ORIGINAL RESEARCH

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

Vincent C. Marconi, MD; Meredith S. Duncan, MA; Kaku So-Armah, PhD; Vincent Lo Re 3rd MD, MSCE; Joseph K. Lim, MD; Adeel A. Butt, MD; Matthew Bidwell Goetz, MD; Maria C. Rodriguez-Barradas, MD; Charles W. Alcorn, MA; Jeffrey Lennox, MD; Joshua A. Beckman, MD; Amy Justice, MD; Matthew Freiberg, MD

***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]

*From the Atlanta Veterans Affairs Medical Center, Atlanta, GA (V.C.M.); Emory University School of Medicine, Atlanta, GA (V.C.M., J.K.L.); Emory University Rollins School of Public Health, Atlanta, GA (V.C.M.); Division of Cardiology, Vanderbilt University Medical Center, Nashville, TN (M.S.D., J.A.B., M.F.); Boston University School of Medicine, Boston, MA (K.S.-A.); University of Pennsylvania Perelman School of Medicine, Philadelphia, PA (V.L.R.); Yale University School of Medicine, New Haven, CT (J.K.L., A.J.); Weill Cornell Medical College, New York, NY and Doha, Qatar (A.A.B.); Veterans Affairs Greater Los Angeles Health Care System, Los Angeles, CA (M.B.G.); Michael E. DeBakey VAMC and Baylor College of Medicine, Houston, TX (M.C.R.-B.); Veterans Affairs Connecticut Healthcare System, West Haven, CT (A.J.); University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA (C.W.A.); Veterans Affairs Pittsburgh Healthcare System, Pittsburgh, PA (A.A.B., C.W.A.); Tennessee Valley Health Care System, Nashville Veterans Affairs, Nashville, TN (M.F.); and David Geffen School of Medicine at UCLA, Los Angeles, CA (M.B.G.).*

This study was presented orally at the Conference on Retroviruses and Opportunistic Infections, February 16, 2017, in Seattle, WA.

**Correspondence to:** Vincent C. Marconi, MD, Health Sciences Research Building, 1760 Haygood Dr NE, Room W325, Atlanta, GA 30322. E-mail: vcmarco@emory.edu

Received October 6, 2017; accepted February 16, 2018.

## Clinical Perspective

### What Is New?

- This study demonstrated, for the first time, that levels of bilirubin can predict cardiovascular disease for individuals with HIV, even after adjusting for known risk factors in this population, and did not appear to be mediated by atazanavir use.
- These findings were also confirmed among age-, sex-, and ethnicity-matched HIV negative controls from the same healthcare system using a total population size of 96 381 patients.
- This inverse relationship between increasing bilirubin levels and cardiovascular disease occurred within the normal physiologic range.
- Even after excluding individuals with liver disease, these findings persisted.

### What Are the Clinical Implications?

- Mounting evidence has demonstrated that HIV-positive individuals have a greater risk of cardiovascular disease than HIV-negative individuals, even after adjusting for known risk factors.
- Inflammation appears to be a mediator of this increased risk.
- Additional studies would be necessary to explore the use of bilirubin as a biomarker for other inflammation-mediated conditions and all-cause mortality among individuals with HIV.
- Total bilirubin could also provide additional prognostic information on morbidity and mortality risk calculators.
- 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.

Bilirubin is known to have antiatherogenic properties, mediated in part by reducing oxidative stress.[7–10] Individuals with Gilbert syndrome have elevated levels of unconjugated bilirubin because of a defect in uridine diphosphate glucuronosyltransferase 1A1 enzyme.[11] Longitudinal studies of this syndrome have demonstrated reductions in coronary artery disease,[12] stroke,[13] and other inflammatory conditions.[14]

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.[15] Several studies have shown atazanavir use can improve endothelial function and reduce lipid levels and blood pressure in HIV-uninfected diabetic patients.[16,17] 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.[18,19]

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.[20,21] 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.[20,21] 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/m$^2$. 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.[22] 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 $\geq$1.45, hepatitis C virus infection, alcohol abuse, or *ICD-9* codes indicative of hepatic decompensation[23]) 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 $\leq$0.4, 0.5 to 0.6, 0.7 to 0.8, and $\geq$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/m$^2$), %* | 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

**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) |
|---|---|---|---|---|---|
| HIV-1 RNA, copies/mL* | | | | | |
| 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 | 19.7 | 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 $\chi^2$ 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).

