Journal of the American Heart Association

ORIGINAL RESEARCH

Association Between Atazanavir-Induced Hyperbilirubinemia and Cardiovascular Disease in Patients Infected with HIV

Michael Li, MD; Walter W. Chan, MD, MPH; Stephen D. Zucker, MD

BACKGROUND: Serum bilirubin is inversely associated with cardiovascular risk. Atazanavir, an HIV protease inhibitor that competitively inhibits bilirubin conjugation, provides a unique opportunity to examine whether selectively increasing bilirubin is cardioprotective. We sought to determine whether patients receiving atazanavir manifest a reduced risk of cardiovascular disease compared with those receiving darunavir, an HIV protease inhibitor that does not increase serum bilirubin.

METHODS AND RESULTS: This was a retrospective cohort study of 1020 patients with HIV. The main outcome was time to myocardial infarction or ischemic stroke. Mean follow-up was 6.6±3.4 years, with 516 receiving atazanavir and 504 darunavir. Atazanavir patients exhibited significantly higher serum total bilirubin (1.7 versus 0.4 mg/dL; $P<0.001$) and longer mean time to ischemic event (10.2 versus 9.4 years; $P<0.001$). On Cox regression, atazanavir treatment (hazard ratio [HR], 0.38; 95% CI, 0.21–0.71; $P=0.002$) and serum bilirubin (HR, 0.60; 95% CI, 0.41–0.89; $P=0.011$) were independently associated with a lower risk of an ischemic event. Notably, when atazanavir and bilirubin were included together in the Cox regression model, atazanavir lost significance (HR, 0.55; 95% CI, 0.24–1.29; $P=0.169$) consistent with bilirubin being an intermediate variable on the causal pathway between atazanavir and its effect on cardiovascular disease. Patients on atazanavir also had a significantly lower risk of developing new cardiovascular disease (HR, 0.53; 95% CI, 0.33–0.86; $P=0.010$) and longer mean time to death (12.2 versus 10.8 years; $P<0.001$).

CONCLUSIONS: Patients with HIV on atazanavir manifest a decreased risk of cardiovascular disease when compared with those on darunavir, an effect that appears to be mediated by serum bilirubin.

Key Words: bilirubin ■ cardiovascular disease ■ HIV

Bilirubin is a potent endogenous antioxidant that has been shown to suppress inflammation by inhibiting leukocyte migration and cytokine signaling,[1-3] and there is growing evidence that bilirubin exerts clinically significant anti-inflammatory effects. Studies have identified an inverse correlation between serum bilirubin concentrations and the development of such inflammatory conditions as cardiovascular disease (CVD), ulcerative colitis, multiple sclerosis, and cancer.[4-10] In addition, individuals possessing the Gilbert's polymorphism, which causes an increase in serum bilirubin through reduced activity of the UDP-glucuronosyltransferase 1A1 enzyme, exhibit a lower incidence of CVD, cancer, asthma, and Crohn's disease.[11-14] However, these association studies do not establish cause and effect, and it remains unclear whether selectively increasing serum bilirubin levels confers protection against inflammatory disorders.

Atazanavir is an HIV protease inhibitor that reliably causes an indirect hyperbilirubinemia by competitively inhibiting UDP-glucuronosyltransferase 1A1,[15,16] thereby inducing a Gilbert's phenotype. HIV infection increases the risk of CVD,[17] and serum bilirubin is inversely associated with CVD in patients with HIV,[18] which has prompted speculation that treatment with atazanavir may confer a protective effect against CVD.

Correspondence to: Michael Li, MD, Division of Gastroenterology, Hepatology, and Endoscopy, Brigham & Women's Hospital, 75 Francis Street, Boston, MA 02115. E-mail: mli31@bwh.harvard.edu

For Sources of Funding and Disclosures, see page 9.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Patients with HIV treated with atazanavir have a lower risk of myocardial infarction or stroke and a lower risk of developing new cardiovascular disease than those treated with darunavir, and this cardioprotective effect appears to be mediated in part by serum bilirubin.

What Are the Clinical Implications?

- Patients with HIV on darunavir-containing regimens with elevated cardiovascular disease risk may benefit from changing to atazanavir treatment.

In support of this proposition, a randomized clinical trial demonstrated a slower rate of carotid artery intima-media thickening in patients with HIV treated with atazanavir versus darunavir, a protease inhibitor that does not affect serum bilirubin.[19] Other effects associated with bilirubin, including a reduction in markers of oxidative stress and inflammation, also have been demonstrated in atazanavir-treated patients.[20,21]

Three prior studies directly examined the influence of atazanavir on the development of CVD. The first identified a significantly lower risk of CVD in patients with elevated bilirubin levels who were taking atazanavir, but not in those taking other antiretroviral agents.[22] The second analyzed a large Veterans Health Administration database and found that, during a mean follow-up of 1 year, the incidence of myocardial infarction (MI) and stroke were lower in atazanavir-treated patients compared with those administered alternative HIV medications, although the results were not correlated with serum bilirubin.[23] A recent prospective study concluded that patients with HIV treated with darunavir exhibited an increased risk of cardiovascular events compared with those receiving atazanavir.[24] Notably, in contrast with multiple prior reports, these investigators did not find an association between serum bilirubin levels and cardiovascular risk.

Speculating that induction of hyperbilirubinemia may reduce cardiovascular risk, in the present study we examined whether patients with HIV receiving atazanavir manifest a lower risk of CVD compared with those who received treatment with darunavir, a protease inhibitor that does not affect bilirubin levels.

