

# In HIV/hepatitis C virus co-infected patients, higher 25-hydroxyvitamin D concentrations were not related to hepatitis C virus treatment responses but were associated with ritonavir use<sup>1–4</sup>

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## ABSTRACT

**Background:** Among patients with hepatitis C virus (HCV) mono-infection, 25-hydroxyvitamin D [25(OH)D] concentrations are positively associated with a response to peg-interferon/ribavirin. Data on the relation between 25(OH)D concentrations and HCV treatment response in HIV-infected patients are limited.

**Objective:** The objective was to determine whether baseline 25(OH)D concentrations predict virologic response in HIV/HCV co-infected patients and to examine variables associated with 25(OH)D concentrations  $\geq 30$  ng/mL.

**Design:** Data and samples from 144 HCV genotype 1, treatment-naive patients from a completed HCV treatment trial were examined in this retrospective study. Early virologic response (EVR) was defined as  $\geq 2$  log<sub>10</sub> reduction in HCV RNA and/or HCV RNA  $< 600$  IU/mL at week 12 of peg-interferon/ribavirin treatment. Baseline 25(OH)D was measured by liquid chromatography/tandem mass spectrometry.

**Results:** Compared with the non-EVR control group ( $n = 68$ ), the EVR group ( $n = 76$ ) was younger, had fewer cirrhotic subjects, had a higher proportion with the *IL28B* CC genotype, had a higher albumin concentration, and had a lower HCV viral load at baseline ( $P \leq 0.05$ ). The difference in baseline 25(OH)D concentrations between EVR and non-EVR patients was not statistically significant (median: 25 ng/mL compared with 20 ng/mL;  $P = 0.23$ ). Similar results were found for sustained virologic response (SVR). In multivariable analysis, white and Hispanic race-ethnicity (OR: 6.26; 95% CI: 2.47, 15.88;  $P = 0.0001$ ) and ritonavir use (OR: 2.68; 95% CI: 1.08, 6.65;  $P = 0.033$ ) were associated with higher 25(OH)D concentrations ( $\geq 30$  ng/mL).

**Conclusion:** Baseline 25(OH)D concentrations did not predict EVR or SVR. Because ritonavir impairs the conversion of 25(OH)D to the active metabolite, utilization of 25(OH)D may have been impaired in subjects taking ritonavir. This trial was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT00078403. *Am J Clin Nutr* 2013;98:423–9.

## INTRODUCTION

Hepatitis C virus (HCV)<sup>5</sup> co-infection occurs in about one-third of subjects infected with HIV in the United States and Europe (1, 2). Liver disease is now a leading cause of mortality in HIV-infected patients, and chronic HCV infection is the most common etiology (3–5). HIV/HCV co-infected patients who achieve a sustained virologic response (SVR) to HCV treatment have increased survival (6) and a reduced risk of subsequent antiretroviral-related toxicities (7); however, responses to the

current standard of care—dual therapy with pegylated interferon (PEG) and ribavirin—are generally poor (8–10).

Several studies have examined viral and host factors predicting HCV virologic response (11–20). Recently, vitamin D status was proposed as a predictor of HCV treatment outcome. The hypothesis that vitamin D might improve treatment responses is based on evidence that vitamin D enhances both innate and adaptive immune responses, reduces inflammation, and retards fibrogenesis (21–27). In HCV mono-infected patients, vitamin D deficiency has been associated with poor treatment response and with more advanced liver fibrosis (28–33), although contrary findings have also been reported (34). Vitamin D supplements have been reported to improve treatment outcomes in HCV mono-infected individuals (35–37). In a study of HIV/HCV co-infected patients carried out in France (38), lower 25-hydroxyvitamin D [25(OH)D] concentrations were associated with more advanced liver fibrosis, but not with HCV treatment failure. In contrast, a study carried out in Austria showed a positive relation between 25(OH)D concentrations and response to HCV treatment (39). To our knowledge, the relation between 25(OH)D concentrations and treatment response has not been examined in HIV/HCV patients in the United States, which has a racially and ethnically diverse population.