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

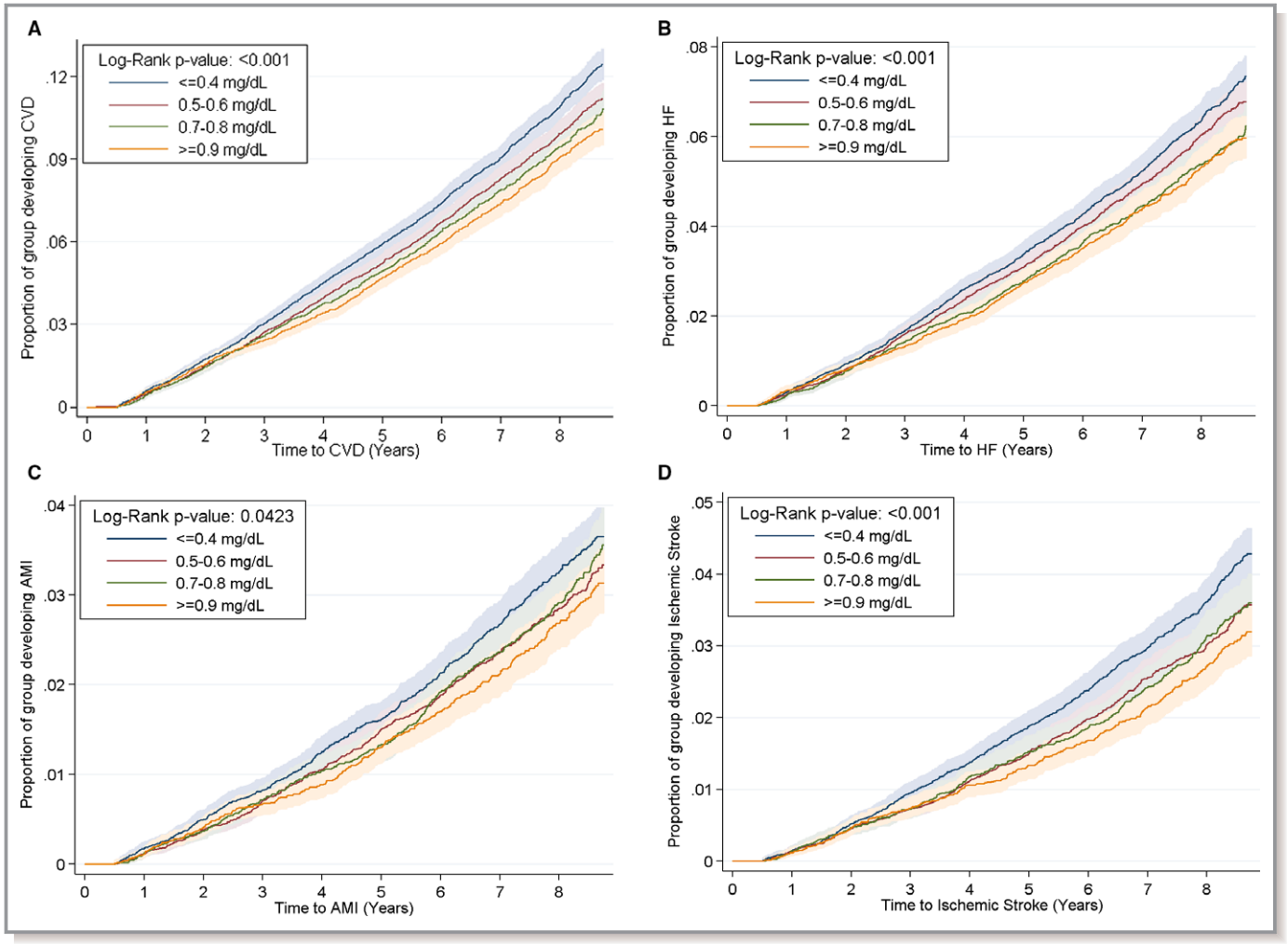


**Figure 1.** Cumulative incidence of cardiovascular disease (CVD) (A), heart failure (HF) (B), acute myocardial infarction (AMI) (C), and ischemic stroke (D) by quartiles of total bilirubin and multivariable adjusted.

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%).[24] 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.[25] 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%,[26] 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,[27] even among those with familial coronary

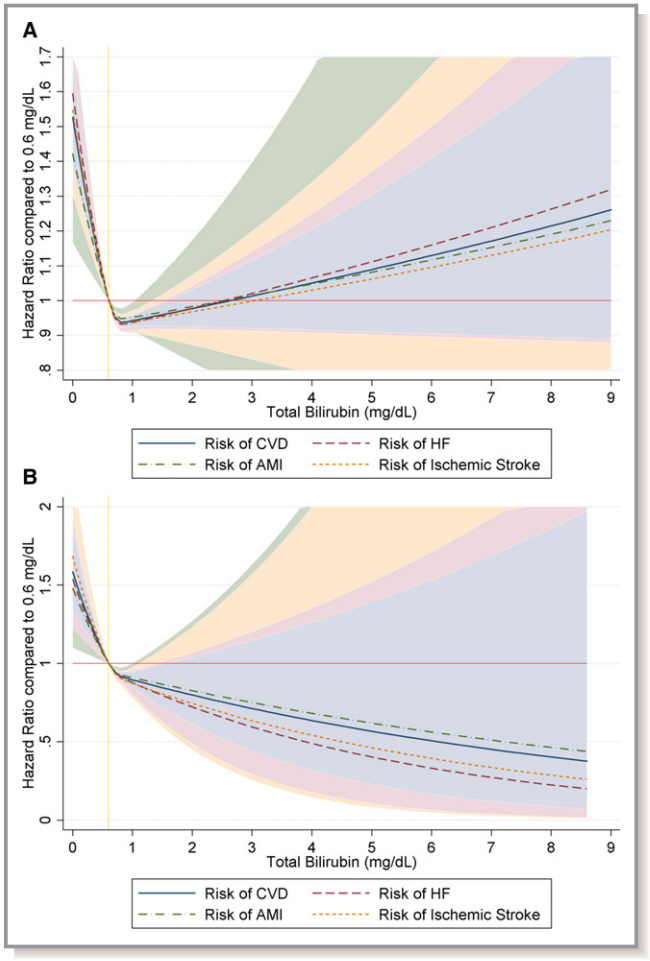


**Figure 2.** Restricted cubic spline plot of total bilirubin and cardiovascular disease (CVD), heart failure (HF), acute myocardial infarction (AMI), and ischemic stroke risk (multivariable adjusted) compared with the population median value (0.6 mg/dL) for the entire cohort (A) and for those individuals without liver disease (B).

artery disease,[28,29] peripheral artery disease,[7] carotid plaque,[30] and myocardial infarction in the Framingham Offspring Study.[31] Similar to the current study, others have also found an inverse relationship with stroke, in which a 0.1-mg/dL increase resulted a 9% reduction in the odds of an event in the National Health and Nutrition Examination Survey.[8] 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.[32] 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.[33] 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.[34,35] 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,[36] neuronal apoptosis,[37] traumatic brain injury,[38] and cerebral ischemia.[39] Increased levels of bilirubin also appear to establish a lean hypolipidemic state by decreasing circulating cholesterol and triacylglycerol concentrations[9] and have been found to inhibit platelet hyperreactivity and thrombosis formation via interaction with collagen and ADP receptors.[10] There is even evidence that bilirubin can inhibit neointima formation after arterial injury, block proliferation and migration of human arterial smooth muscle cells,[40] and promote angiogenesis.[41]

