ART was categorized by regimen within a window of 180 days before baseline through 7 days after baseline, including a nucleoside reverse transcriptase inhibitor (NRTI) plus a protease inhibitor, an NRTI plus a non-NRTI, other (ie, use of protease inhibitor, NRTI, or non-NRTI medications but not in combination, as described in the other 2 categories), and no ART (reference group). HIV-specific biomarkers CD4 and viral load were collected at baseline and throughout follow-up until the end of 2011.

Statistical Analysis
We performed descriptive analyses stratified by bilirubin quartile. We used survival analyses to construct cumulative incidence plots stratified by total bilirubin quartile for each outcome. Log-rank tests were performed to determine whether time to event differed by total bilirubin quartile. To further explore this relationship between baseline bilirubin and risk of CVD, restricted cubic splines were developed to allow a nonlinear association between the log-hazards of CVD outcomes and bilirubin. We then performed multivariable Cox proportional hazards regression to examine the association between baseline quartiles of total bilirubin and risk of total CVD and its components in the full cohort and for HIV⁺ participants only. In the full cohort, models were adjusted for HIV status, demographic characteristics, and traditional CVD risk factors, as well as liver fibrosis and substance dependence or abuse. Models limited to HIV-positive veterans were additionally adjusted for HIV-1 RNA, CD4 cell count, and ART regimen. Next, we incorporated all recorded values of total bilirubin on those in our full sample during the follow-up period using total bilirubin as a time-varying covariate and the same adjustment variables previously mentioned, and we again assessed the association between total bilirubin and incident CVD.

Sensitivity analyses were performed on participants without liver disease (defined as FIB-4 ≥1.45, hepatitis C virus infection, alcohol abuse, or ICD-9 codes indicative of hepatic decompensation) using quartiles of baseline total bilirubin and time-updated total bilirubin as our exposure of interest. Because atazanavir has been shown to increase total bilirubin levels, supplemental analyses assessed whether rates and risk of CVD differed among those HIV-positive individuals receiving atazanavir compared with those receiving other ART regimens.

Missing data were accounted for by creating 5 complete data sets through the use of multiple imputation. Continuous variables were imputed through the use of predictive mean matching to produce biologically plausible imputed values, whereas multinomial logistic regression was used to impute categorical variables. All analyses were performed in Stata 13.1 (Stata Corp), and a 2-sided $P<0.05$ was used to determine statistical significance.

Results
Participant Characteristics
In total, there were 96 381 participants (30 427 HIV⁺ and 65 954 HIV⁻) who were followed up for a mean of 5.7 years after enrollment. The mean age was 48 years, with 97% of the cohort being men and 48% being black. During the follow-up period, we observed 6603 total CVD events, 3843 HF events, 1931 AMI events, and 2112 IS events. All participant characteristics (Table 1) were statistically different across quartiles of total bilirubin ($P<0.05$).

Cumulative Incidence and Cox Proportional Hazards Regression Models
The thresholds for bilirubin quartiles in this cohort were ≤0.4, 0.5 to 0.6, 0.7 to 0.8, and ≥0.9 mg/dL. Cumulative incidence of CVD, HF, AMI, and IS events differed across quartiles of total bilirubin (all log-rank $P<0.05$), with the highest incidence occurring in lowest quartile and lowest incidence in the highest quartile (Figure 1). Using splines to allow for nonlinear relationships, we found that CVD risk exponentially decreased...
Table 1. Participant Characteristics by Bilirubin Quartile