Apart from its possible effect on treatment outcome and liver disease progression, the vitamin D status of HIV/HCV-positive

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<sup>2</sup> The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

<sup>3</sup> Supported by the NIH (U01AI068636, U01AI068634, DA031095, DK090317, and K24 AI78884).

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<sup>5</sup> Abbreviations used: ACTG, AIDS Clinical Trials Group; EVR, early virologic response; GFR, glomerular filtration rate; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; PEG, pegylated interferon; SVR, sustained virologic response; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

Received August 9, 2012. Accepted for publication April 22, 2013.

First published online June 5, 2013; doi: 10.3945/ajcn.112.048785.

patients is important because vitamin D promotes the absorption of dietary calcium and strengthens bone. HIV/HCV co-infected patients often have low bone density (40–42) and are exposed to antiretroviral drugs that disturb vitamin D and calcium metabolism: efavirenz reduces 25(OH)D concentrations (43), ribavirin reduces serum calcium concentrations (44), ritonavir impairs bioactivation of vitamin D (45), and tenofovir causes elevations in parathyroid hormone that are especially pronounced in patients with 25(OH)D concentrations <30 ng/mL (46–49).

The most widely used clinical indicator of vitamin D status is 25(OH)D. This molecule is the precursor of the active metabolite 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D], which is a steroid hormone. The optimal concentration of 25(OH)D for an individual is difficult to establish. The Institute of Medicine determined that 20 ng/mL is adequate for 97.5% of healthy adults (50); however, HIV practice guidelines recommend concentrations >30 ng/mL (51).

In this study, we examined the relation between baseline 25(OH)D concentrations and response to PEG/ribavirin treatment in HCV treatment-naïve patients with genotype 1 HCV from the completed AIDS Clinical Trials Group (ACTG) A5178 study (52). We also identified factors associated with 25(OH)D concentrations  $\geq$ 30 ng/mL in this study group.

## SUBJECTS AND METHODS

### Subjects

The design of A5178 was previously described (52). Briefly, it was a multicenter prospective trial conducted by the ACTG to elucidate the role of maintenance PEG therapy in HCV treatment nonresponders with HIV co-infection (52). The trial enrolled 330 subjects from August 2004 to April 2007 from 36 ACTG sites within the United States. In Step 1, all subjects were treated with pegylated-interferon- $\alpha$  2a 180  $\mu$ g/wk plus weight-based ribavirin for 12 wk to distinguish treatment responders from nonresponders by early virologic response (EVR), defined as a  $\geq$ 2 log<sub>10</sub> decrease from baseline or undetectable HCV RNA (<600 IU/mL) at 12 wk. Patients with an EVR received a total of 72 wk of PEG/ribavirin therapy, whereas patients without an EVR were followed in a maintenance trial. All A5178 subjects provided written informed consent, and 87% provided consent for genetic testing through a separate protocol. The institutional review boards of all sites participating in the parent study approved the study protocol. Guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of this research. The current study was a retrospective analysis of 144 subjects with genotype 1 HCV who were treatment-naïve and who consented to *IL28B* genotyping as part of a separate study (19). The study group included 47 non-Hispanic whites, 76 non-Hispanic blacks, and 21 Hispanics (19 of whom were white and 2 of whom were black). In several analyses whites were grouped with Hispanics, in keeping with the methods used in the parent study. Only one Asian was eligible for the study, and this person was excluded to reduce heterogeneity.

### Vitamin D concentrations

The 25(OH)D concentration was measured centrally in baseline serum samples by HPLC tandem mass spectrometry (Quest

Diagnostics). The interassay CV across the analytic range of the assay is 7%. Serum samples were pristine and stored at  $-70^{\circ}\text{C}$  at local sites until shipment on dry ice to the central repository and ultimately the central laboratory. 25(OH)D was analyzed as a continuous variable and as a dichotomous variable by using a cutoff of  $\geq$ 30 ng/mL (51).

