Cystatin C-Based Renal Function Changes After Antiretroviral Initiation: A Substudy of a Randomized Trial

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Background. The effects of antiretrovirals on cystatin C-based renal function estimates are unknown.

Methods. We analyzed changes in renal function using creatinine and cystatin C-based estimating equations in 269 patients in A5224s, a substudy of study A5202, in which treatment-naive patients were randomized to abacavir/lamivudine or tenofovir/emtricitabine with open-label atazanavir/ritonavir or efavirenz.

Results. Changes in renal function significantly improved (or declined less) with abacavir/lamivudine treatment compared with tenofovir/emtricitabine using the Cockcroft-Gault formula ($P = .016$) and 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI; $P = .030$) and 2012 CKD-EPI cystatin C-creatinine ($P = .025$). Renal function changes significantly improved (or declined less) with efavirenz compared with atazanavir/ritonavir ($P < .001$ for all equations). Mean (95% confidence interval) renal function changes specifically for tenofovir/emtricitabine combined with atazanavir/ritonavir were −8.3 (−14.0, −2.6) mL/min with Cockcroft-Gault; −14.9 (−19.7, −10.1) mL/min per 1.73^2 with Modification of Diet in Renal Disease; −12.8 (−16.5, −9.0) mL/min per 1.73^2 with 2009 CKD-EPI; +8.9 (4.2, 13.7) mL/min per 1.73^2 with 2012 CKD-EPI cystatin C; and −1.2 (−5.1, 2.6) mL/min per 1.73^2 with 2012 CKD-EPI cystatin C-creatinine. Renal function changes for the other treatment arms were more favorable but similarly varied by estimating equation.

Conclusions. Antiretroviral-associated changes in renal function vary in magnitude and direction based on the estimating equation used.

Keywords. atazanavir; creatinine; cystatin C; HIV-1; nephropathy; tenofovir.

Antiretroviral therapy (ART) may negatively affect renal function through drug toxicity mechanisms [1] or improve renal function by ameliorating the detrimental effects of untreated human immunodeficiency virus (HIV) on the kidney [2, 3]. Several studies have suggested that use of tenofovir disoproxil fumarate is associated with worse changes in estimated glomerular filtration rate (eGFR) compared with other nucleoside reverse-transcriptase inhibitors (NRTIs), and that this effect is magnified with concomitant use of protease inhibitors (PIs) [4–8]. However, not all studies have confirmed this relationship [9–11]. In the AIDS Clinical Trials Group (ACTG) 5202 trial, worse changes in eGFR (estimated as creatinine clearance using the Cockcroft-Gault equation [12]) were found with the use of tenofovir/emtricitabine compared with abacavir/lamivudine, especially when tenofovir/emtricitabine was used in combination with atazanavir/ritonavir [13]. Other observational studies have suggested that the antiretroviral PI combination atazanavir/ritonavir also negatively affects renal function [14, 15].

There is growing interest in the use of serum cystatin C as a new marker of renal function. Compared with serum

creatinine, cystatin C is not affected by muscle mass and is completely eliminated by the kidney through glomerular filtration. Perhaps because of this improved ability to measure glomerular filtration, cystatin C seems to have greater utility over creatinine in predicting adverse outcomes in both the general population [16–18] and in the HIV-infected population [19, 20]. As such, newer GFR-estimating equations have been developed using cystatin C, including the 2012 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) cystatin C equation, which uses cystatin C only without creatinine, and the 2012 CKD-EPI cystatin C-creatinine equation, which incorporates both markers [21]. In the general US population [18], identifying renal dysfunction with the 2012 CKD-EPI cystatin C-creatinine combined equation appears to be more predictive of cardiovascular disease, end stage renal disease, and overall mortality compared with the 2012 CKD-EPI cystatin C equation and the older 2009 CKD-EPI equation [22], the latter of which includes only serum creatinine. A recent study in women infected with HIV suggested that both of the newer 2012 cystatin C-based equations were more accurate than the 2009 CKD-EPI equation in identifying those patients with renal dysfunction with greater risk of mortality [20].

A recent American study using iohexol clearance as the reference measurement of GFR assessed the accuracy of these newer cystatin C-based equations in patients infected with HIV, most of whom were receiving antiretroviral medications, and found that that the 2012 CKD-EPI cystatin C-creatinine combined equation most accurately estimated GFR compared with the 2012 CKD-EPI cystatin C equation and the original 2009 CKD-EPI equation [23]. Another American study corroborated these findings by again finding that the 2012 CKD-EPI cystatin C-creatinine combined equation was more accurate than the other 2 CKD-EPI equations when compared with direct GFR measurement using iohexol clearance [24]. However, in a similar study conducted in Europe, no appreciable differences were found between the combined 2012 CKD-EPI equation and the 2009 CKD-EPI equation [25]. Of note, both of these HIV studies suggest that all 3 CKD-EPI equations were significantly more accurate than the Modification of Diet in Renal Disease (MDRD) equation [22], which is important given that much of our understanding of the effects of antiretrovirals on renal function from observational cohort studies used this latter equation [7, 26].

Therefore, we assessed changes in renal function using 5 different estimating equations in ACTG 5224s, a substudy of ACTG 5202, in which cystatin C was systematically measured, and assessed the nephrotoxicity profiles with commonly used once-daily regimens.

METHODS

Study Design and Procedures

The ACTG A5224s was a metabolic substudy of A5202 (Clinical Trials.gov NCT00118898) in which ART-naive study participants from ACTG sites in the United States and Puerto Rico aged ≥16 years and with an HIV-1 RNA level >1000 copies/mL were randomized to a blinded NRTI component, abacavir/lamivudine or tenofovir/emtricitabine, with either the open-label PI atazanavir/ritonavir or the non-NRTI (NNRTI) efavirenz. A secondary objective of A5224s was to compare the effects of initiating abacavir/lamivudine with those of tenofovir/emtricitabine on renal function after 96 weeks. A secondary renal objective was to compare the effects of atazanavir/ritonavir with efavirenz on these endpoints after 96 weeks. As previously described [27], the NRTI assignment was prematurely unblinded for patients with A5202 screening HIV-1 RNA at least 100 000 copies/mL because of higher rates of virologic failure with abacavir/lamivudine regimens.