| Baseline Characteristic | Quartile 1: Bilirubin ≤0.4 mg/dL (n=24 229) | Quartile 2: Bilirubin 0.5–0.6 mg/dL (n=23 641) | Quartile 3: Bilirubin 0.7–0.8 mg/dL (n=17 463) | Quartile 4: Bilirubin ≥0.9 mg/dL (n=18 524) | Missing Bilirubin (n=12 524) |
|-------------------------|--------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Age, y                  | Mean (SD) 48.4 (9.4)                        | 48.6 (9.7)                                    | 48.7 (9.8)                                    | 48.6 (10.0)                                   | 46.6 (10.1)                                   |
|                         | Median 48.0                                | 49.0                                          | 49.0                                          | 49.0                                          | 47.0                                          |
| Male sex, %             | 95.3                                        | 96.7                                          | 97.9                                          | 98.4                                          | 97.4                                          |
| Race/ethnicity, %       | Black 51.3                                  | 48.7                                          | 48.6                                          | 45.3                                          | 45.4                                          |
|                         | White 38.1                                  | 38.9                                          | 38.1                                          | 39.6                                          | 37.4                                          |
|                         | Hispanic 6.3                                | 7.6                                           | 8.0                                           | 9.3                                           | 8.4                                           |
|                         | Other 4.3                                   | 4.8                                           | 5.3                                           | 5.9                                           | 8.9                                           |
| HIV positive, %         | 37.6                                        | 34.0                                          | 30.9                                          | 32.2                                          | 15.3                                          |
| Framingham risk factors, % |                |                                                |                                                |                                                |                                                |
| SBP, %*                 | SBP <140 mm Hg, no antihypertensive agents 39.4 | 39.2                                          | 38.8                                          | 40.1                                          | 58.4                                          |
|                         | SBP <140 mm Hg, taking antihypertensive agents 35.2 | 35.2                                          | 35.6                                          | 34.7                                          | 15.6                                          |
|                         | SBP ≥140 mm Hg, taking antihypertensive agents 19.3 | 19.5                                          | 19.3                                          | 19.2                                          | 11.7                                          |
|                         | SBP ≥140 mm Hg, no antihypertensive agents 6.1 | 6.1                                           | 6.3                                           | 6.0                                           | 14.4                                          |
| Diabetes mellitus, %    | 14.4                                        | 14.0                                          | 13.9                                          | 13.3                                          | 4.0                                           |
| Lipids, mg/dL*          | Total cholesterol ≥200 mg/dL 37.2            | 37.1                                          | 36.9                                          | 35.9                                          | 41.4                                          |
|                         | HDL cholesterol ≥60 mg/dL 14.8               | 13.2                                          | 12.8                                          | 13.5                                          | 14.6                                          |
|                         | HDL cholesterol 40–59 mg/dL 45.5             | 44.6                                          | 43.2                                          | 43.2                                          | 50.3                                          |
|                         | HDL cholesterol <40 mg/dL 39.8               | 42.3                                          | 44.0                                          | 43.3                                          | 35.1                                          |
| Smoking, %*             | Current 59.4                                | 53.5                                          | 49.1                                          | 44.5                                          | 54.0                                          |
|                         | Former 13.7                                 | 16.7                                          | 17.6                                          | 18.6                                          | 15.3                                          |
|                         | Never 27.0                                  | 29.8                                          | 33.4                                          | 36.9                                          | 30.7                                          |
| Other risk factors, %   | FIB-4, %*                                   |                                                |                                                |                                                |                                                |
|                         | FIB-4 <1.45 80.4                            | 75.6                                          | 73.0                                          | 64.7                                          | 80.6                                          |
|                         | 1.45 ≤ FIB-4 ≤ 3.25 17.5                    | 21.2                                          | 22.7                                          | 24.4                                          | 17.4                                          |
|                         | FIB-4 >3.25 2.1                             | 3.2                                           | 4.2                                           | 10.9                                          | 1.9                                           |
| HCV infection, %        | 16.5                                        | 19.1                                          | 20.2                                          | 24.4                                          | 6.3                                           |
| Obese (BMI ≥30 kg/m²), %* | 30.3                                | 32.5                                          | 33.6                                          | 30.2                                          | 30.2                                          |
| History of alcohol abuse, % | 28.4                                      | 26.5                                          | 25.4                                          | 26.5                                          | 22.2                                          |
| History of cocaine abuse, % | 18.6                                      | 16.6                                          | 15.6                                          | 15.2                                          | 15.3                                          |
| HIV-specific variables  | CD4 cell count, mm*                         |                                                |                                                |                                                |                                                |
|                         | Mean (SD) 415.7 (305.7)                      | 420.9 (297.3)                                 | 442.2 (303.0)                                 | 438.1 (291.8)                                 | 468.2 (306.0)                                 |
|                         | Median 365.0                                | 375.0                                         | 398.0                                         | 398.5                                         | 244.0                                         |

Continued
Bilirubin Is Associated With CVD for HIV+ and HIV−. Trends and statistical significance were similar, although incidence rates were higher with increasing bilirubin (Figure 2A). The same association was seen with HF, AMI, and IS. In all cases, the risk decreased until ≈0.9 mg/dL and subsequently increased as total bilirubin levels increased to values indicative of liver failure. When individuals with known liver disease were excluded (Figure 2B), the risk continued to decrease throughout the range of increasing bilirubin levels.