### Viral load tests and treatment outcomes

HCV RNA was tested by using the Roche Cobas Amplicor assay with a lower detection limit of 600 IU/mL for the quantitative assay (during the first 12 wk) and 60 IU/mL for the qualitative assay (to assess SVR). HIV RNA was tested by using Roche Ultrasensitive HIV reverse transcriptase–polymerase chain reaction with a lower limit of quantification of 50 copies/mL. EVR was defined as a  $\geq$ 2 log<sub>10</sub> reduction in HCV RNA and/or HCV RNA <600 IU/mL at week 12 by using the quantitative assay. SVR was defined as undetectable HCV RNA at 24 wk after treatment cessation.

### Covariates

The following covariates were of interest to characterize the study population: sex, age at enrollment, race-ethnicity (non-Hispanic blacks vs. white and Hispanics), BMI, current or prior intravenous drug use, cirrhosis, season when baseline sample was collected, region of site enrollment in the United States [north, >40°N/south, <40°N], and baseline clinical laboratory values, including estimated glomerular filtration rate (GFR), platelet count, alkaline phosphatase, albumin, HOMA-IR, CD4 cell count, HIV-1 RNA concentration, HCV viral load, CD4 nadir, *IL28B* genotype, use of highly active antiretroviral therapy (HAART; defined as a combination of  $\geq$ 3 antiretroviral drugs, one of which could be ritonavir as a pharmacokinetic booster). GFR was estimated by using the Chronic Kidney Disease Epidemiology Collaboration equation (53). HOMA-IR was calculated by using the subject's fasting glucose and fasting insulin concentrations:  $\text{HOMA-IR} = (\text{fasting glucose} \times \text{fasting insulin})/405$ .

### Statistical analysis

Wilcoxon's rank-sum test was used to test differences in continuous measures between 2 groups, including comparison of 25(OH)D concentrations between EVRs and non-EVRs, and Van Elteren's test was used for stratified rank-based comparisons. Fisher's exact tests were used for comparisons of binary and categorized variables between 2 groups, except for *IL28B*, for which the Cochran-Armitage trend test was used. Logistic regression models were developed to assess factors associated with 25(OH)D. The following factors were considered: sex, age, race, ethnicity, BMI, intravenous drug use, cirrhosis, region, season of sample collection, use of HAART, use of specific antiretroviral drugs (efavirenz, ritonavir, and tenofovir), platelet counts, GFR, baseline CD4<sup>+</sup>T cell count, HIV viral load, and *IL28B* genotype. The analyses were done by using SAS (version 9; SAS Institute). A statistical significance level of 0.05, 2-sided, was used. Because of the exploratory nature of the analysis, no adjustment for multiple testing was made.

## RESULTS

### Comparison of EVR and non-EVR groups

The 144 study subjects were predominantly male and non-Hispanic black and had a median age of 48 y (**Table 1**). Most subjects (79.2%) were receiving HAART, and 38.2% were taking ritonavir, which was used only as a pharmacologic booster. The median 25(OH)D concentration was 22.5 ng/mL [quartile 1 = 14.0; quartile 3 = 29.0]. Slightly more than half of the subjects met the criteria for EVR. Compared with the non-EVR group ( $n = 68$ ), the EVR group ( $n = 76$ ) was younger, had a higher percentage of non-Hispanic whites, had a lower percentage with liver cirrhosis, had a higher albumin concentration, had a lower HCV viral load, and had a higher percentage with the favorable *IL28B* CC genotype (Table 1).