Renal function was assessed using the following 5 estimating equations: Cockcroft-Gault, 4-variable MDRD, 2009 CKD-EPI, 2012 CKD-EPI cystatin C, and 2012 CKD-EPI cystatin C-creatinine. Serum creatinine measurements and urine analyses for dipstick protein were performed locally at the laboratory of the participating ACTG site. Serum cystatin C (Siemens N Latex kit, lower limit of detection 0.05 mg/L, run on the Siemens Nephelometer II) was measured centrally at Quest Diagnostics. Neither the serum creatinine nor the serum cystatin C levels were calibrated against an international standard. These renal parameters were measured under fasting conditions for at least 8 hours at study entry (baseline), at week 24, at week 48, and every 48 weeks afterwards through 96 weeks past the last A5202 participant enrollment.

Abbott Pharmaceuticals, Bristol-Myers Squibb, Gilead Sciences, and GlaxoSmithKline provided the study medications. The decision to publish the manuscript was solely that of the academic authors. All the authors participated in the trial design, data analysis, and preparation of the manuscript, and all the authors vouch for the completeness and accuracy of the reported data.

Study Participants

To be included in the parent A5202 trial [27], participants were required to have a screening creatinine clearance by Cockcroft-Gault >60 mL/min. The protocol initially did not exclude participants with active hepatitis B but was later amended to exclude participants with a positive hepatitis B surface antigen result within 6 months of study entry. To be included in A5224s, participants also could not have uncontrolled thyroid disease or American Diabetes Association-defined diabetes mellitus. The human subjects' ethics committee at each participating center approved the study protocol, and written informed consent was obtained from all participants in compliance with the human experimentation guidelines of the US Department of Health and Human Services and the Declaration of Helsinki.

Statistical Analysis

The prespecified secondary study objectives of A5224s were to compare renal function changes from baseline to week 96

between pooled and randomized NRTI components (abacavir/lamivudine vs tenofovir/emtricitabine with third drug combined) and between NNRTI and PI components (atazanavir/ritonavir vs efavirenz with NRTI combined). All analyses were performed using intent-to-treat principles based on randomized treatment assignment. All available data and modifications to randomized treatment were included in these analyses. For all comparisons, a factorial analysis approach was used, and, after assessing for treatment effect modification by the other component, the NRTI effect was assessed by combining efavirenz and atazanavir/ritonavir arms and vice versa. The P values below .05 (<.10 for assessing treatment effect modification) were considered statistically significant, and nominal values are reported without adjustment for multiple comparisons. Analyses were performed using SAS, version 9.2 (SAS Institute Inc., Cary, NC).

Comparisons of mean changes between regimen components used 2-sample t tests in the absence of regimen interactions and adjusted linear regression if interactions existed. Linear regression, adjusted for NRTI and NNRTI/PI components, was also used to assess the association of both baseline renal function stratum (<90 vs ≥90 mL/min for estimated creatinine clearance or <90 vs ≥90 mL/min per 1.73^2 for each of the 4 eGFR measures) and screening HIV-1 RNA level stratum (<100 000 vs ≥100 000 copies/mL) with change in renal function at week 96. To assess baseline factors independently associated with renal function change, multivariable linear regression models were constructed initially consisting of factors with univariate P values <.20 and then, using backwards selection, retained factors with a P value <.05. The prespecified baseline factors were age, sex, race/ethnicity, HIV-1 RNA, CD4 cell count, urine dipstick protein (negative vs nonnegative), systolic blood pressure, diastolic blood pressure, viral hepatitis B or C coinfection, homeostasis model assessment-insulin resistance (using fasting insulin and glucose measures) [28], weight, body mass index, and total body lean mass from dual-energy x-ray absorptiometry measurements.

The sample size estimate was based on the primary A5224s objective of changes in fat distribution [29]. Complete details of the randomization procedures are described elsewhere [13].

RESULTS

Participant Characteristics
A total of 271 participants from 37 ACTG sites enrolled in A5224s. Two participants were subsequently found to be ineligible; thus, 269 were included in the analysis population. Enrollment spanned from October 5, 2005 to November 7, 2007. The disposition of these participants during the trial has been described previously. The baseline characteristics of the randomized participants are summarized in Table 1. The baseline levels of renal function using all 5 estimating equations were well balanced by randomization amongst the 4 arms. However, the 5 renal estimating equations varied for baseline levels of renal function, with MDRD and 2012 CKD-EPI cystatin C equations resulting in the lowest renal function estimates and with Cockcroft-Gault resulting in the highest.

Changes in Renal Function Over Time
The mean (95% confidence interval [CI]) renal function estimates over time using the 5 different estimating equations are shown in Figure 1. The magnitudes and directions of the change in eGFR depended on the estimating equation used. For example, with the MDRD and the 2009 CKD-EPI equations, renal function either did not appreciably change or declined with all treatments. On the other hand, both 2012 cystatin C-based equations resulted in changes in eGFR that increased or did not appreciably change with treatment. In general, tenofovir/emtricitabine with atazanavir/ritonavir resulted in the worst (least positive or most negative) changes in eGFR of the 4 treatment arms at 96 weeks with all 5 equations.

The changes in eGFR using all 5 equations were greater (or less negative) with abacavir/lamivudine compared with tenofovir/emtricitabine and were also greater (or less negative) with efavirenz than with atazanavir/ritonavir (see Supplementary Table 1). Of note, 2 participants developed a creatinine clearance <60 mL/min using Cockcroft-Gault, and 3, 2, 1, and 0 participants, respectively, developed eGFR <60 mL/min per 1.73^2 at week 96 with MDRD, 2009 CKD-EPI, 2012 CKD-EPI cystatin C, and 2012 CKD-EPI cystatin C-creatinine.