METHODS

Patients and Study Design

This was a multicenter, retrospective cohort study of all patients with HIV who received outpatient care in the Partners HealthCare system and who received treatment with atazanavir or darunavir between 2003 and 2019. Patients were identified through the Partners Research Patient Data Registry, a centralized clinical data warehouse that gathers clinical information from the multiple hospital systems within Partners HealthCare. Patients were excluded if they were (1) younger than 18 years of age, (2) not on continuous treatment with either medication for at least 1 year, (3) did not receive care after the Epic electronic health record system was implemented in 2015, or (4) did not receive outpatient care in the Partners HealthCare system (Figure 1).

Approval was obtained from the Partners Human Research Committee. Given the retrospective nature of the study, consent for participation was waived. The data that support the findings of the study are available from the corresponding author upon reasonable request.

Outcomes and Covariates

The primary outcome was time to first ischemic event, which was defined as either an ischemic cardiac event or an ischemic stroke. An ischemic cardiac event was defined as the new development of unstable angina, ST-segment–elevation MI and non–ST-segment–elevation MI. Patients with type II MI (ie, demand ischemia) were not included in the primary outcome. Only patients with ischemic stroke were included (those with hemorrhagic stroke were excluded as the purpose of this study was to investigate events related to inflammation and atherosclerosis). Outcomes were determined through individual chart examination of Epic diagnosis codes and clinical notes and further validated by review of cardiac catheterization or brain imaging reports whenever possible (available in 87% of instances). Secondary outcomes were (1) all-cause mortality and (2) time to new diagnosis of CVD that, in addition to the primary outcomes, included coronary artery disease, peripheral vascular disease, carotid artery stenosis, and transient ischemic attack. The diagnosis of new CVD was established through a review of diagnosis codes and clinical notes as well as medications, imaging reports, stress test results, and catheterization reports.

Patients not reaching outcome were censored at termination of treatment, death, or last clinic visit. In addition to patients who were started on atazanavir or darunavir during the study period, those who were already receiving atazanavir or darunavir at the time of entry into the Partners HealthCare system were also included in the analyses, with the date of their first encounter in the Partners' system considered to be the start date. Baseline demographic data included age, sex, ethnicity, smoking status, concomitant antiretroviral use, whether patients had been antiretroviral

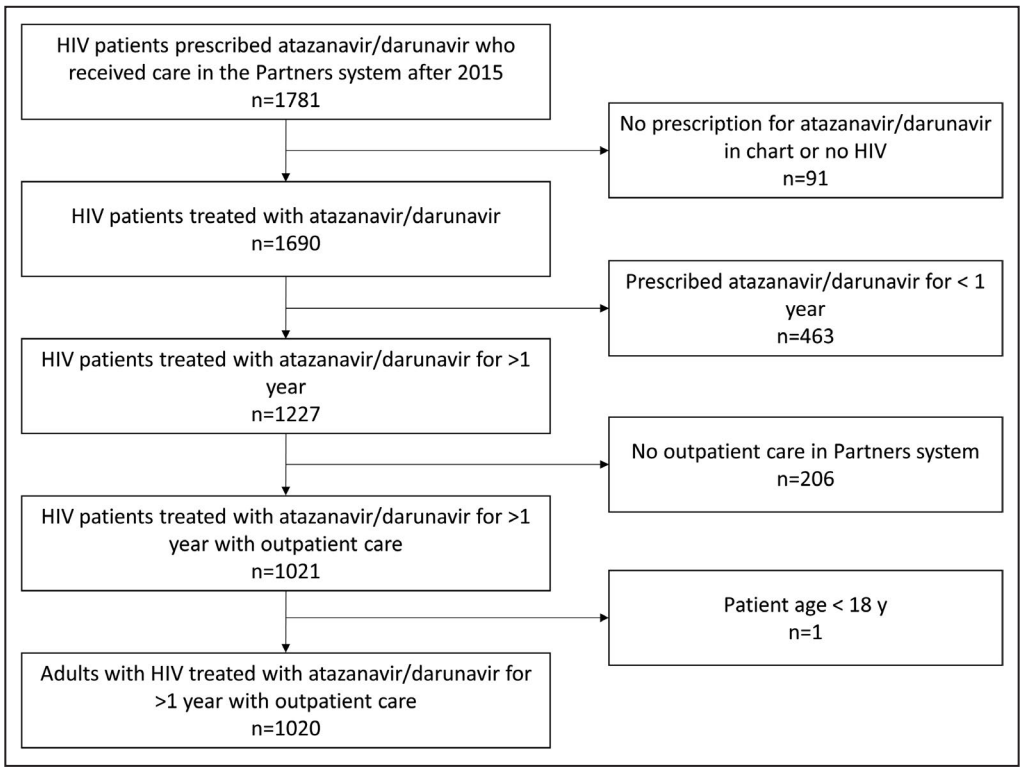

Figure 1. Flowchart of patient identification and exclusion.

naïve at study entry, diabetes mellitus, hyperlipidemia, pre-existing chronic kidney disease (defined as an estimated glomerular filtration rate below 60 mL/min per 1.73 m^2 for 3 months), and pre-existing CVD (defined and determined as previously described for secondary outcomes).

Clinical parameters (blood pressure, body mass index, virologic failure) and laboratory values (serum total and direct bilirubin, serum lipid levels [total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol], CD4 count, HIV viral load) obtained at the closest time point before study completion or treatment discontinuation to account for potential medication effects from long-term atazanavir or darunavir therapy. To account for the normal variance in serum bilirubin concentrations, if multiple bilirubin levels were available within the year before discontinuation of treatment, the median of the last 3 serum bilirubin values was used. Virologic failure was defined as viremia >200 copies per mL on at least 2 consecutive measurements. Serum bilirubin concentrations and virologic failure at the time of any primary event were used in the statistical analyses.