The difference in baseline 25(OH)D concentrations between EVR and non-EVR patients was not statistically significant ( $P = 0.23$ ): median of 25.0 ng/mL (quartile 1 = 13.0; quartile 3 = 30.5) compared with 20.0 ng/mL (quartile 1 = 14.0; quartile 3 = 27.5), respectively. The trends were similar between blacks (non-Hispanic) and non-blacks (whites and Hispanics). Median 25(OH)D concentrations were 19.0 and 17.0 ng/mL among EVR and non-EVR blacks, respectively, and 28.0 and 26.0 ng/mL among EVR and non-EVR non-blacks. Hence, adjustment for race-ethnicity in the analysis did not change the conclusion ( $P = 0.78$ ). An analysis that adjusted for ritonavir use also did not change the conclusion ( $P = 0.29$ ). The 25(OH)D concentrations did not differ significantly ( $P = 0.51$ ) between SVR ( $n = 43$ ) and non-SVR ( $n = 101$ ) patients: median of 26.0 ng/mL (quartile 1 = 12.0; quartile 3 = 31.0) compared with 21.0 ng/mL (quartile 1 = 14.0; quartile 3 = 28.0), respectively. To examine the relation between 25(OH)D concentrations and treatment outcome further, additional analyses were performed in which 25(OH)D was analyzed as a dichotomized variable. None showed a statistically significant relation between 25(OH)D measurements and EVR or SVR.

### Variables associated with 25(OH)D concentrations $\geq 30$ ng/mL

Three of 18 variables differed significantly between the group with 25(OH)D concentrations  $\geq 30$  ng/mL ( $n = 35$ ) and the group with lower values ( $n = 109$ ) (**Table 2**). The group with higher 25(OH)D measurements had a lower percentage of non-Hispanics blacks (22.9% compared with 62.4%), more HAART users (94.3% compared with 74.3%), and, specifically, more ritonavir users (57.1% compared with 32.1%). These 3 variables were included in a multivariable logistic regression analysis (**Table 3**). In the multivariable analysis, a 25(OH)D concentration  $\geq 30$  ng/mL was positively associated with white and Hispanic race-ethnicity and ritonavir exposure.

## DISCUSSION

We found no significant association between the baseline serum 25(OH)D concentration and EVR or SVR in this clinical trial of PEG/ribavirin treatment of HIV/HCV co-infected subjects. The median value among EVRs was 25.0 ng/mL compared with 20.0 ng/mL among non-EVRs, and this difference was not statistically significant. The lack of association between serum 25(OH)D concentration and HCV virologic response was not

confounded by race or any other measured covariate in this analysis. One interpretation of these results was that vitamin D status is unrelated to treatment response in HIV/HCV co-infected patients; however, there were several caveats.

The study had a relatively small sample size of 144 patients. Our study also had the limitation of being a retrospective study from a completed trial. In addition, vitamin D status was determined by a single baseline measurement of 25(OH)D. In the future, longitudinal measurements and/or genetic analysis of polymorphisms that affect 25(OH)D concentrations might provide a clearer understanding of the relation between 25(OH)D concentrations and treatment responses.

A further consideration is that 25(OH)D is only a surrogate marker of vitamin D status. Using skeletal health as the outcome, the Institute of Medicine determined that 50% of the adult population is vitamin D replete at a 25(OH)D concentration of 16 ng/mL, whereas the other 50% require up to 20 ng/mL, or more, to achieve optimal vitamin D status. If these estimates are correct, they indicate that almost 50% of the population changes from a state of vitamin D repletion to one of vitamin D deficiency as 25(OH)D concentrations fall by 4 ng/mL. As a result, 25(OH)D concentrations in the range 16–20 ng/mL are expected to have limited ability to predict vitamin D status. In future studies, measurements of additional factors that indicate vitamin D status, such as parathyroid hormone, calcium, phosphate, and markers of bone turnover, might allow patients with and without vitamin D deficiency to be distinguished more accurately than is possible when 25(OH)D is used as the sole determinant.

The need to use a combination of factors to establish vitamin D status is especially great in patients receiving HAART because antiviral drugs cause complex alterations in vitamin D and calcium metabolism. This is illustrated by published data demonstrating that parathyroid hormone elevations occur in tenofovir users who have 25(OH)D concentrations  $< 30$  ng/mL (46–49). In the current study, a different type of alteration was observed in ritonavir users. Ritonavir exposure was associated with higher 25(OH)D concentrations. A recent study of 672 HIV-positive patients found this same association between higher 25(OH)D concentrations and ritonavir exposure (54). Ritonavir blocks the activity of the  $1\alpha$  hydroxylase that converts 25(OH)D into its biologically active form,  $1,25(\text{OH})_2\text{D}$  (45). Because the physiologic effects of vitamin D require adequate concentrations of  $1,25(\text{OH})_2\text{D}$ , we propose that ritonavir (and possibly other drugs) distorts the relation between 25(OH)D measurements and vitamin D status by blocking the conversion of 25(OH)D to  $1,25(\text{OH})_2\text{D}$ . More information about the effect of antiviral drugs on vitamin D and calcium metabolism could improve the design of future studies of the relation between vitamin D status and treatment outcome in HIV/HCV co-infected patients.