In adjusted regression models, CVD risk differed by bilirubin quartile (P<0.001), and higher bilirubin quartiles were associated with lower CVD risk compared with the lowest bilirubin quartile (Table 2). A test for trend across quartiles was also statistically significant (P<0.001), with risk of CVD decreasing by 8% for each increase in total bilirubin quartile. In separate adjusted models for HF, AMI, and IS, this same inverse relationship was observed (Table 2), although the trend was not as consistent for AMI or IS.

Among HIV-positive participants, regression models were further adjusted for baseline HIV−1 RNA, CD4 count, and ART regimen (Table 3). The relative risk of CVD events by bilirubin quartiles was similar, although incidence rates were higher among HIV-positive participants compared with the full cohort. Trends and statistical significance for HF, AMI, and IS were similar between the full cohort and the HIV-positive participants, except for the lack of association between total bilirubin quartiles and AMI risk among HIV-positive participants.

In the full cohort, when time-updating total bilirubin throughout the follow-up period, we observed the following CVD risk compared with the referent group composed of those with a total bilirubin ≤0.4 mg/dL: total bilirubin 0.5 to 0.6 mg/dL, hazard ratio [HR] = 0.82 (95% confidence interval [CI], 0.76–0.88); total bilirubin 0.7 to 0.8 mg/dL, HR = 0.79 (95% CI, 0.73–0.86); and total bilirubin ≥0.9 mg/dL, HR = 0.81 (95% CI, 0.75–0.87).

### Sensitivity and Supplemental Analyses

To determine whether liver disease could have affected the relationship between bilirubin and CVD, a subgroup analysis was performed on the entire cohort, excluding individuals with hepatitis C virus, an FIB-4 ≥1.45, alcohol abuse at baseline, or ICD-9 codes indicative of hepatic decompensation (Table 4). Rates of CVD were lower for each quartile, but the trend in relative risk reduction with increasing bilirubin quartile was maintained (P<0.001). On time-updating total bilirubin in the subset of individuals without liver disease,22 we observed the following CVD risk compared with the referent group composed of those with a total bilirubin ≤0.4 mg/dL: total bilirubin 0.5 to 0.6 mg/dL, HR = 0.77 (95% CI, 0.68–0.87); total bilirubin 0.7 to 0.8 mg/dL, HR = 0.76 (95% CI, 0.67–0.87); and total bilirubin ≥0.9 mg/dL, HR = 0.70 (95% CI, 0.62–0.80).

Last, the incidence of CVD events was assessed among HIV-positive participants by atazanavir use to explore whether the observed inverse association between bilirubin levels and CVD could be explained by an atazanavir-mediated increase in bilirubinemia and an atazanavir-mediated reduction in CVD risk or whether atazanavir had an additive effect to reduce the

### Table 1. Continued

| Baseline Characteristic | Quartile 1: Bilirubin ≤0.4 mg/dL (n=24 229) | Quartile 2: Bilirubin 0.5–0.6 mg/dL (n=23 641) | Quartile 3: Bilirubin 0.7–0.8 mg/dL (n=17 463) | Quartile 4: Bilirubin ≥0.9 mg/dL (n=18 524) | Missing Bilirubin (n=12 524) |
|-------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|-----------------------------|
| Mean (SD)               | 73 709.8 (246 747.5)                         | 91 714.1 (1 800 754.0)                      | 58 102.7 (224 687.8)                        | 45 276.0 (195 459.9)                       | 59 227.0 (162 158.5)       |
| Median                  | 1823.0                                      | 1875.0                                      | 1186.0                                      | 400.0                                       | 1669.0                      |
| ART regimen, %          |                                             |                                             |                                             |                                             |                             |
| NRTI + PI               | 14.2                                        | 16.6                                        | 20.3                                        | 30.9                                        | 7.7                         |
| NRTI + NNRTI            | 25.9                                        | 22.2                                        | 18.7                                        | 11.9                                        | 8.2                         |
| Other                   | 4.5                                         | 5.2                                         | 5.0                                         | 6.0                                         | 2.1                         |
| No ART                  | 55.5                                        | 56.0                                        | 55.0                                        | 51.3                                        | 82.0                        |
| Atazanavir use          | 1.0                                         | 1.1                                         | 1.0                                         | 2.9                                         | 0.4                         |