Statistical Analysis

Baseline patient demographics and covariates are reported as means and standard deviations for continuous normal data, medians and interquartile ranges for continuous non-normal data, and frequencies and percentages for categorical data. Univariable analyses were performed using a 2-sided Student *t* test or Wilcoxon rank sum test for continuous variables, χ^2 test for categorical variables, and Kaplan–Meier analysis for time-to-event data. Multivariable analysis using Cox proportional hazards regression was employed to control for potential confounders. As missing data were rare for any specific variable apart from direct bilirubin, then any missing values were excluded from statistical analysis. A mediation analysis was performed to test whether any relationship between atazanavir exposure and cardiovascular outcomes was attributable to total bilirubin as an intermediate variable on the causal pathway.[25,26] For survival analysis, patients were censored at the time of last follow-up, death, or medication discontinuation. Statistical significance was defined as $P \leq 0.05$. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

A total of 937 patients treated with atazanavir and 844 patients treated with darunavir were identified in the initial search of the Research Patient Data Registry, of which 761 met at least 1 of the exclusion criteria (Figure 1). The remaining 516 patients who received atazanavir and 504 who received darunavir were included in the analysis. Table 1 lists the baseline

Table 1. Demographics of Patient Cohorts

Characteristic	Atazanavir (n=516)	Darunavir (n=504)	*P* Value
Age at enrollment, y	45.4±9.2	47.5±10.6	<0.001
Age at end of study, y	52.9±9.8	53.2±11.0	0.664
Follow-up, y	7.5±3.5	5.7±2.9	<0.001
Patients with ambulatory infectious diseases follow-up	492 (95.4)	482 (95.6)	0.826
Patients lost to follow-up	10 (1.9)	20 (4.0)	0.055
Patients censored because of medication discontinuation	440 (85.3)	283 (56.2)	<0.001
Males	351 (68.0)	330 (65.5)	0.388
Ethnicity			
White	268 (52.0)	222 (44.1)	0.041
Black	157 (30.4)	177 (35.1)	
Hispanic	83 (16.1)	89 (17.7)	
Other/unknown	8 (1.6)	16 (3.2)	
Body mass index	27.7±5.6	27.6±6.1	0.674
Systolic blood pressure	126.9±16.5	126.9±16.4	0.969
Diastolic blood pressure	78.50±10.6	77.42±10.3	0.100
Lipids			
Cholesterol, mg/dL	179.5±41.5	179.6±46.7	0.968
Triglycerides, mg/dL	161.6±104.4	160.8±101.5	0.900
HDL, mg/dL	47.2±16.3	47.5±17.7	0.755
LDL, mg/dL	100.6±35.4	101.4±37.3	0.712
Serum bilirubin			
Total bilirubin, mg/dL	1.7 (1–2.5)	0.4 (0.3–0.5)	<0.001
Direct bilirubin, mg/dL	0.2 (0.2–0.3)	0.1 (0.1–0.2)	<0.001
Cardiovascular risk factors			
Smoking	214 (41.5)	181 (35.9)	0.068
Hypertension	259 (50.2)	228 (45.2)	0.113
Diabetes mellitus	76 (14.7)	87 (17.3)	0.270
Hyperlipidemia	232 (45.0)	205 (40.7)	0.167
Pre-existing chronic kidney disease	59 (11.4)	76 (15.1)	0.086
Pre-existing cardiovascular disease	28 (5.4)	39 (7.7)	0.136
HIV parameters			
CD4 count, per μL	603.1±333.6	534.1±340.4	0.001
Viral load, copies/mL	20 (0–50)	20 (0–30)	0.140
Below detectable viral load	367 (71.3)	371 (73.6)	0.402
Virologic failure	66 (12.8)	45 (9.0)	0.050
Antiretroviral naïve	62 (12.0)	30 (5.0)	0.001
Atazanavir/darunavir initiated at enrollment	412 (79.8)	406 (80.6)	0.776
Other HIV medications at enrollment			
Ritonavir	466 (90.3)	478 (94.8)	0.006
Truvada	314 (60.9)	338 (67.1)	0.039
Abacavir	24 (4.7)	14 (2.8)	0.114
Outcomes			
Myocardial infarction	12 (2.3)	19 (3.8)	0.179
Ischemic stroke	6 (1.2)	20 (4.0)	0.005
New diagnosis of cardiovascular disease	35 (7.1)	43 (9.2)	0.243
Death	6 (1.2)	22 (4.4)	0.002

Data are mean±SD, median (interquartile range), or number (percentage). HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

characteristics by cohort, which were generally well matched. Notable differences between those receiving atazanavir versus darunavir were mean age at the start of treatment (45.4 versus 47.5 years; $P<0.001$), mean follow-up (7.5 versus 5.7 years; $P<0.001$), rates of medication discontinuation (85.3% versus 56.2%; $P<0.001$), ethnicity ($P=0.041$), CD4 count (603.1 versus 534.1; $P=0.001$), proportion who were antiretroviral naïve (12.0% versus 6.0%; $P<0.001$), and virologic failure at end of follow-up (12.8% versus 9.0%; $P=0.050$). As expected, the median serum total bilirubin concentration was higher in the atazanavir compared with the darunavir cohort (1.7 versus 0.4 mg/dL; $P<0.001$), with a marked broadening of the bilirubin distribution among atazanavir-treated patients (Figure 2).

Consistent with previous studies,[8,27] total bilirubin was higher in men compared with women in the atazanavir (1.8 versus 1.4 mg/dL; $P<0.001$) and darunavir (0.4 versus 0.3 mg/dL; $P<0.001$) groups and was also higher in White patients compared with Black patients (2.0 versus 1.3 mg/dL [$P<0.001$] in the atazanavir group and 0.4 versus 0.3 mg/dL [$P<0.001$] in the darunavir group).