Effects of Interactions Between Treatment Components, Initial HIV-1 RNA, and Initial Renal Function
Significant interactions were found for change in eGFR from baseline to week 96 between NRTI treatment groups and the NNRTI/PI treatment groups with Cockcroft-Gault, MDRD, and the 2009 CKD-EPI equations, but not with the two 2012 CKD-EPI equations (see Supplementary Table 1 and Figures 2 and 3). In particular, tenofovir/emtricitabine with atazanavir/ritonavir had significantly worse eGFR changes compared with tenofovir/emtricitabine with efavirenz. Significant treatment interactions between treatment group and initial HIV-1 RNA strata were only found for efavirenz vs atazanavir/ritonavir using 2009 CKD-EPI and 2012 CKD-EPI cystatin C-creatinine; in particular, atazanavir/ritonavir was associated with worse changes in eGFR compared with efavirenz within the <100 000 copies/mL stratum but not the ≥100 000 copies/mL stratum. There were no significant 3-way interactions between the NRTI treatment components, the NNRTI/PI component, and initial HIV-1 RNA strata (data not shown).

There were no significant interactions between either the NRTI components or the NNRTI/PI components and initial renal function strata using any of the 5 renal function equations. In general, combined across arms, those within the lower initial

Table 1. Baseline Characteristics of the 269 A5224s Study Participants[a,b]

| Baseline Characteristic | Efavirenz | | Atazanavir/Ritonavir | | |
	Tenofovir/ emtricitabine (n = 69)	Abacavir/ lamivudine (n = 70)	Tenofovir/ emtricitabine (n = 65)	Abacavir/ lamivudine (n = 65)	Total (n = 269)
Age, y	39 (10)	39 (10)	38 (10)	37 (10)	38 (10)
Men, n (%)	58 (84)	56 (80)	56 (86)	59 (91)	229 (85)
Race or ethnicity, n (%)					
Black, non-Hispanic	22 (32)	20 (29)	21 (32)	27 (42)	90 (33)
White, non-Hispanic	37 (54)	34 (49)	26 (40)	29 (45)	126 (47)
Hispanic	8 (12)	14 (20)	14 (22)	8 (12)	44 (16)
Other	2 (2)	2 (2)	4 (6)	1 (1)	9 (4)
Body mass index, kg/m^2	24.7 (4.0)	25.5 (4.6)	26.2 (5.4)	25.7 (4.5)	25.5 (4.7)
CD4 cell count/μL	248 (160)	231 (167)	226 (142)	238 (189)	236 (165)
HIV-1 RNA level,[b] log$_{10}$ copies/mL	4.6 (0.7)	4.6 (0.6)	4.6 (0.7)	4.7 (0.7)	4.6 (0.7)
<100 000 copies/mL, n (%)	56 (81)	59 (84)	52 (80)	48 (74)	215 (80)
≥100 000 copies/mL, n (%)	13 (19)	11 (16)	13 (20)	17 (26)	54 (20)
Hepatitis B surface antigen positive, n (%)	5 (7)	3 (4)	0 (0)	1 (2)	9 (3)
Hepatitis C antibody positive, n (%)	5 (7)	8 (11)	3 (5)	7 (11)	23 (9)
Systolic blood pressure, mm Hg	119 (15)	122 (14)	121 (10)	120 (13)	121 (13)
Diastolic blood pressure, mm Hg	75 (9)	77 (10)	75 (7)	75 (11)	76 (9)
HOMA-IR	1.2 (1.7)	1.6 (1.5)	1.5 (2.1)	1.4 (1.4)	1.4 (1.7)
Creatinine clearance using Cockcroft-Gault, mL/min	118.3(24.5)	122.2 (35.3)	124.0 (35.4)	124.0 (33.0)	122.1 (32.2)
<90 mL/min, n (%)	8 (12)	15 (22)	12 (19)	8 (13)	43 (16)
eGFR using MDRD, mL/min per 1.73^2	100.6 (18.4)	100.0 (23.6)	100.0 (25.8)	103.0 (20.3)	100.9 (22.0)
<90 mL/min per 1.73^2, n (%)	21 (31)	25 (37)	24 (39)	16 (25)	86 (33)
eGFR using 2009 CKD-EPI, mL/min per 1.73^2	107.1 (15.1)	105.7 (19.4)	106.3 (20.5)	109.3 (16.6)	107.1 (17.9)
<90 mL/min per 1.73^2, n (%)	10 (15)	17 (25)	16 (26)	9 (14)	53 (20)
eGFR using 2012 CKD-EPI CysC, mL/min per 1.73^2	101.8 (19.2)	95.0 (19.7)	99.5 (21.1)	101.4 (20.6)	99.4 (20.2)
<90 mL/min per 1.73^2, n (%)	18 (26)	27 (40)	20 (32)	17 (27)	82 (31)
eGFR using 2012 CKD-EPI CysC-Cr, mL/min per 1.73^2	104.8 (15.6)	99.7 (17.0)	102.9 (19.2)	105.3 (17.0)	103.2 (17.2)
<90 mL/min per 1.73^2, n (%)	11 (16)	17 (25)	15 (24)	13 (20)	56 (21)
Urine dipstick proteinuria,[c] n (%)	16 (24)	22 (31)	20 (33)	17 (27)	75 (29)

Abbreviations: CKD-EPI, Chronic Kidney Diseases Epidemiology Collaboration; Cr, creatinine; CysC, cystatin C; eGFR, estimated glomerular filtration rate; HIV, human immunodeficiency virus; HOMA-IR, homeostasis model assessment-insulin resistance; MDRD, Modification of Diet in Renal Diseases.