Although, in this analysis, there did not appear to be an independent effect of atazanavir with CVD[18] risk, 2 studies (ACTG 5257 and 5260) have shown a reduction in cardiovascular biomarkers and slower progression of carotid intimal-medial thickness[19] with atazanavir compared with raltegravir and boosted darunavir, which was partially mediated by bilirubin. A study from Spain showed similar findings.[42] Three cross-sectional studies,[43] 2 that compared those with normal levels to levels >2.5 mg/dL, did not find any benefit.[44,45] 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,[16] whereas the second study showed only a reduction in low-density lipoprotein levels and blood pressure.[17] 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.[46–48] 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.

**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.

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,[14] and rheumatoid arthritis.[49] 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.[50] 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.[51] Because the vast majority of participants were men, we

**Table 3.** Absolute Risk, Incidence Rates, and Relative Hazards of Cardiovascular Events by Baseline Bilirubin Quartile Among HIV-Positive Participants

| Total Bilirubin, mg/dL | Events/N | Incidence Rate (95% CI) | HR (95% CI) | *P* Value |
|---|---|---|---|---|
| CVD | | | | |
| Quartile 1: ≤0.4 | 764/9105 | 15.51 (14.45–16.65) | 1 (Reference) | <0.001 |
| Quartile 2: 0.5–0.6 | 627/8038 | 13.93 (12.89–15.07) | 0.85 (0.76–0.94) | |
| Quartile 3: 0.7–0.8 | 410/5398 | 13.15 (11.94–14.48) | 0.77 (0.68–0.88) | |
| Quartile 4: ≥0.9 | 416/5968 | 12.92 (11.74–14.23) | 0.74 (0.65–0.84) | |
| Missing* | 138/1918 | 14.99 (12.69–17.72) | … | |
| Trend across quartiles | 2355/30 427 | … | 0.90 (0.86–0.94) | <0.001 |
| HF | | | | |
| Quartile 1: ≤0.4 | 421/9105 | 8.40 (7.63–9.24) | 1 (Reference) | <0.001 |
| Quartile 2: 0.5–0.6 | 384/8038 | 8.41 (7.61–9.29) | 0.92 (0.80–1.06) | |
| Quartile 3: 0.7–0.8 | 214/5398 | 6.75 (5.91–7.72) | 0.72 (0.60–0.85) | |
| Quartile 4: ≥0.9 | 234/5968 | 7.16 (6.30–8.14) | 0.71 (0.60–0.84) | |
| Missing* | 70/1918 | 7.49 (5.93–9.47) | … | |
| Trend across quartiles | 1323/30 427 | … | 0.88 (0.83–0.93) | <0.001 |
| AMI | | | | |
| Quartile 1: ≤0.4 | 246/9105 | 4.88 (4.31–5.53) | 1 (Reference) | 0.086 |
| Quartile 2: 0.5–0.6 | 197/8038 | 4.28 (3.72–4.92) | 0.81 (0.67–0.98) | |
| Quartile 3: 0.7–0.8 | 136/5398 | 4.27 (3.61–5.06) | 0.80 (0.64–0.99) | |
| Quartile 4: ≥0.9 | 146/5968 | 4.45 (3.78–5.23) | 0.82 (0.65–1.03) | |
| Missing* | 34/1918 | 3.62 (2.58–5.06) | … | |
| Trend across quartiles | 759/30 427 | … | 0.93 (0.87–1.00) | 0.055 |
| Ischemic stroke | | | | |
| Quartile 1: ≤0.4 | 241/9105 | 4.78 (4.21–5.42) | 1 (Reference) | 0.007 |
| Quartile 2: 0.5–0.6 | 188/8038 | 4.08 (3.53–4.70) | 0.81 (0.67–0.97) | |
| Quartile 3: 0.7–0.8 | 133/5398 | 4.17 (3.52–4.94) | 0.81 (0.66–1.01) | |
| Quartile 4: ≥0.9 | 113/5968 | 3.43 (2.86–4.13) | 0.66 (0.52–0.85) | |
| Missing* | 51/1918 | 5.42 (4.12–7.13) | … | |
| Trend across quartiles | 726/30 427 | … | 0.88 (0.82–0.95) | 0.001 |