Regarding outcomes in the total study population, 31 patients experienced a new ischemic cardiac event, 26 patients were diagnosed with an ischemic stroke, 78 patients developed new CVD, and 28 patients died during the entire follow-up period. Crude incidence rates per thousand patient-years in the atazanavir versus the darunavir cohort for ischemic cardiac events and ischemic stroke were significantly lower in the atazanavir group (4.65 versus 13.55; incidence rate ratio, 0.34; 95% CI, 0.20–0.60; $P<0.001$). On survival analysis, the atazanavir cohort had a significantly longer time to first MI or stroke than did the darunavir cohort (log-rank $P<0.001$; Figure 3A).

After adjusting for established cardiovascular risk factors (ie, hypertension, hyperlipidemia, diabetes mellitus, smoking, sex, ethnicity) as well as differences in baseline characteristics of the cohorts (ie, virologic failure, ritonavir use, age) (Table 2), using Cox regression analysis, atazanavir treatment was significantly associated with a decreased risk of ischemic cardiac event or stroke when compared with darunavir (hazard ratio [HR], 0.38; 95% CI, 0.21–0.71; $P=0.002$). Similarly, when the serum total bilirubin level was substituted for atazanavir as a continuous variable in the regression model (Table 2), it also was significantly associated with a decreased risk of MI or stroke (HR, 0.60; 95% CI, 0.41–0.89; $P=0.011$). To test the hypothesis that atazanavir exerts a protective effect against CVD by augmenting serum bilirubin levels, both atazanavir and serum bilirubin were included simultaneously as variables in the regression model in a mediation analysis (Table 2). After the addition of bilirubin to the model, atazanavir treatment was no longer significantly associated with a decreased risk of ischemic events (HR, 0.55; 95% CI, 0.24–1.29; $P=0.169$), which is consistent with bilirubin serving as an intermediary in the causal pathway between atazanavir and CVD.[26]

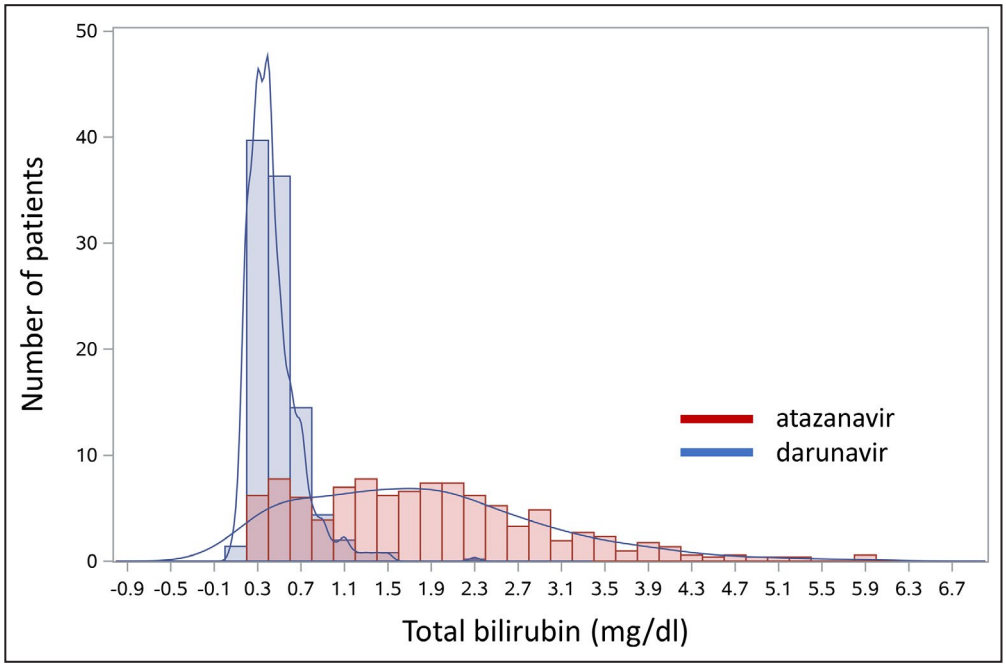

Figure 2. Distribution of serum total bilirubin in patients receiving atazanavir or darunavir
Overlay histogram indicating number of patients at each serum bilirubin concentration.