Our results add to the growing literature on 25(OH)D concentrations and virologic responses to HCV therapy (28–33, 35–37–39). Previously, an investigation in HIV/HCV co-infected patients conducted in Austria reported a positive relation between 25(OH)D concentrations and SVR (39), whereas a study conducted in France found no association (38), in keeping with our results, which were obtained in a US study group that was 52.8% black and 14.6% Hispanic. The inconsistent results across studies suggest that the effect of baseline 25(OH)D on treatment outcomes may be modest, if it exists. However, given the very high costs and toxicity of

**TABLE 1**  
Baseline characteristics by EVR status<sup>1</sup>

	EVR status			P value
	Total (n = 144)	EVR (n = 76)	Non-EVR (n = 68)	
Sex, male [n (%)]	119 (82.6)	66 (86.8)	53 (77.9)	0.19 <sup>2</sup>
Age (y)				0.006 <sup>3</sup>
Median	48	47	49	
Q1, Q3	41.5, 52.0	40, 50	44, 53	
Race-ethnicity [n (%)]				0.014 <sup>2</sup>
White, non-Hispanic	47 (32.6)	33 (43.4)	14 (20.6)	
Black, non-Hispanic	76 (52.8)	33 (43.4)	43 (63.2)	
Hispanic	21 (14.6)	10 (13.2)	11 (16.2)	
BMI (kg/m <sup>2</sup> )				0.77 <sup>3</sup>
Median	25.7	25.5	25.9	
Q1, Q3	22.8, 29.3	23.0, 28.5	22.7, 29.6	
IV drug use history [n (%)]	91 (63.2)	44 (57.9)	47 (69.1)	0.17 <sup>2</sup>
Cirrhosis [n (%)]	18 (12.5)	5 (6.6)	13 (19.1)	0.041 <sup>2</sup>
Platelet count (10 <sup>3</sup> /mm <sup>3</sup> )				0.14 <sup>3</sup>
Median	206	209	198	
Q1, Q3	159, 250	172, 257	155, 236	
Alkaline phosphatase (U/L) <sup>4</sup>				0.82 <sup>3</sup>
Median	94	92	96	
Q1, Q3	78, 120	74, 124	79, 114	
Albumin (g/dL) <sup>4</sup>				0.003 <sup>3</sup>
Median	4.15	4.30	4	
Q1, Q3	3.90, 4.40	4.00, 4.50	3.80, 4.30	
GFR (mL · min <sup>-1</sup> · 1.73 m <sup>-2</sup> ) <sup>5</sup>				0.82 <sup>3</sup>
Median	101.5	99.8	104.4	
Q1, Q3	84.3, 113.5	87.1, 111.8	81.7, 113.8	
HOMA-IR (mg/dL · μU · mL <sup>-1</sup> · 405 <sup>-1</sup> ) <sup>6</sup>				0.44 <sup>2</sup>
Median	3.15	3.37	2.76	
Q1, Q3	1.76, 5.10	1.88, 5.51	1.59, 4.65	
Baseline CD4 (cells/mm <sup>3</sup> )				0.97 <sup>3</sup>
Median	507	507	504	
Q1, Q3	380, 703	384, 645	356, 719	
Nadir CD4 (cells/mm <sup>3</sup> )				0.12 <sup>3</sup>
Median	247	205	258	
Q1, Q3				
Baseline HIV-1 RNA [n (%)]				1.00 <sup>2</sup>
Undetectable, <50 copies/mL	101 (70.1)	53 (69.7)	48 (70.6)	
Baseline log <sub>10</sub> HCV RNA (IU/mL)				0.043 <sup>2</sup>
Median	6.54	6.50	6.60	
Q1, Q3	6.15, 6.88	5.88, 6.87	6.40, 6.89	
IL28B genotype [n (%)] <sup>7</sup>				0.024 <sup>8</sup>
TT	23 (21.5)	7 (12.5)	16 (31.4)	
CT	49 (45.8)	27 (48.2)	22 (43.1)	
CC	35 (32.7)	22 (39.3)	13 (25.5)	
Season, summer [n (%)] <sup>9</sup>	73 (50.7)	37 (48.7)	36 (52.9)	0.62 <sup>2</sup>
Antiretroviral medication use [n (%)]				
HAART	114 (79.2)	62 (81.6)	52 (76.5)	0.54 <sup>2</sup>
Ritonavir	55 (38.2)	31 (40.8)	24 (35.3)	0.61 <sup>2</sup>
25(OH)D (ng/mL)				0.23 <sup>3</sup>
Median	22.5	25	20	
Q1, Q3	14.0, 29.0	13.0, 30.5	14.0, 27.5	