[a] All data presented as mean (standard deviation) or as number (percent). Numbers of missing data for the renal function estimates and for urine dipstick proteinuria were <10 each for the entire study population.

[b] HIV-1 RNA strata defined as either <100 000 vs ≥ 100 000 copies/mL at screening.

[c] Urine dipstick proteinuria defined as any result that was nonnegative.

renal function stratum of <90 mL/min (for creatinine clearance) or <90 mL/min per 1.73^2 (for eGFR) had significantly greater improvements (or less declines) in renal function (mean [95% CI]) compared with those in the higher initial renal function stratum using MDRD (3.0 [−0.7, 6.8] vs −8.4 [−11.0, −5.7]), 2009 CKD-EPI (2.3 [−1.4, 6.0] vs −6.3 [−8.2, −4.3]), 2012 CKD-EPI cystatin C (26.1 [22.7, 29.5] vs 6.5 [4.2, 8.8]), and 2012 CKD-EPI cystatin C-creatinine (14.1 [10.5, 17.7] vs 3.3 [1.3, 5.2]) (all P < .001); the improvements in the lower renal function stratum were nonsignificantly greater than those in the higher stratum using Cockcroft-Gault (6.3 [−0.5, 13.0] vs 1.0 [−1.9, 3.9]; P = .16).