SI conversion factors: To convert HDL to millimoles per liter, multiply by 0.0259. All characteristics were statistically different across quartiles of total bilirubin (P<0.05) using χ² test or Kruskal-Wallis test. ART indicates antiretroviral therapy; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HCV, hepatitis C virus; HDL, high-density lipoprotein; NNRTI, non-NRTI; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; and SBP, systolic blood pressure.

*All variables had complete data, except the following: SBP data were available for 92 964; total cholesterol data were available for 79 330; HDL cholesterol data were available for 75 546; smoking data were available for 65 120; FIB-4 data were available for 77 349; BMI data were available for 91 536; CD4 cell count data were available for 25 198 (HIV positive); and HIV-1 RNA data were available for 25 829 (HIV positive).
incidence of CVD (Table 5). There were 425 HIV-positive participants receiving atazanavir at baseline (1.4%), whereas 12,853 (42.2%) were receiving an ART regimen excluding atazanavir. CVD incidence rates were slightly higher among those receiving atazanavir (16.5 [95% CI, 11.07–24.64] versus 14.6 [95% CI, 13.86–15.47] events per 1000 person-years), but this difference did not reach statistical significance.

Discussion

In this study of bilirubin and CVD risk, we found that VACS participants with mildly elevated bilirubin levels had a lower risk of incident CVD, HF, AMI, and IS events, even after adjusting for known risk factors. Among HIV-positive participants, this inverse association persisted for HF and IS but was not significant for AMI. The association remained even for individuals without liver disease.

To date, this is the first report of the relationship between bilirubin levels and incident CVD events for individuals living with HIV. In clinical studies of HIV-positive individuals, mildly elevated levels of bilirubin have been beneficial for multiple conditions. Individuals with Gilbert syndrome have a lower risk of ischemic heart disease (2%) compared with the general population (12.1%). In particular, those homozygous for the UGT1A1*28 allele had one third of the risk of coronary heart disease than those who were not homozygotes in the Framingham Heart Study. Although the (TA)-repeat variation in the UGT1A1 gene is the main genetic factor for determining bilirubin concentrations, the penetrance ranges from 1% to 67%, further confounding the genetic association, protein expression, and CVD outcomes. Genetic association studies have not conclusively found an association between the (TA)-repeat variation or a causal relationship between bilirubin and CVD outcomes; however, a causal relationship cannot be excluded on the basis of these studies.

In the general population, multiple studies have confirmed the strong negative association of bilirubin levels with atherosclerosis, even among those with familial coronary...
artery disease, peripheral artery disease, carotid plaque, and myocardial infarction in the Framingham Offspring Study. Similar to the current study, others have also found an inverse relationship with stroke, in which a 0.1-mg/dL increase resulted in a 9% reduction in the odds of an event in the National Health and Nutrition Examination Survey. This reduction is similar to the changes seen in this study at the lower quartiles. The decreasing trend observed with increasing quartiles suggests that the nonlinear trend observed in the time-updated analysis in the full cohort may have been driven by individuals with abnormally high bilirubin levels, which are indicative of liver failure, or the effect is maximal or saturated at lower bilirubin levels. An earlier study showed a similar U-shaped relationship in a cohort of British men. On the other hand, the PREVEND (Prevention of Renal and Vascular End-Stage Disease) study demonstrated a log-linear relationship for both CVD and stroke that persisted through the entire range of bilirubin levels. Although it was unclear whether participants with liver disease were excluded from the main study, in the meta-analysis from this article, there appeared to be a narrower variance of the CVD risk reduction for studies that adjusted for other liver markers.