<sup>1</sup> CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; EVR, early virologic response; GFR, glomerular filtration rate; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; IV, intravenous; Q1, first quartile (25th percentile); Q3, third quartile (75th percentile); 25(OH)D, 25-hydroxyvitamin D.

<sup>2</sup> Fisher's exact test.

<sup>3</sup> Wilcoxon's test.

<sup>4</sup> Total n = 142 (n = 75 for EVR and n = 67 for non-EVR).

<sup>5</sup> CKD-EPI equation.

<sup>6</sup> Total n = 70 (n = 37 for EVR and 33 for non-EVR).

<sup>7</sup> Total n = 107 (n = 56 for EVR and n = 51 for non-EVR).

<sup>8</sup> Exact Cochran-Armitage trend test.

<sup>9</sup> June–November.

**TABLE 2**  
Baseline characteristics by 25(OH)D concentrations<sup>1</sup>

	Dichotomized 25(OH)D <sup>2</sup>			P value
	Total (n = 144)	<30 ng/mL (n = 109)	≥30 ng/mL (n = 35)	
Sex, male [n (%)]	119 (82.6)	89 (81.7)	30 (85.7)	0.80 <sup>3</sup>
Age (y)				
Median	48	48	47	0.92 <sup>4</sup>
Q1, Q3	41.50, 52.00	32, 66	35, 59	
Race [n (%)]				
Black non-Hispanic	76 (52.8)	68 (62.4)	8 (22.9)	<0.001 <sup>3</sup>
Hispanic, regardless of race	21 (14.6)	14 (12.8)	7 (20.0)	0.29 <sup>3</sup>
BMI (kg/m <sup>2</sup> )				
Median	25.74	25.83	25.50	0.73 <sup>4</sup>
Q1, Q3	22.84, 29.33	23.05, 29.03	22.64, 30.20	
IV drug use history [n (%)]	91 (63.2)	71 (65.1)	20 (57.1)	0.42 <sup>3</sup>
Cirrhosis [n (%)]	18 (12.5)	14 (12.8)	4 (11.4)	1.00 <sup>3</sup>
Region, north [n (%)]	60 (41.7)	49 (45.0)	11 (31.4)	0.17 <sup>3</sup>
Season, summer <sup>5</sup>	73 (50.7)	52 (47.7)	21 (60.0)	0.25 <sup>3</sup>
Antiretroviral medication use [n (%)]				
Efavirenz	39 (27.1)	32 (29.4)	7 (20.0)	0.38 <sup>3</sup>
Ritonavir	55 (38.2)	35 (32.1)	20 (57.1)	0.010 <sup>3</sup>
Tenofovir	32 (22.2)	23 (21.1)	9 (25.7)	0.64 <sup>3</sup>
HAART	114 (79.2)	81 (74.3)	33 (94.3)	0.015 <sup>3</sup>
Platelet count (10 <sup>3</sup> /mm <sup>3</sup> )				
Median	206.5	206	219	0.25 <sup>4</sup>
Q1, Q3	159, 250	156, 237	166, 265	
GFR (mL · min <sup>-1</sup> · 1.73 m <sup>-2</sup> ) <sup>6</sup>				
Median	101.53	100.29	102.97	0.79 <sup>4</sup>
Q1, Q3	84.27, 113.50	89.38, 112.53	79.89, 113.68	
Baseline CD4 count (cells/mm <sup>3</sup> )				
Median	507	495	549	0.38 <sup>4</sup>
Q1, Q3	380, 703	384, 645	356, 719	
Baseline HIV-1 RNA (copies/mL)				
Undetectable (<50)	101 (70.1)	77 (70.6)	24 (68.6)	0.83 <sup>3</sup>
<i>IL28B</i> genotype [n (%)] <sup>7</sup>				
TT	23 (21.5)	18(22.8)	5 (17.9)	0.65 <sup>8</sup>
CT	49 (45.8)	36 (45.6)	13 (46.4)	
CC	35 (32.7)	25 (31.6)	10 (35.7)	