Models adjusted for age, sex, race-ethnicity, systolic blood pressure, smoking, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, hepatitis C, liver fibrosis measured by FIB-4, alcohol abuse/dependence, cocaine, obesity, CD4 cell count, viral load, and antiretroviral therapy regimen. *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.

## Acknowledgments

We thank the patients and clinical staff in the Veteran Affairs clinical sites part of VACS (Veterans Aging Cohort Study).

**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[a] | CVD Events* | Rate (95% CI)[a] | 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.

## Sources of Funding

This work was supported by: Agency for Healthcare Research and Quality (R01-HS018372); National Institute on Alcohol Abuse and Alcoholism (U24-AA020794, U01-AA020790, U01-AA020795, U01-AA020799, U24-AA022001, U24 AA022007, U10 AA013566-completed); National Heart, Lung, and Blood Institute (R01-HL095136, R01-HL090342); National Institute of Allergy and Infectious Diseases (U01-A1069918); Fogarty International Center (R25TW009337); National Institute of Mental Health (P30-MH062294); National Institute on Drug Abuse (R01DA035616); National Cancer Institute (R01 CA173754); the Veterans Health Administration Office of Research and Development (VA REA 08-266, VA IRR Merit Award); and Office of Academic Affiliations (Medical Informatics Fellowship), Emory Center for AIDS Research (P30AI050409) for Marconi, National Heart, Lung, and Blood Institute (K01HL134147) for So-Armah and (5R01HL125032) for Freiberg.

**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.

## Disclosures

Marconi has received investigator-initiated research grants (to the institution, unrelated to current work) from Bayer, Gilead Sciences, and ViiV. The remaining authors have no disclosures to report.

## References

1. Marin B, Thiebaut R, Bucher HC, Rondeau V, Costagliola D, Dorrucci M, Hamouda O, Prins M, Walker S, Porter K, Sabin C, Chene G. Non-AIDS-defining

deaths and immunodeficiency in the era of combination antiretroviral therapy. *AIDS*. 2009;23:1743–1753.

2. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, Butt AA, Bidwell Goetz M, Leaf D, Oursler KA, Rimland D, Rodriguez Barradas M, Brown S, Gibert C, McGinnis K, Crothers K, Sico J, Crane H, Warner A, Gottlieb S, Gottdiener J, Tracy RP, Budoff M, Watson C, Armah KA, Doebler D, Bryant K, Justice AC. HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med*. 2013;173:614–622.
3. Mitka M. Exploring statins to decrease HIV-related heart disease risk. *JAMA*. 2015;314:657–659.
4. Post WS, Budoff M, Kingsley L, Palella FJ Jr, Witt MD, Li X, George RT, Brown TT, Jacobson LP. Associations between HIV infection and subclinical coronary atherosclerosis. *Ann Intern Med*. 2014;160:458–467.
5. So-Armah K, Freiberg MS. Cardiovascular disease risk in an aging HIV population: not just a question of biology. *Curr Opin HIV AIDS*. 2014;9:346–354.
6. Gilbert JM, Fitch KV, Grinspoon SK. HIV-related cardiovascular disease, statins, and the reprieve trial. *Top Antivir Med*. 2015;23:146–149.
7. Perlstein TS, Pande RL, Beckman JA, Creager MA. Serum total bilirubin level and prevalent lower-extremity peripheral arterial disease: National Health and Nutrition Examination Survey (NHANES) 1999 to 2004. *Arterioscler Thromb Vasc Biol*. 2008;28:166–172.
8. Perlstein TS, Pande RL, Creager MA, Weuve J, Beckman JA. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999–2004. *Am J Med*. 2008;121:781–788.e781.
9. Bulmer AC, Verkade HJ, Wagner KH. Bilirubin and beyond: a review of lipid status in Gilbert's syndrome and its relevance to cardiovascular disease protection. *Prog Lipid Res*. 2013;52:193–205.
10. Kundur AR, Singh I, Bulmer AC. Bilirubin, platelet activation and heart disease: a missing link to cardiovascular protection in Gilbert's syndrome? *Atherosclerosis*. 2015;239:73–84.
11. Burchell B, Hume R. Molecular genetic basis of Gilbert's syndrome. *J Gastroenterol Hepatol*. 1999;14:960–966.
12. Gupta N, Singh T, Chaudhary R, Garg SK, Sandhu GS, Mittal V, Gupta R, Bodin R, Sule S. Bilirubin in coronary artery disease: cytotoxic or protective? *World J Gastrointest Pharmacol Ther*. 2016;7:469–476.
13. Pineda S, Bang OY, Saver JL, Starkman S, Yun SW, Liebeskind DS, Kim D, Ali LK, Shah SH, Ovbiagele B. Association of serum bilirubin with ischemic stroke outcomes. *J Stroke Cerebrovasc Dis*. 2008;17:147–152.
14. Rigato I, Ostrow JD, Tiribelli C. Bilirubin and the risk of common non-hepatic diseases. *Trends Mol Med*. 2005;11:277–283.
15. Rodriguez-Novoa S, Barreiro P, Jimenez-Nacher I, Soriano V. Overview of the pharmacogenetics of HIV therapy. *Pharmacogenomics J*. 2006;6:234–245.
16. Dekker D, Dorresteijn MJ, Pijnenburg M, Heemskerk S, Rasing-Hoogveld A, Burger DM, Wagener FA, Smits P. The bilirubin-increasing drug atazanavir improves endothelial function in patients with type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2011;31:458–463.
17. Milian J, Goldfine AB, Zuflacht JP, Parmer C, Beckman JA. Atazanavir improves cardiometabolic measures but not vascular function in patients with long-standing type 1 diabetes mellitus. *Acta Diabetol*. 2015;52:709–715.
18. Kelesidis T, Tran TT, Stein JH, Brown TT, Moser C, Ribaudo HJ, Dube MP, Murphy R, Yang OO, Currier JS, McComsey GA. Changes in inflammation and immune activation with atazanavir-, raltegravir-, darunavir-based initial antiviral therapy: ACTG 5260s. *Clin Infect Dis*. 2015;61:651–660.
19. Stein JH, Ribaudo HJ, Hodis HN, Brown TT, Tran TT, Yan M, Brodell EL, Kelesidis T, McComsey GA, Dube MP, Murphy RL, Currier JS. A prospective, randomized clinical trial of antiretroviral therapies on carotid wall thickness. *AIDS*. 2015;29:1775–1783.
20. Fultz SL, Skanderson M, Mole LA, Gandhi N, Bryant K, Crystal S, Justice AC. Development and verification of a "virtual" cohort using the National VA Health Information System. *Med Care*. 2006;44:S25–S30.
21. Justice AC, Dombrowski E, Conigliaro J, Fultz SL, Gibson D, Madenwald T, Goulet J, Simberkoff M, Butt AA, Rimland D, Rodriguez-Barradas MC, Gibert CL, Oursler KA, Brown S, Leaf DA, Goetz MB, Bryant K. Veterans Aging Cohort Study (VACS): overview and description. *Med Care*. 2006;44:S13–S24.
22. Lo Re V III, Kallan MJ, Tate JP, Lim JK, Goetz MB, Klein MB, Rimland D, Rodriguez-Barradas MC, Butt AA, Gibert CL, Brown ST, Park LS, Dubrow R, Reddy KR, Kostman JR, Justice AC, Localio AR. Predicting risk of end-stage liver disease in antiretroviral-treated human immunodeficiency virus/hepatitis C virus-coinfected patients. *Open Forum Infect Dis*. 2015;2:ofv109.