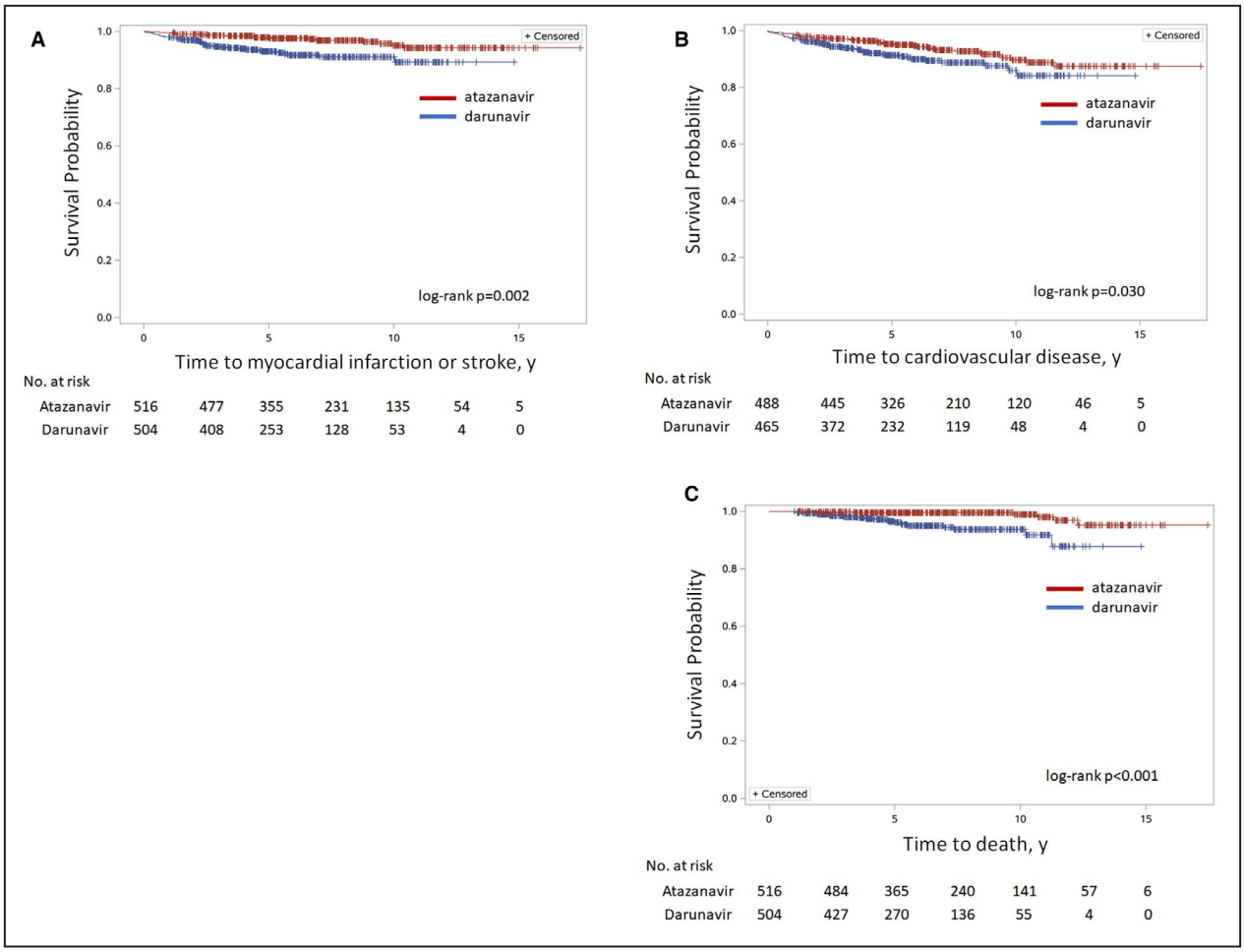

Figure 3. Kaplan–Meier curves.
A, Time to myocardial infarction or stroke. **B**, Time to new diagnosis of cardiovascular disease. **C,** Time to death.

Notably, a Cox regression model built using a stepwise forward-selection technique eliminated age, sex, smoking, and diabetes mellitus as covariates, but did not meaningfully alter the findings; atazanavir treatment remained strongly associated with a diminished risk of ischemic cardiac events or stroke (HR,

Table 2. Cox Regression Models and Time to Ischemic Event

Covariate	Model Excluding Bilirubin, HR (95% CI)	*P* Value	Model Excluding Atazanavir, HR (95% CI)	*P* Value	All-Inclusive Model, HR (95% CI)	*P* Value
Atazanavir treatment	0.38 (0.21–0.71)	0.0022	…	…	0.55 (0.24–1.29)	0.1694
Total bilirubin	…	…	0.58 (0.39–0.86)	0.0067	0.77 (0.47–1.28)	0.3113
Hyperlipidemia	4.40 (2.05–9.42)	0.0001	4.34 (2.02–9.30)	0.0002	4.34 (2.02–9.33)	0.0002
Chronic kidney disease	2.17 (1.19–3.96)	0.0114	2.25 (1.23–4.09)	0.0082	2.19 (1.20–3.98)	0.0102
Virologic failure	2.97 (1.25–7.03)	0.0135	1.94 (0.83–4.57)	0.1286	2.42 (0.97–6.06)	0.0586
Ritonavir	3.60 (1.29–10.06)	0.0143	4.26 (1.58–11.54)	0.0043	3.63 (1.30–10.13)	0.0138
Hypertension	2.02 (1.02–3.99)	0.0445	1.81 (0.91–3.58)	0.0888	1.80 (0.91–3.59)	0.0929
Cardiovascular disease	2.05 (1.01–4.19)	0.0474	1.93 (0.94–3.96)	0.0750	1.98 (0.97–4.07)	0.0619
Black	0.59 (0.31–1.10)	0.0963	0.56 (0.30–1.05)	0.0706	0.93 (0.63–1.37)	0.7060
Male sex	0.75 (0.40–1.41)	0.3650	0.78 (0.41–1.47)	0.4345	0.83 (0.44–1.57)	0.5710
Age at start of study	1.01 (0.98–1.05)	0.4030	1.01 (0.98–1.05)	0.3879	1.01 (0.98–1.05)	0.4738
Diabetes mellitus	0.88 (0.47–1.68)	0.7064	0.89 (0.47–1.68)	0.7076	0.89 (0.47–1.69)	0.7197
Smoking	1.04 (0.58–1.86)	0.8897	0.98 (0.55–1.74)	0.9370	1.05 (0.59–1.88)	0.8750

HR indicates hazard ratio.

0.39; 95% CI, 0.21–0.71; *P*=0.002). The addition of bilirubin to the model was again found to negate this association (HR, 0.57; 95% CI, 0.25–1.32; *P*=0.191). Although some studies have suggested that abacavir may alter cardiovascular risk,[28,29] the addition of abacavir to the regression models did not substantially alter the results. As it has previously been suggested that it is unconjugated, rather than conjugated, bilirubin that exerts a protective effect against CVD, we performed an analysis of the subset of 469 patients (230 in the atazanavir cohort and 239 in the darunavir cohort) in whom serum bilirubin levels were fractionated. When either direct (conjugated) or indirect (unconjugated) serum bilirubin were substituted for total bilirubin in the Cox regression model, indirect bilirubin was significantly associated with lower risk of MI or ischemic stroke (HR, 0.51; 95% CI, 0.31–0.84; *P*=0.008), whereas direct bilirubin was not (HR, 0.12; 95% CI, 0.01–3.40; *P*=0.216). These data support that the observed effect of bilirubin on CVD is primarily attributable to the unconjugated fraction. In addition, when indirect bilirubin and atazanavir were jointly included in the Cox regression model, atazanavir again lost significance with respect to risk of an ischemic event (HR, 0.47; 95% CI, 0.17–1.30; *P*=0.145) consistent with indirect bilirubin serving as an intermediary in the causal pathway between atazanavir and CVD.