There are several mechanisms whereby bilirubin levels may directly affect CVD outcomes. As an end product of heme metabolism, bilirubin is toxic and insoluble. As such, it must be glucuronidated before being excreted in the bile. Alternatively, bilirubin can serve as a potent antioxidant by reducing reactive oxygen species and is subsequently oxidized back to biliverdin. This pathway appears to affect oxidative stress–induced membrane lipid degradation more than its effect on water-soluble proteins (in contrast to glutathione). Although concentrations of bilirubin are relatively lower than glutathione, the continuous recycling of biliverdin to bilirubin amplifies its effect by ≥10 000 times. Therefore, even minor increases in bilirubin levels could account for a significant increase in antioxidant potency and, therefore, affect CVD outcomes. In mouse models, depletion of bilirubin levels has resulted in intracerebral hemorrhage, neuronal apoptosis, traumatic brain injury, and cerebral ischemia. Increased levels of bilirubin also appear to establish a lean hypolipidemic state by decreasing circulating cholesterol and triacylglycerol concentrations and have been found to inhibit platelet hyperreactivity and thrombosis formation via interaction with collagen and ADP receptors. There is even evidence that bilirubin can inhibit neo-intima formation after arterial injury, block proliferation and migration of human arterial smooth muscle cells, and promote angiogenesis.

Although, in this analysis, there did not appear to be an independent effect of atazanavir with CVD risk, 2 studies (ACTG 5257 and 5260) have shown a reduction in cardiovascular biomarkers and slower progression of carotid intimal-medial thickness with atazanavir compared with raltegravir and boosted darunavir, which was partially mediated by bilirubin. A study from Spain showed similar findings. Three cross-sectional studies, 2 that compared those with normal levels to levels >2.5 mg/dL, did not find any benefit. Atazanavir has even been tested in HIV-negative diabetic patients to determine its effect on carotid intimal thickness. In one of these trials, endothelial function improved, whereas the second study showed only a reduction in low-density lipoprotein levels and blood pressure. To potentially explain these mixed findings, atazanavir and other protease inhibitors have been shown to actually increase endothelial dysfunction, oxidative stress, and von Willebrand factor, thereby counteracting some of the benefits of the elevated bilirubin levels. Because CVD rates did not differ by atazanavir status in the VACS analysis, it is unlikely that the bilirubin effect is merely a surrogate for a protective effect afforded by atazanavir use.

Figure 2. Restricted cubic spline plot of total bilirubin and cardiovascular disease (CVD), heart failure (HF), acute myocardial infarction (AMI), and ischemic stroke risk (multivariable adjusted) compared with the population median value (0.6 mg/dL) for the entire cohort (A) and for those individuals without liver disease (B).
If replicated successfully, these results have potential clinical implications not only for CVD risk assessment and reduction but also in other chronic conditions, including cancers, demyelinating neuropathies, seasonal affective disorder, and rheumatoid arthritis. This work provides epidemiologic rationale for future studies to investigate how the antioxidant effect of bilirubin could be harnessed to reduce chronic disease morbidity risk. Future studies should explore the use of bilirubin as a biomarker for other inflammation-mediated conditions and all-cause mortality. Total bilirubin could also provide additional prognostic information in morbidity and mortality risk estimators, like the VACS index. Interestingly, large increases in bilirubin were not required to see an effect on CVD risk reduction, and most of the change happened well within the normal physiologic range and specifically from the first to the second quartile.