Associations Between Baseline Factors and Changes in Renal Function At Week 96

We performed multivariable regression models adjusted for treatment components to evaluate the associations of baseline factors with renal function change at week 96 using the 3 CKD-EPI equations (Table 2). Assignment to abacavir/ lamivudine vs tenofovir/emtricitabine was variably associated with 96-week changes in renal function, whereas assignment to atazanavir/ritonavir remained independently associated with worse renal function change compared with efavirenz in these multivariable models. However, other baseline factors (including race/ethnicity, initial HIV-1 RNA level, initial presence
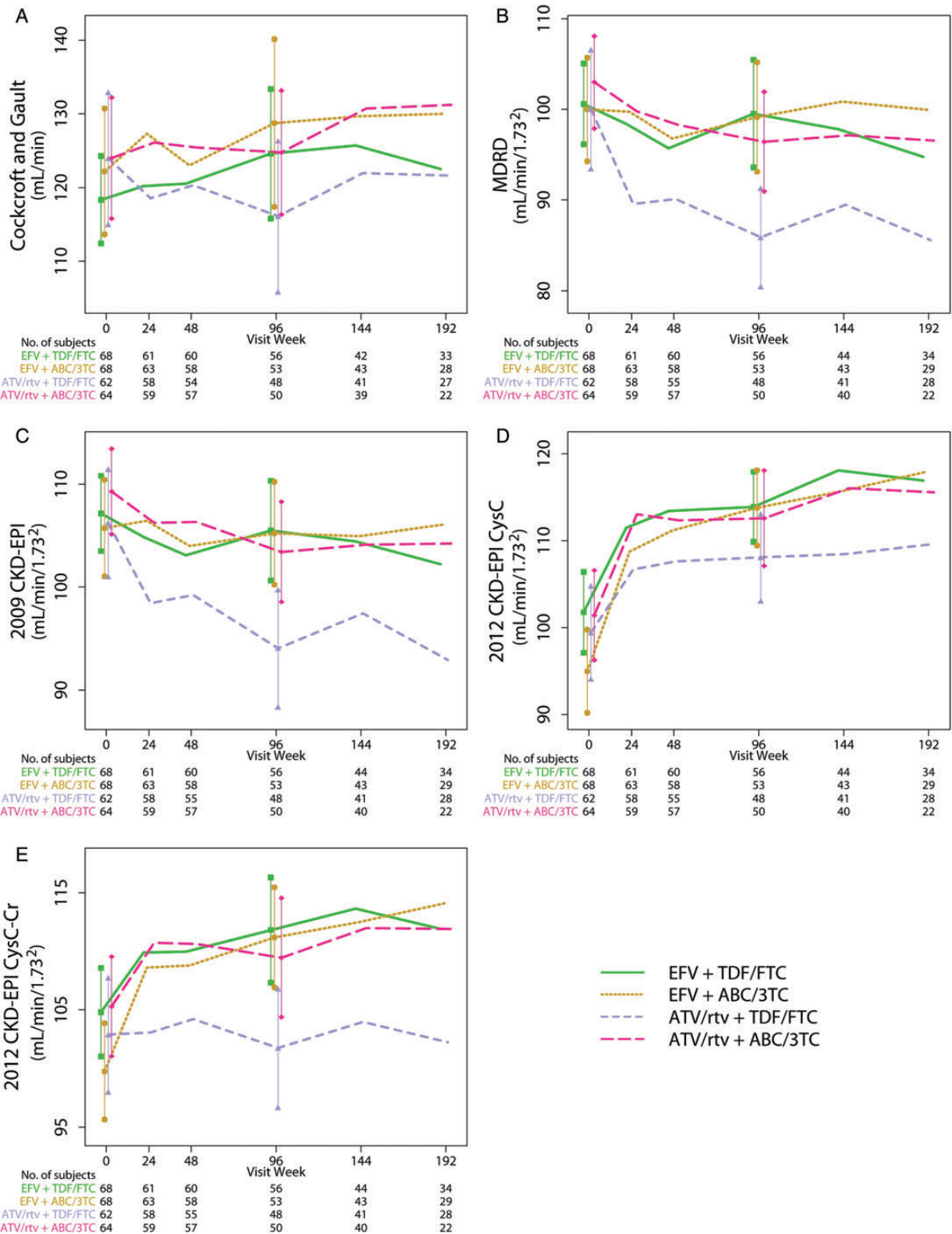
Figure 1. Renal function estimates (mean [95% confidence interval]) by treatment assignment over time using each of the 5 estimating formula. *A*, Estimated creatinine clearance using the Cockcroft-Gault equation. *B*, Estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) equation. *C*, eGFR using the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. *D*, eGFR using the 2012 CKD-EPI cystatin C (CysC) equation. *E*, eGFR using the 2012 CKD-EPI cystatin C-creatinine (CysC-Cr) equation. Abbeviations: ABC/3TC, abacavir/lamivudine; ATV/rtv, atazanavir/ritonavir; EFV, efavirenz; TDF/FTC, tenofovir/emtricitabine.
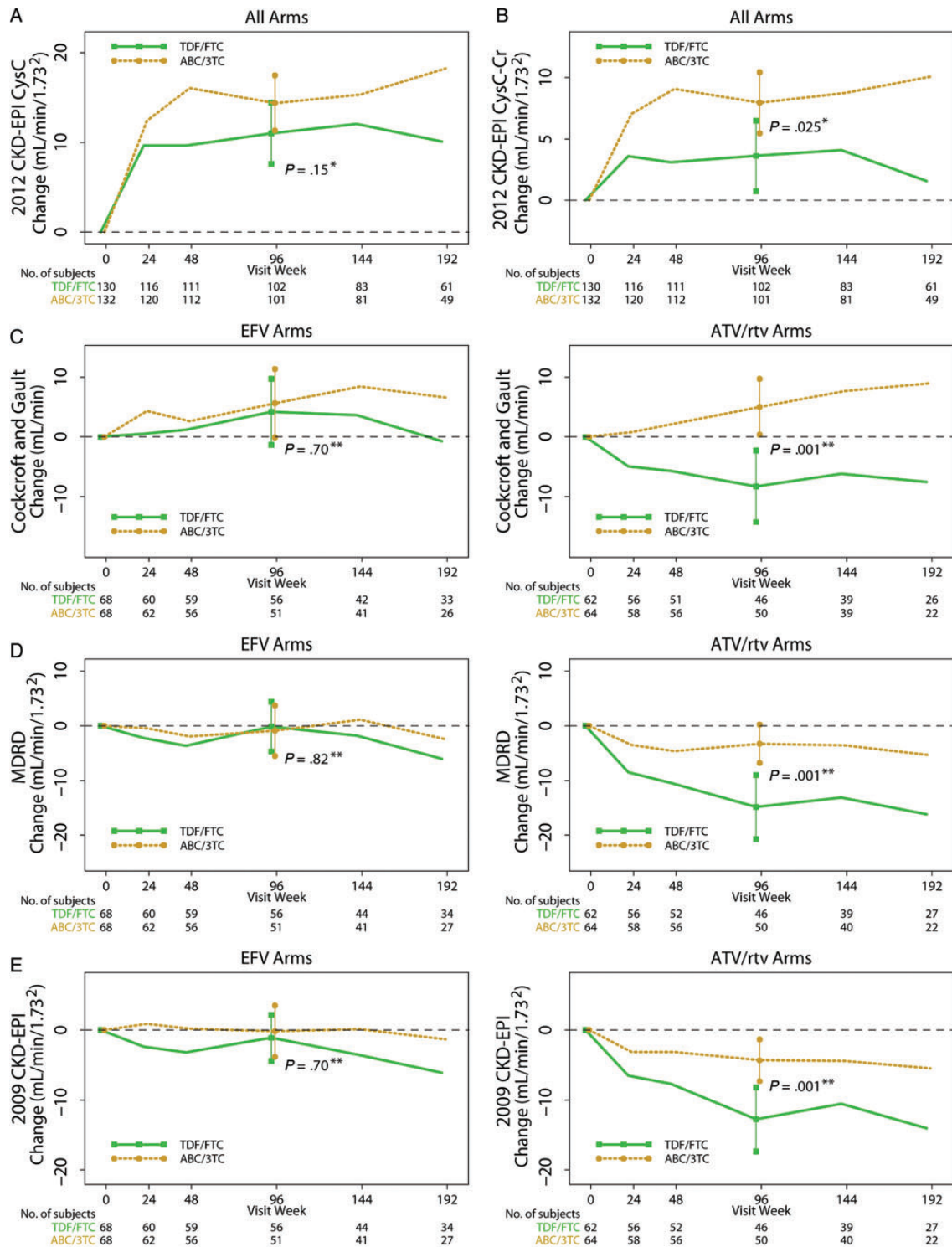
Figure 2. Changes in renal function estimates (mean [95% confidence interval]) by nucleoside reverse-transcriptase treatment component. No significant interactions were noted between nucleoside reverse-transcriptase inhibitor (NRTI) and non-NRTI (NNRTI)/protease inhibitor (PI) treatment components using the 2012 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) cystatin C equation (*A*) or the 2012 CKD-EPI cystatin C-creatinine equation (*B*). Thus, we pooled the NRTI groups in the figure panels for these 2 equations. However, because treatment interactions were noted using the Cockcroft-Gault equation (*C*), Modification of Diet in Renal Disease (MDRD) equation (*D*), and the 2009 CKD-EPI equation (*E*), the changes in renal function when the NRTI components are combined with either an NNRTI or PI are shown separately to highlight these differences. *P value for 2-sample *t* test; **P value for linear regression model adjusting for the other treatment component and the component interaction. Abbreviations: ABC/3TC, abacavir/lamivudine; ATV/rtv, atazanavir/ritonavir; Cr, creatinine; CysC, cystatin C; EFV, efavirenz; TDF/FTC, tenofovir/emtricitabine.
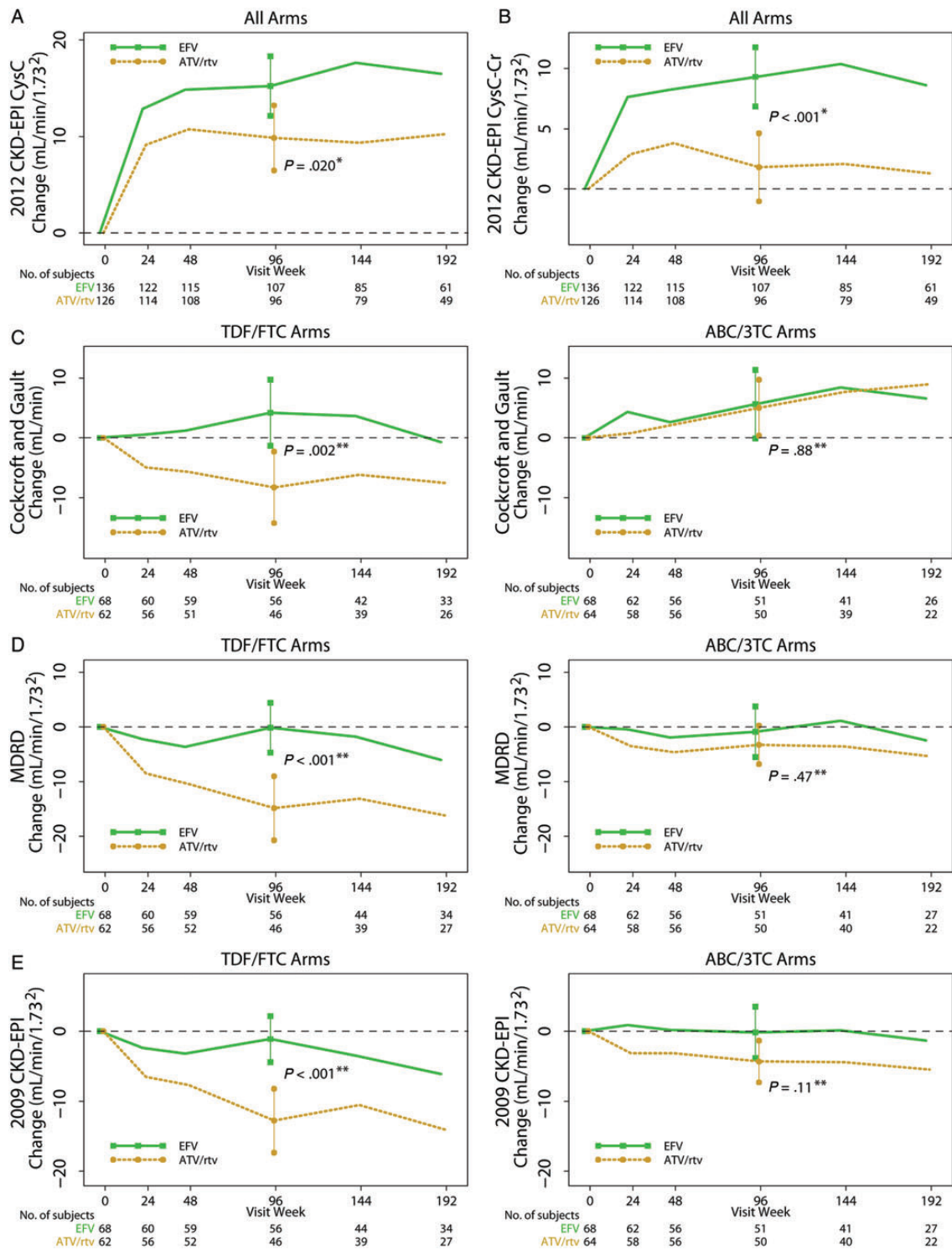
Figure 3. Changes in renal function estimates [mean (95% confidence interval)] by nonnucleoside reverse-transcriptase inhibitor (NNRTI) vs protease inhibitor (PI) component. No significant interactions were noted between NRTI and NNRTI/PI treatment components using the 2012 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) cystatin C equation (*A*) or the 2012 CKD-EPI cystatin C-creatinine equation (*B*). Thus, we pooled the NNRTI/PI groups in the figure panels for these 2 equations. However, because treatment interactions were noted using the Cockcroft-Gault equation (*C*), Modification of Diet in Renal Disease (MDRD) equation (*D*), and the 2009 CKD-EPI equation (*E*), the changes in renal function when the NNRTI/PI components are combined with each of the 2 NRTI components are shown separately to highlight these differences. *P value for 2-sample *t* test; **P value for linear regression model adjusting for the other treatment component and the component interaction. ABC/3TC, abacavir/lamivudine; ATV/rtv, atazanavir/ritonavir; Cr, creatinine; CysC, cystatin C; EFV, efavirenz; TDF/FTC, tenofovir/emtricitabine.