When examining the secondary outcomes of time to diagnosis of new CVD and time to all-cause mortality, log-rank testing demonstrated that the atazanavir cohort had significantly longer time to CVD (*P*=0.030; Figure 3B) and to all-cause mortality (*P*<0.001; Figure 3C) than did the darunavir cohort. On Cox regression analysis using the same covariates as in our primary model (apart from the exclusion of pre-existing CVD), atazanavir treatment was associated with a significantly lower risk of developing new CVD compared with darunavir (HR, 0.53; 95% CI, 0.33–0.86; *P*=0.010) as was bilirubin itself (HR, 0.73; 95% CI, 0.56–0.95; *P*=0.017). After accounting for bilirubin, atazanavir no longer significantly decreased this risk (HR, 0.69; 95% CI, 0.35–1.34; *P*=0.267), again supporting that bilirubin lies on the causal pathway between atazanavir treatment and decreased risk of CVD. Although multivariable analysis of time to death was constrained by the low number of deaths (28 total) in the overall study, using a simplified Cox regression model adjusting only for age, atazanavir treatment was associated with a significantly decreased risk of death (HR, 0.19; 95% CI, 0.07–0.49; *P*=0.001), as was bilirubin when examined separately (HR, 0.37; 95% CI, 0.18–0.74; *P*=0.005). Once again after accounting for bilirubin, the atazanavir effect on risk of death was negated (HR, 0.33; 95% CI, 0.10–1.16; *P*=0.085).

DISCUSSION

A number of epidemiological analyses have consistently demonstrated an inverse association between serum bilirubin levels and the risk of CVD,[5-7] with some investigations additionally correlating the presence of the Gilbert's polymorphism with these findings.[11,12,30] What is less well established is whether the induction of a sustained increase in circulating bilirubin levels can result in a beneficial therapeutic effect on cardiovascular risk. In the present study involving a large, diverse HIV cohort, patients treated with atazanavir exhibited a sustained increase in serum bilirubin levels in conjunction with a significantly decreased risk of ischemic events when compared with those receiving darunavir, even after adjusting for established cardiovascular risk factors. Moreover, atazanavir treatment also was significantly associated with improved survival. As most HIV parameters including viral load and percent of patients with undetectable viral loads were no different between the atazanavir and darunavir cohorts (in line with previously published studies[31,32]), we speculate that the lower rate of death in patients receiving atazanavir results from a protective effect of this medication against CVD and potentially other comorbidities associated with chronic HIV infection.

As bilirubin has been shown to prevent atherosclerosis in animal models by inhibiting inflammatory cell migration into sites of vascular injury,[33] we speculate that the cardioprotective effects of atazanavir are mediated by its ability to effectively increase the concentration of bilirubin in the systemic circulation. Support for this hypothesis is derived from our finding that inclusion of bilirubin in Cox regression models negates the influence of atazanavir on CVD, consistent with bilirubin acting as at least a partial intermediary of this phenomenon. We further show that the protective effect of bilirubin is specifically associated with the unconjugated fraction, which is consistent with experimental data demonstrating that conjugated bilirubin does not regulate leucocyte migration.[3] On the other hand, it is important to point out that the HR for bilirubin also does not remain significant when atazanavir is included in the Cox regression model and that the HR for atazanavir is only partially mitigated from 0.38 to 0.55 and does not approach 1 (although notably losing significance with bilirubin in the model). These observations raise the possibility that hyperbilirubinemia is not the sole determinant of the protective effect of atazanavir against CVD and that there may be an as of yet unidentified cardioprotective mechanism of atazanavir that is not related to its effect on bilirubin. Alternatively, it could reflect that darunavir exerts a deleterious cardiovascular effect, as postulated by Ryom et al.[24] The results also could be confounded by the high prevalence of the Gilbert's polymorphism

(approximately 40%) in the general population,[34] as these individuals are not only at lower risk of CVD[30,35] but also develop substantially higher levels of serum bilirubin when receiving atazanavir.[16]

Our results are consistent with prior reports suggesting that atazanavir protects against CVD. In a randomized clinical trial, Stein et al[19] showed that carotid intima-media thickening (a surrogate marker of cardiovascular risk) progressed at a slower rate in patients treated with atazanavir compared with those receiving darunavir. Similarly, Estrada et al[20] and Kelesidis et al[21] found that serological markers of oxidative stress and inflammation are decreased in individuals on atazanavir. LaFleur et al[23] retrospectively examined cardiovascular outcomes in a large Veterans Health Administration cohort and found a reduced incidence of MI and stroke, but not mortality, in patients with HIV receiving an atazanavir-containing versus non-atazanavir-containing regimen. Limitations of this study include a homogenous patient population (>90% male) and relatively short duration of follow-up (1 year), and these investigators did not correlate findings with serum bilirubin. In a large observational study, Ryom et al[24] concluded that the cumulative use of ritonavir-boosted darunavir, but not atazanavir, was associated with an increased incidence of CVD, although no interaction between atazanavir (or darunavir) and plasma bilirubin concentrations on rates of CVD was identified. Notably, a randomized controlled trial comparing the metabolic effects of atazanavir versus darunavir demonstrated that atazanavir treatment is associated with worse dyslipidemia, increased abdominal adiposity, and higher insulin resistance.[36,37] These metabolic effects would likely make it more difficult to demonstrate a lowered risk of CVD attributed to atazanavir exposure and strengthens our conclusions.