**Limitations**

This study had a few limitations. Because VACS is an observational study, causality cannot be determined nor can all confounders be identified and completely controlled. Because the vast majority of participants were men, we

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**Table 2. Absolute Risk, Incidence Rates, and Relative Hazards of Cardiovascular Events by Baseline Bilirubin Quartile**

| Total Bilirubin, mg/dL | Events/N | Incidence Rate (95% CI) | HR (95% CI) | P Value |
|-----------------------|----------|------------------------|-------------|---------|
| **CVD**               |          |                        |             |         |
| Quartile 1: ≤0.4      | 1944/24 229 | 13.93 (13.33–14.57) | 1 (Reference) | <0.001 |
| Quartile 2: 0.5–0.6   | 1709/23 641 | 12.47 (11.89–13.08) | 0.86 (0.80–0.91) |         |
| Quartile 3: 0.7–0.8   | 1197/17 463 | 11.84 (11.19–12.53) | 0.82 (0.76–0.88) |         |
| Quartile 4: ≥0.9      | 1136/18 524 | 11.09 (10.47–11.76) | 0.77 (0.71–0.82) |         |
| Missing*              | 617/12 524 | 8.76 (8.09–9.47)     | ...         |         |
| Trend across quartiles| 6603/96 381 | ...                  | 0.92 (0.90–0.94) | <0.001 |
| **HF**                |          |                        |             |         |
| Quartile 1: ≤0.4      | 1127/24 229 | 7.95 (7.50–8.42)     | 1 (Reference) | <0.001 |
| Quartile 2: 0.5–0.6   | 1027/23 641 | 7.39 (6.95–7.85)     | 0.88 (0.80–0.96) |         |
| Quartile 3: 0.7–0.8   | 677/17 463 | 6.60 (6.12–7.12)     | 0.80 (0.72–0.89) |         |
| Quartile 4: ≥0.9      | 665/18 524 | 6.41 (5.94–6.92)     | 0.76 (0.69–0.84) |         |
| Missing*              | 347/12 524 | 4.87 (4.39–5.41)     | ...         |         |
| Trend across quartiles| 3843/96 381 | ...                  | 0.91 (0.88–0.94) | <0.001 |
| **AMI**               |          |                        |             |         |
| Quartile 1: ≤0.4      | 564/24 229 | 3.94 (3.63–4.28)     | 1 (Reference) | 0.021  |
| Quartile 2: 0.5–0.6   | 490/23 641 | 3.50 (3.20–3.82)     | 0.85 (0.76–0.96) |         |
| Quartile 3: 0.7–0.8   | 372/17 463 | 3.60 (3.25–3.99)     | 0.89 (0.76–1.04) |         |
| Quartile 4: ≥0.9      | 336/18 524 | 3.22 (2.89–3.58)     | 0.81 (0.70–0.93) |         |
| Missing*              | 169/12 524 | 2.36 (2.03–2.75)     | ...         |         |
| Trend across quartiles| 1931/96 381 | ...                  | 0.94 (0.90–0.98) | 0.007  |
| **Ischemic stroke**   |          |                        |             |         |
| Quartile 1: ≤0.4      | 647/24 229 | 4.53 (4.20–4.89)     | 1 (Reference) | <0.001 |
| Quartile 2: 0.5–0.6   | 625/23 641 | 3.75 (3.44–4.09)     | 0.80 (0.71–0.90) |         |
| Quartile 3: 0.7–0.8   | 386/17 463 | 3.74 (3.38–4.13)     | 0.81 (0.71–0.91) |         |
| Quartile 4: ≥0.9      | 344/18 524 | 3.30 (2.97–3.66)     | 0.72 (0.63–0.83) |         |
| Missing*              | 209/12 524 | 2.92 (2.55–3.34)     | ...         |         |
| Trend across quartiles| 2112/96 381 | ...                  | 0.90 (0.87–0.94) | <0.001 |

Models adjusted for age, sex, race-ethnicity, systolic blood pressure, smoking, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, HIV, hepatitis C, liver fibrosis measured by FIB-4, alcohol abuse/dependence, cocaine, and obesity. P-value test for overall significance of total bilirubin categories. AMI indicates acute myocardial infarction; CI, confidence interval; CVD, cardiovascular disease; HF, heart failure; and HR, hazard ratio.

*Missing category used only for calculation of incidence rates. For models, missing bilirubin levels were imputed.
cannot generalize these findings to a population of women. Direct versus indirect bilirubin was not examined. CVD events were not further categorized into type-specific disease codes.