<sup>1</sup> CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GFR, glomerular filtration rate; HAART, highly active antiretroviral therapy; IV, intravenous; Q1, first quartile (25th percentile); Q3, third quartile (75th percentile); 25(OH)D, 25-hydroxyvitamin D.

<sup>2</sup> Cutoff = 30 ng/mL.

<sup>3</sup> Fisher's exact test.

<sup>4</sup> Wilcoxon's test.

<sup>5</sup> June–November.

<sup>6</sup> CKD-EPI equation.

<sup>7</sup> Total n = 107; n = 79 for 25(OH)D <30 ng/mL and n = 28 for 25(OH)D ≥30 ng/mL.

<sup>8</sup> Exact Cochran-Armitage trend test.

HCV treatment, even a modest beneficial effect could be clinically and economically significant if it can be obtained through the use of vitamin D supplements. With further investigation, it may be possible to identify the characteristics of patients most likely to benefit from higher 25(OH)D concentrations and from adjunctive treatment with vitamin D supplements before and during HCV treatment; moreover, given the high prevalence of bone disease in HCV-infected patients (42, 55–59), HIV-infected patients (60), and HIV/HCV co-infected patients (40, 41), it may be prudent to consider vitamin D supplements for all patients with 25(OH)D concentrations <20–30 ng/mL, regardless of the anticipated effect on HCV treatment response.

In summary, this study of 144 HIV/HCV co-infected patients did not show a significant relation between baseline 25(OH)D concentrations and EVR or SVR. Ritonavir exposure and white

**TABLE 3**  
Factors associated with 25(OH)D ≥30 ng/mL in multivariate analysis<sup>1</sup>

	OR	95% CI	P value
White and Hispanic race-ethnicity	6.26	2.47, 15.88	0.0001
HAART use	3.23	0.65, 16.11	0.15
Ritonavir use	2.68	1.08, 6.65	0.033

<sup>1</sup> Total n = 144; n = 109 for 25(OH)D <30 ng/mL and n = 35 for 25(OH)D ≥30 ng/mL. HAART, highly active antiretroviral therapy; 25(OH)D, 25-hydroxyvitamin D.

race were associated with 25(OH)D concentrations  $\geq 30$  ng/mL. Future studies are needed to identify methods for accurately determining the physiologic vitamin D status of patients receiving antiviral therapy for HIV and to clarify the effect of the new direct-acting antiviral drugs for HCV on vitamin D and calcium metabolism.

We thank the ACTG A5178 study team, the study subjects, and the many individuals involved in the conduct and/or care of patients enrolled in this study at each of the participating institutions.

The authors' responsibilities were as follows—ADB, MJG, RTC, and MK: designed the research; MK and KH: performed the statistical analysis; ADB: wrote the first draft of the manuscript; CMW: directed the interpretation of GFR data; ADB and MJG: had primary responsibility for the final content; and all authors: edited and approved the final version. The authors declared that no competing interests existed.

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