Strengths of our study include the large and diverse patient cohort with relatively long follow-up (mean 6.6 years) and low drop-out rate (2.9%). Baseline characteristics were generally well matched, and >95% of patients had regular outpatient follow-up with an infectious disease specialist. Primary outcomes were validated by cardiac catheterization and brain imaging in 87% of instances. Moreover, by directly comparing outcomes for atazanavir with another contemporary protease inhibitor (darunavir), the likelihood of confounding by indication is minimized, as the rationale for prescribing atazanavir and darunavir are nearly identical. Reasons for converting patients from atazanavir to darunavir are limited and include the need for proton pump inhibitor treatment and patient preference (generally because of overt jaundice). Notably, these indications are unrelated to our measured outcomes and, if anything, would be expected to bias against our findings. Most other HIV parameters including viral load and percent of patients with undetectable viral loads were no different between the atazanavir and darunavir cohorts. This is consistent with previously published studies that demonstrated that atazanavir and darunavir-containing regimens achieve equivalent rates of virologic control.[31,32]

An important limitation of our study is the observational nature, which raises the possibility of unmeasured confounders. HIV-2 infection may be one of these as HIV subtype was not recorded, although as HIV-2 infection in the United States is rare it is unlikely that it is a clinically meaningful confounder. Furthermore, there were some differences in the study population notably with atazanavir-exposed patients being younger and having longer follow-up. Although these raise the possibility of indication bias, we speculate that the age difference is attributable to atazanavir patients being more frequently treatment naïve (which is consistent with darunavir being an option for treatment-resistant disease) and that the difference in follow-up time is attributable to atazanavir being approved by the US Food and Drug Administration 3 years earlier than darunavir. Significantly more patients receiving atazanavir had their medication discontinued compared with darunavir (85.3% versus 56.2%), which would also raise concern for residual confounding except that this observation is consistent with prior reports of higher discontinuation rates with atazanavir.[31] This higher discontinuation rate has been attributed to atazanavir-induced hyperbilirubinemia and, in more recent clinical practice, by perceived better compliance with the single-pill formulation of darunavir, which is not available for atazanavir. Indeed, there was significantly more virologic failure in patients receiving atazanavir versus darunavir (12.8% versus 9.0%; P=0.05; see Table 1), which could be explained by lower compliance in the atazanavir cohort. Finally, events that occurred outside of the Partners system were captured through reviews of outpatient notes and Care Everywhere (a national interoperability system that links our electronic health record to other Epic-based electronic health records), although the possibility exists that events could have been missed if the data were not accessible through these methods.

In summary, patients with HIV treated with atazanavir were found to have a reduced incidence of CVD and improved survival compared with those receiving darunavir. Our data support that bilirubin is a mediator of this effect and suggest that the induction of a sustained increase in serum bilirubin may represent a viable therapeutic approach to reducing cardiovascular risk.

ARTICLE INFORMATION

Received March 18, 2020; accepted August 10, 2020.

Affiliations

From the Division of Gastroenterology, Hepatology, and Endoscopy, Brigham & Women's Hospital, Boston, MA; and Harvard Medical School, Boston, MA.

Sources of Funding

None.

Disclosures

None.