**Conclusions**
Recent HIV guidelines have emphasized the need to enhance the detection and management of those individuals at risk of CVD through behavioral modification and preventive therapy, such as aspirin and lipid-lowering agents. It is important to determine whether incorporating bilirubin levels into current risk stratification models could improve prognostication of inflammatory diseases. In addition, further exploration into the mechanisms related to the antioxidant properties of bilirubin could provide new insights into therapeutic strategies.

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Table 4. Absolute Risk, Incidence Rates, and Relative Hazards of CVD Events by Baseline Bilirubin Quartile for Individuals Without Liver Disease

| Total Bilirubin, mg/dL | N  | CVD Events* | Rate (95% CI)* | HR (95% CI) | P Value |
|------------------------|----|-------------|----------------|-------------|---------|
| Total cohort           |    |             |                |             |         |
| Quartile 1: ≤0.4       | 11 738 | 797          | 11.61 (10.83–12.44) | 1 (Reference) | <0.001  |
| Quartile 2: 0.5–0.6    | 11 003 | 630          | 9.69 (8.96–10.47)  | 0.84 (0.76–0.94) |         |
| Quartile 3: 0.7–0.8    | 8117  | 420          | 8.81 (8.01–9.70)   | 0.81 (0.72–0.92) |         |
| Quartile 4: ≥0.9       | 7839  | 317          | 7.04 (6.31–7.86)   | 0.68 (0.59–0.77) |         |
| Missing†               | 876   | 41           | 7.70 (5.67–10.46)  | …           |         |
| Trend across quartiles | 39 573 | 2205         | …               | 0.89 (0.85–0.92) | <0.001  |

HIV-positive veterans

| Quartile 1: ≤0.4       | 4019  | 253          | 11.42 (10.10–12.92) | 1 (Reference) | 0.032   |
| Quartile 2: 0.5–0.6    | 3115  | 162          | 8.92 (7.65–10.41)   | 0.80 (0.66–0.98) |         |
| Quartile 3: 0.7–0.8    | 1995  | 104          | 8.66 (7.14–10.49)   | 0.83 (0.66–1.04) |         |
| Quartile 4: ≥0.9       | 1960  | 84           | 7.38 (5.96–9.14)    | 0.70 (0.54–0.91) |         |
| Missing†               | 164   | 8            | 8.80 (4.40–17.59)   | …           |         |
| Trend across quartiles | 11 253 | 612          | …               | 0.90 (0.83–0.97) | 0.006   |

Liver disease was defined as hepatitis C virus positive, FIB-4 >1.45, alcohol abuse at baseline, or presence of International Classification of Diseases, Ninth Revision (ICD-9) codes for hepatic decompensation. Models adjusted for age, sex, race-ethnicity, systolic blood pressure, smoking, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, HIV, cocaine, and obesity; models limited to HIV-positive veterans are additionally adjusted for CD4 cell count, viral load, and antiretroviral therapy regimen. P-value test for overall significance of total bilirubin categories. CI indicates confidence interval; CVD, cardiovascular disease; and HR, hazard ratio.

*These columns are based on the 39 638 individuals who are free of liver disease at baseline. Because FIB-4 values are missing for 19 032 individuals, models additionally include individuals whose imputed FIB-4 values are <1.45.

†Missing category used only for calculation of incidence rates. For models, missing bilirubin levels were imputed.

Author Contributions

All authors contributed to study design. Alcorn contributed to data collection; Marconi, Duncan, So-Armah, Justice, and Freiberg contributed to data quality and analysis; all authors contributed to article development and have critically reviewed the article and approved the final version.

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Table 5. Incidence of CVD Events by HIV Status, Baseline ART, and Baseline Atazanavir Use

| Group                    | N   | CVD Events | Rate (95% CI) |
|--------------------------|-----|------------|---------------|
| Uninfected               | 65 954 | 4248      | 11.07 (10.74–11.41) |
| HIV*, no ART            | 17 149 | 1066      | 13.50 (12.71–14.33) |
| HIV*, ART with atazanavir | 425  | 24         | 16.52 (11.07–24.64) |
| HIV*, ART and no atazanavir | 12 853 | 1265    | 14.64 (13.86–15.47) |

ART indicates antiretroviral therapy; CI, confidence interval; and CVD, cardiovascular disease.

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