Table 2. Multivariable Models of the Effects of Baseline Factors on Change in Renal Function at 96 Weeks With the 3 CKD-EPI Estimating Equations[a]

Baseline Factor	2009 CKD-EPI		2012 CKD-EPI CysC		2012 CKD-EPI CysC-Cr	
	Estimated mean change (95% CI)	P value	Estimated mean change (95% CI)	P value	Estimated mean change (95% CI)	P value
ATV/rtv (vs EFV)	−11.7 (−16.7, −6.6)	<.001	−6.3 (−10.7, −1.8)	.006	−8.0 (−11.6, −4.4)	<.001
ABC/3TC (vs TDF/FTC)	0.9 (−4.0, 5.8)	.70	4.3 (−0.1, 8.7)	.058	4.9 (1.3, 8.5)	.008
ABC/3TC*ATV/rtv interaction (vs not ABC/3TC and ATV/rtv)	7.5 (0.4, 14.6)	.039				
Race/ethnicity						
Non-Hispanic Black vs non-Hispanic White			−3.4 (−8.5, 1.6)	.047		
Hispanic (any race) vs non-Hispanic White			−7.8 (−14.2, −1.4)			
HIV-1 RNA (per 1 log$_{10}$ copies/mL higher)			5.8 (2.5, 9.2)	.001	3.3 (0.6, 6.0)	.017
Negative urine dipstick protein (vs nonnegative urine dipstick protein)			−6.9 (−12.0, −1.8)	.008		
Systolic blood pressure (per 10 mmHg higher)			−2.0 (−3.7, −0.3)	.025		

Abbreviations: ABC, abacavir; ATV/rtv, atazanavir/ritonavir; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; Cr, creatinine; CysC, cystatin C; EFV, efavirenz; HIV, human immunodeficiency virus; HOMA-IR, homeostasis model assessment-insulin resistance; 3TC, lamivudine; TDF, tenofovir.