REFERENCES

1. Sedlak TW, Snyder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. *Pediatrics*. 2004;113:1776–1782.
2. Vogel ME, Zucker SD. Bilirubin acts as an endogenous regulator of inflammation by disrupting adhesion molecule-mediated leukocyte migration. *Inflamm Cell Signal*. 2016;3:e1178.
3. Keshavan P, Deem TL, Schwemberger SJ, Babcock GF, Cook-Mills JM, Zucker SD. Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. *J Immunol*. 2005;174:3709–3718.
4. Peng F, Deng X, Yu Y, Chen X, Shen L, Zhong X, Qiu W, Jiang Y, Zhang J, Hu X. Serum bilirubin concentrations and multiple sclerosis. *J Clin Neurosci*. 2011;18:1355–1359.
5. Djoussé L, Levy D, Cupples LA, Evans JC, D'Agostino RB, Ellison RC. Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. *Am J Cardiol*. 2001;87(10):1196–1200.
6. Horsfall LJ, Nazareth I, Petersen I. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. *Circulation*. 2012;126:2556–2564.
7. Novotný L, Vítek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. *Exp Biol Med (Maywood)*. 2003;228:568–571.
8. Zucker SD, Horn PS, Sherman KE. Serum bilirubin levels in the U.S. population: gender effect and inverse correlation with colorectal cancer. *Hepatology*. 2004;40:827–835.
9. Temme EH, Zhang J, Schouten EG, Kesteloot H. Serum bilirubin and 10-year mortality risk in a Belgian population. *Cancer Causes Control*. 2001;12:887–894.
10. Schieffer KM, Bruffy SM, Rauscher R, Koltun WA, Yochum GS, Gallagher CJ. Reduced total serum bilirubin levels are associated with ulcerative colitis. *PLoS One*. 2017;12:e0179267.
11. Fretzayas A, Moustaki M, Liapi O, Karpathios T. Gilbert syndrome. *Eur J Pediatr*. 2012;171:11–15.
12. 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.
13. Horsfall LJ, Hardy R, Wong A, Kuh D, Swallow DM. Genetic variation underlying common hereditary hyperbilirubinaemia (Gilbert's syndrome) and respiratory health in the 1946 British birth cohort. *J Hepatol*. 2014;61:1344–1351.
14. de Vries HS, Te Morsche RH, Jenniskens K, Peters WH, de Jong DJ. A functional polymorphism in UGT1A1 related to hyperbilirubinemia is associated with a decreased risk for Crohn's disease. *J Crohns Colitis*. 2012;6:597–602.
15. Gammal RS, Court MH, Haidar CE, Iwuchukwu OF, Gaur AH, Alvarellos M, Guillemette C, Lennox JL, Whirl-Carrillo M, Brummel SS, et al. Clinical pharmacogenetics implementation consortium (CPIC) guideline for UGT1A1 and Atazanavir prescribing. *Clin Pharmacol Ther*. 2016;99(4):363–369.
16. Lankisch TO, Moebius U, Wehmeier M, Behrens G, Manns MP, Schmidt RE, Strassburg CP. Gilbert's disease and atazanavir: from phenotype to UDP-glucuronosyltransferase haplotype. *Hepatology*. 2006;44:1324–1332.
17. Triant VA. Cardiovascular disease and HIV infection. *Curr HIV/AIDS Rep*. 2013;10:199–206.
18. Marconi VC, Duncan MS, So-Armah K, Re VL, Lim JK, Butt AA, Goetz MB, Rodriguez-Barradas MC, Alcorn CW, Lennox J, et al. Bilirubin is inversely associated with cardiovascular disease among HIV-positive and HIV-negative individuals in VACS (Veterans Aging Cohort Study). *J Am Heart Assoc*. 2018;7(10):e007792.
19. Stein JH, Ribaudo HJ, Hodis HN, Brown TT, Tran TT, Yan M, Brodell EL, Kelesidis T, McComsey GA, Dube MP, et al. A prospective, randomized clinical trial of antiretroviral therapies on carotid wall thickness. *AIDS*. 2015;29:1775–1783.
20. Estrada V, Monge S, Gómez-Garre MD, Sobrino P, Masiá M, Berenguer J, Portilla J, Viladés C, Martínez E, Blanco JR, et al. 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.
21. Kelesidis T, Tran TT, Stein JH, Brown TT, Moser C, Ribaudo HJ, Dube MP, Murphy R, Yang OO, Currier JS, et al. Changes in inflammation and immune activation with Atazanavir-, Raltegravir-, Darunavir-based initial antiviral therapy: ACTG 5260s. *Clin Infect Dis*. 5260s;61:651–660.
22. Crane HM, Nance RM, Heckbert SR, Ritchings C, Rosenblatt L, Budoff M, Wood BR, Tirschwell DL, Kim HN, Mathews WC, et al. Association between bilirubin, Atazanavir, and cardiovascular disease events among people living with HIV across the United States. *J Acquir Immune Defic Syndr*. 2019;81:e141–e147.
23. LaFleur J, Bress AP, Rosenblatt L, Crook J, Sax PE, Myers J, Ritchings C. Cardiovascular outcomes among HIV-infected veterans receiving atazanavir. *AIDS*. 2017;31:2095–2106.
24. Ryom L, Lundgren JD, El-Sadr W, Reiss P, Kirk O, Law M, Phillips A, Weber R, Fontas E, Mocroft A. et al. Cardiovascular disease and use of contemporary protease inhibitors: the D:A: D international prospective multicohort study. *Lancet HIV*. 2018;5:e291–e300.
25. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. *Annu Rev Psychol*. 2007;58:593–614.
26. Ananth CV, Schisterman EF. Confounding, causality, and confusion: the role of intermediate variables in interpreting observational studies in obstetrics. *Am J Obstet Gynecol*. 2017;217:167–175.
27. Carmel R, Wong ET, Weiner JM, Johnson CS. Racial differences in serum total bilirubin levels in health and in disease (pernicious anemia). *JAMA*. 1985;253:3416–3418.
28. Choi AI, Vittinghoff E, Deeks SG, Weekley CC, Li Y, Shlipak MG. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. *AIDS*. 2011;25:1289–1298.
29. Nan C, Shaefer M, Urbaityte R, Oyee J, Hopking J, Ragone L, Perger T, Win B, Vangerow H, McCoig C, et al. Abacavir use and risk for myocardial infarction and cardiovascular events: pooled analysis of data from clinical trials. *Open Forum Infect Dis*. 2018;5.
30. Schwertner HA, Vítek L. Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. *Atherosclerosis*. 2008;198:1–11.
31. Lennox JL, Landovitz RJ, Ribaudo HJ. Three nonnucleoside reverse transcriptase inhibitor-sparing antiretroviral regimens for treatment-naive volunteers infected with HIV-1. *Ann Intern Med*. 2015;162:461–462.
32. Antoniou T, Szadkowski L, Walmsley S, Cooper C, Burchell AN, Bayoumi AM, Montaner JS, Loutfy M, Klein MB, Machouf N, et al. Comparison of atazanavir/ritonavir and darunavir/ritonavir based antiretroviral therapy for antiretroviral naïve patients. *BMC Infect Dis*. 2017;17:266.
33. Vogel ME, Idelman G, Konaniah ES, Zucker SD. Bilirubin prevents atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice by inhibiting endothelial VCAM-1 and ICAM-1 signaling. *J Am Heart Assoc*. 2017;6:e004820. DOI: 10.1161/JAHA.116.004820.
34. Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci U S A*. 1998;95:8170–8174.
35. 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.
36. Martinez E, Gonzalez-Cordon A, Ferrer E, Domingo P, Negredo E, Gutierrez F, Portilla J, Curran A, Podzamczer D, Murillas J, et al. Early lipid changes with atazanavir/ritonavir or darunavir/ritonavir. *HIV Med*. 2014;15:330–338.
37. Martinez E, Gonzalez-Cordon A, Ferrer E, Domingo P, Negredo E, Gutierrez F, Portilla J, Curran A, Podzamczer D, Ribera E, et al. Differential body composition effects of protease inhibitors recommended for initial treatment of HIV infection: a randomized clinical trial. *Clin Infect Dis*. 2015;60:811–820.