23. Lo Re V III, Lim JK, Goetz MB, Tate J, Bathulapalli H, Klein MB, Rimland D, Rodriguez-Barradas MC, Butt AA, Gibert CL, Brown ST, Kidwai F, Brandt C, Dorey-Stein Z, Reddy KR, Justice AC. Validity of diagnostic codes and liver-related laboratory abnormalities to identify hepatic decompensation events in the Veterans Aging Cohort Study. *Pharmacoepidemiol Drug Saf*. 2011;20:689–699.
24. Vitek L, Jirsa M, Brodanova M, Kalab M, Marecek Z, Danzig V, Novotny L, Kotal P. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis*. 2002;160:449–456.
25. Lin JP, O'Donnell CJ, Schwaiger JP, Cupples LA, Lingenhel A, Hunt SC, Yang S, Kronenberg F. Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. *Circulation*. 2006;114:1476–1481.
26. Kringen MK, Piehler AP, Grimholt RM, Opdal MS, Haug KB, Urdal P. Serum bilirubin concentration in healthy adult North-Europeans is strictly controlled by the UGT1A1 TA-repeat variants. *PLoS One*. 2014;9:e90248.
27. Novotny L, Vitek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. *Exp Biol Med (Maywood)*. 2003;228:568–571.
28. Hopkins PN, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1996;16:250–255.
29. Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin Chem*. 1994;40:18–23.
30. Ishizaka N, Ishizaka Y, Takahashi E, Yamakado M, Hashimoto H. High serum bilirubin level is inversely associated with the presence of carotid plaque. *Stroke*. 2001;32:580–583.
31. Djousse L, Rothman KJ, Cupples LA, Levy D, Ellison RC. Effect of serum albumin and bilirubin on the risk of myocardial infarction (the Framingham Offspring Study). *Am J Cardiol*. 2003;91:485–488.
32. Breimer LH, Wannamethee G, Ebrahim S, Shaper AG. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. *Clin Chem*. 1995;41:1504–1508.
33. Kunutsor SK, Bakker SJ, Gansevoort RT, Chowdhury R, Dullaart RP. Circulating total bilirubin and risk of incident cardiovascular disease in the general population. *Arterioscler Thromb Vasc Biol*. 2015;35:716–724.
34. Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR, Snyder SH. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proc Natl Acad Sci USA*. 2009;106:5171–5176.
35. Florczyk UM, Jozkowicz A, Dulak J. Biliverdin reductase: new features of an old enzyme and its potential therapeutic significance. *Pharmacol Rep*. 2008;60:38–48.
36. Wang J, Zhuang H, Dore S. Heme oxygenase 2 is neuroprotective against intracerebral hemorrhage. *Neurobiol Dis*. 2006;22:473–476.
37. Chen J, Tu Y, Connolly EC, Ronnett GV. Heme oxygenase-2 protects against glutathione depletion-induced neuronal apoptosis mediated by bilirubin and cyclic GMP. *Curr Neurovasc Res*. 2005;2:121–131.
38. Chang EF, Wong RJ, Vreman HJ, Igarashi T, Galo E, Sharp FR, Stevenson DK, Noble-Haeusslein LJ. Heme oxygenase-2 protects against lipid peroxidation-mediated cell loss and impaired motor recovery after traumatic brain injury. *J Neurosci*. 2003;23:3689–3696.
39. Dore S, Sampei K, Goto S, Alkayed NJ, Guastella D, Blackshaw S, Gallagher M, Traystman RJ, Hurn PD, Koehler RC, Snyder SH. Heme oxygenase-2 is neuroprotective in cerebral ischemia. *Mol Med*. 1999;5:656–663.
40. Peyton KJ, Shebib AR, Azam MA, Liu XM, Tulis DA, Durante W. Bilirubin inhibits neointima formation and vascular smooth muscle cell proliferation and migration. *Front Pharmacol*. 2012;3:48.
41. Ikeda Y, Hamano H, Satoh A, Horinouchi Y, Izawa-Ishizawa Y, Kihira Y, Ishizawa K, Aihara K, Tsuchiya K, Tamaki T. Bilirubin exerts pro-angiogenic property through Akt-eNOS-dependent pathway. *Hypertens Res*. 2015;38:733–740.
42. Estrada V, Monge S, Gomez-Garre MD, Sobrino P, Masia M, Berenguer J, Portilla J, Vilades C, Martinez E, Blanco JR; CoRIS and the HIV Biobank integrated in the Spanish AIDS Research NetworkCoRis. Relationship between plasma bilirubin level and oxidative stress markers in HIV-infected patients on atazanavir- vs. efavirenz-based antiretroviral therapy. *HIV Med*. 2016;17:653–661.
43. Hileman C, Longenecker C, Carman T, Milne G, Labbato DE, Storer N, White C, McComsey G. Relationship between total bilirubin and endothelial function, inflammation and oxidative stress in HIV-infected adults on stable antiretroviral therapy. *HIV Med*. 2012;13:609–616.
44. Barber TJ, Moyle G, Hill A, Singh GJ, Boffito M, Nelson M. Effect of hyperbilirubinaemia on neurocognitive, renal, bone and cardiovascular markers in HIV infection treated with boosted protease inhibitors. *J Int AIDS Soc*. 2014;17:19827.
45. Barber TJ, Moyle G, Hill A, Jagjit Singh G, Scourfield A, Yapa HM, Waters L, Asboe D, Boffito M, Nelson M. A cross-sectional study to evaluate the association of hyperbilirubinaemia on markers of cardiovascular disease,