[a] Results are presented after adjustment for treatment components, and only variables with significant associations using at least 1 CKD estimating equation are shown in the table. Other variables assessed in the models but not found to be statistically significantly associated with week 96 renal function change with any CKD estimating equation include the following: age, male vs female, body mass index, HIV-1 RNA stratum (<100 000 vs ≥100 000 copies/mL), CD4 cell count, diastolic blood pressure, hepatitis B or C coinfections, HOMA-IR, and weight.

[b] All renal function data in units of mL/min per 1.73^2.

of urine dipstick proteinuria, and initial systolic blood pressure) were variably associated with week 96 renal function change depending on which CKD-EPI renal function estimating equation was used.

DISCUSSION

Because currently recommended antiretroviral regimens for initial treatment have become similarly and consistently efficacious in controlling viral replication, treatment-associated complications, including nephrotoxicities, have become increasingly important in choosing therapy. To our knowledge, this is the first study utilizing the 2012 cystatin C-based GFR estimating formulae in determining the changes in renal function with initiation of ART in patients infected with HIV. As such, we have shown that the changes in eGFR associated with initiation of these regimens, and, in turn, the interpretations of their nephrotoxicity profiles depend greatly on which renal function estimating equation is used.

Our results confirm those from other studies that have found worse changes in eGFR with the initiation of tenofovir compared with other NRTIs, especially in those receiving concomitant PIs [4, 6–8], although not all studies have found poorer renal function changes with tenofovir compared with abacavir in randomized trials [9, 11, 30]. The parent trial of this renal substudy, ACTG 5202, found a significant relative decline in estimated creatinine clearance as estimated by Cockcroft-Gault with tenofovir/emtricitabine in combination with atazanavir/ritonavir compared with its combination with efavirenz [13]. We found similar differences in the A5224s substudy when using the 2009 CKD-EPI and 2012 CKD-EPI cystatin C-creatinine equations, although we did not find significant differences when using the 2012 CKD-EPI cystatin C equation. We also noted significant interactions between the nucleoside components and the third treatment component (efavirenz vs atazanavir/ritonavir) with Cockcroft-Gault, MDRD, and 2009 CKD-EPI, but not with either of the two 2012 equations incorporating cystatin C. In particular, the 96-week mean (95% CI) renal function changes for tenofovir/emtricitabine combined with atazanavir/ritonavir widely ranged from −14.9 (−19.7, −10.1) mL/min per 1.73^2 with MDRD to +8.9 (+4.2, +13.7) mL/min per 1.73^2 with 2012 CKD-EPI cystatin C. The ranges of renal function changes within the other treatment arms were similarly varied. Thus, the choice of renal function equation may indeed influence our understanding of the relative renal safety profiles of these antiretroviral regimens.

The mechanism by which tenofovir with PIs may lead to renal toxicity has been presumed to be due to accumulation of tenofovir in renal proximal tubule cells secondary to inhibition of efflux transporters by PIs, especially in genetically

predisposed individuals [31, 32], although this has not been confirmed in all studies [33].

Atazanavir/ritonavir has also been recently linked with chronic kidney disease [14, 15], which has been speculated to be due to intrarenal crystallization of atazanavir with associated interstitial nephritis [34, 35]. The current study does indeed suggest that use of atazanavir/ritonavir is associated with worse renal function changes compared with efavirenz using any of the 5 estimating equations. As demonstrated in vitro, ritonavir may potentially increase serum creatinine concentrations via inhibition of creatinine efflux through the multidrug and toxin extrusion 1 (MATE1) transporter in the proximal tubule cell [36]. However, our data do not appear to be solely due to any potentially isolated effect of MATE1 inhibition by ritonavir. Using the 2012 CKD-EPI cystatin C equation (which does not include creatinine), eGFR increased in all groups, but there were still lower improvements in those assigned to abacavir/lamivudine with atazanavir/ritonavir compared with those assigned to abacavir/lamivudine with efavirenz (10.7 vs 18.0 mL/min per 1.73^2) and in those assigned to tenofovir/emtricitabine with atazanavir/ritonavir compared with those assigned to tenofovir/emtricitabine with efavirenz (8.9 vs 12.7 mL/min per 1.73^2) (see Supplementary Table 1). As shown in Figure 3A, we see that there remains a significant difference between eGFR changes using the 2012 CKD-EPI cystatin C equation when pooling the NRTI components. The differences in eGFR between atazanavir/ritonavir and efavirenz using an equation not incorporating serum creatinine are of similar magnitude to those when using creatinine-based equations. As such, atazanavir/ritonavir likely has an effect on eGFR independent of any possible serum creatinine increase due simply to MATE1 inhibition.

Interactions between the atazanavir/ritonavir or efavirenz components with the nucleoside treatment component were found, however, with the 3 equations incorporating only creatinine and not with those including cystatin C. In addition, significantly less beneficial changes in eGFR with atazanavir/ritonavir were found in those whose screening HIV-1 RNA levels were <100 000 copies/mL. It is possible that the potential adverse renal toxicity of atazanavir/ritonavir is unmasked in the lower viral load stratum because this group is less likely to benefit from improvements in renal function due to reduction in initial viremia, as might be expected in the higher stratum [37].

The key new finding in this study is that the apparent differences in renal function within treatment groups are highly dependent on the eGFR estimating equation used. No previous studies have used cystatin C-based estimating equations to assess the effects of antiretroviral initiation on renal function. A recent evaluation by Inker et al [23] in HIV-infected patients receiving virologically suppressive antiretroviral treatment suggested that the 2012 CKD-EPI equation incorporating both cystatin C and creatinine was marginally more accurate for estimating GFR than the 2012 CKD-EPI equation using only cystatin C and the 2009 CKD-EPI equation using only creatinine compared with iohexol clearance as the reference standard. Inker et al [21] has previously suggested that the greater accuracy of the 2012 CKD-EPI cystatin C-creatinine equation may be due to reduced variances of postulated non-GFR determinants of these 2 renal markers, such as inflammation, when used together as opposed to using each alone. Gagneux-Brunon et al [25] performed a similar study in an HIV-infected European cohort, which had appreciably different demographic characteristics than the one studied by Inker et al [21], and did not find significant differences amongst the 3 CKD-EPI equations. However, both studies demonstrated that the MDRD equation was significantly less accurate than any of the CKD-EPI equations. It should be noted that these newer equations have not been validated against direct GFR measurement methods in HIV-infected, treatment-naive patients. As such, the improvements in renal function, especially those found in the first 24 weeks (as shown in Figures 2 and 3), with the cystatin C-based equations may not only reflect true improvements in GFR but might also be influenced by reductions in inflammation and viremia or by improvements in CD4 cell counts, all of which have been variably associated with cystatin C levels [38]. In fact, the recent study by Bhasin et al [24] suggests that the 2012 CKD-EPI cystatin C equation, but not the other 2 CKD-EPI equations, was biased against true GFR measurement by greater T cell activation, higher HIV-1 RNA levels, and use of ART.

Using the 2012 CKD-EPI cystatin C-creatinine equation, we consider that treatment with tenofovir/emtricitabine with atazanavir/ritonavir has no noticeable effect on renal function at 96 weeks (mean change −1.2 [95% CI, −5.1, 2.6] mL/min per 1.73^2) and that treatment with the other once-daily regimens in this study would lead to improved renal function. On the other hand, using either the MDRD equation or the 2009 CKD-EPI equation, we conclude that there are significant declines from baseline in eGFR with tenofovir/emtricitabine combined with atazanavir/ritonavir with essentially neutral effects with the other 3 treatment arms. It is plausible to find that renal function generally improves with any ART given the known detrimental effects of untreated HIV on renal function, even in those without classic risk factors for HIV-associated nephropathy such as patients of black race [37]. Indeed, we found that the improvements in renal function were better in those with initial eGFR lower than 90 mL/min per 1.73^2, which supports the concept that untreated HIV does indeed have negative effects on renal function. Thus, the neutral changes found with tenofovir/emtricitabine using the 2012 CKD-EPI cystatin C-creatinine equation may be due to opposing effects of toxicity from this particular regimen and the benefits of virologic suppression.

Recent evidence suggests that the 2012 CKD-EPI cystatin C-creatinine equation is the most accurately available method to identify patients, including those with HIV infection, with

reduced renal function who have the highest risk of long-term adverse outcomes [18, 20]. Since our data suggest that eGFR using the 2012 CKD-EPI combined equation improves at 96 weeks with use of tenofovir/emtricitabine with efavirenz, abacavir/lamivudine with efavirenz, or abacavir/lamivudine with atazanavir/ritonavir, then long-term outcomes may improve in those receiving any of these three regimens. However, eGFR using the 2012 CKD-EPI combined equation did not change at 96 weeks with use of tenofovir/emtricitabine with atazanavir/ritonavir; this may suggest that the contribution of renal function to long-term outcomes may not be impacted (either positively or negatively) with the use of this particular regimen. However, additional research is needed to determine whether using the 2012 CKD-EPI cystatin C-creatinine equation for routine clinical monitoring of renal function in those receiving ART actually leads to changes in management that would prevent future complications in those with reduced renal function. It should be noted that, regardless of the estimating equation used, the development of eGFR <60 mL/min per 1.73^2 at 96 weeks was infrequent in this trial.

We also developed 3 different multivariable models to identify baseline factors associated with 96-week renal function changes using the 3 CKD-EPI equations. We found that the baseline factors assessed were variably associated with changes in eGFR depending on the equation used. These results again suggest that the choice of eGFR estimating equation directly impacts our understanding of which factors are responsible for changes in renal function in HIV-infected patients initiating treatment.

The limitations of this study should be acknowledged. We did not directly measure renal function to validate and identify the most accurate estimating equation for these patients initiating antiretrovirals. Although our study is one of the longest to assess changes in renal function with initiation of ART, we cannot make any conclusions regarding differences between regimens beyond 96 weeks. Our results may not be generalizable to patients with pretreatment creatinine clearance <60 mL/min or to those with diabetes, groups that were excluded from this substudy. Although we measured cystatin C centrally, we did not do so for creatinine. We also acknowledge that neither creatinine nor cystatin C were calibrated against international standards, which may lead to analytical drift of the measurements [39]. However, because we assessed changes in renal function, the potential variability of these results due to the lack of either centralized or standardized measurement is somewhat minimized. Another limitation was the lack of blinding for the efavirenz and atazanavir/ritonavir treatment components, although the nucleoside treatment components were blinded. Finally, these analyses were performed without adjustment for multiple comparisons, thereby increasing the possibility of type I errors for falsely detecting differences.

In summary, we found that initiation of ART with tenofovir/emtricitabine and atazanavir/ritonavir led to less beneficial changes in eGFR at 96 weeks compared with abacavir/lamivudine and efavirenz, respectively. However, the magnitudes, directions, and statistical significances of these changes in renal function varied with the estimating equation used. If the newer cystatin C-based equations are indeed confirmed to be more clinically useful, then the renal profiles of antiretroviral regimens should be reinterpreted and, as such, would have important implications for HIV clinical care.

Supplementary Data

Notes

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