neurocognitive function, bone mineral density and renal markers in HIV-1 infected subjects on protease inhibitors. *HIV Clin Trials*. 2016;17:123–130.

46. Liu XM, Durante ZE, Peyton KJ, Durante W. Heme oxygenase-1-derived bilirubin counteracts HIV protease inhibitor-mediated endothelial cell dysfunction. *Free Radic Biol Med*. 2016;94:218–229.
47. Jiang B, Hebert VY, Li Y, Mathis JM, Alexander JS, Dugas TR. HIV antiretroviral drug combination induces endothelial mitochondrial dysfunction and reactive oxygen species production, but not apoptosis. *Toxicol Appl Pharmacol*. 2007;224:60–71.
48. Beckman JA, Wood BR, Ard KL, Price CN, Solomon DA, Zuflacht JP, Milian J, Prenner JC, Sax PE. Conflicting effects of atazanavir therapy on atherosclerotic risk factors in stable HIV patients: a randomized trial of regimen switch to atazanavir. *PLoS One*. 2017;12:e0181993.
49. Fischman D, Valluri A, Gorrepati VS, Murphy ME, Peters I, Cheriyath P. Bilirubin as a protective factor for rheumatoid arthritis: an NHANES study of 2003–2006 data. *J Clin Med Res*. 2010;2:256–260.
50. Salinas JL, Rentsch C, Marconi VC, Tate J, Budoff M, Butt AA, Freiberg MS, Gibert CL, Goetz MB, Leaf D, Rodriguez-Barradas MC, Justice AC, Rimland D. Baseline, time-updated, and cumulative HIV care metrics for predicting acute myocardial infarction and all-cause mortality. *Clin Infect Dis*. 2016;63:1423–1430.
51. Kunutsor SK. Serum total bilirubin levels and coronary heart disease: causal association or epiphenomenon? *Exp Gerontol*. 2015;72:63–66.