Title
Vitamin D status and immune function reconstitution in HIV-infected men initiating therapy

Journal
AIDS, 32(8)

ISSN
0269-9370

Authors
Abraham, Alison G
Zhang, Long
Calkins, Keri
et al.

Publication Date
2018-05-15

DOI
10.1097/qad.0000000000001782

Peer reviewed

HHS Public Access

Author manuscript

AIDS. Author manuscript; available in PMC 2019 May 15.

Published in final edited form as:

AIDS. 2018 May 15; 32(8): 1069–1076. doi:10.1097/QAD.0000000000001782.

Vitamin D status and immune function reconstitution in HIV-infected men initiating therapy in the Multicenter AIDS Cohort Study

Alison G Abraham, PhD[1,8], **Long Zhang**[1], **Keri Calkins**[1], **Adrienne Tin**[1], **Andrew Hoofnagle**[2], **Frank J. Palella Jr.**[3], **Michelle M. Estrella**[4], **Lisa P Jacobson**[1], **Mallory D. Witt**[5], **Lawrence A Kingsley**[6], and **Todd T. Brown**[7]

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

[2]Department of Laboratory Medicine, University of Washington

[3]Northwestern University Feinberg School of Medicine

[4]Kidney Health Research Collaborative at University of California, San Francisco and San Francisco VA Medical Center

[5]Los Angeles Biomedical Research Institute at Harbor-UCLA & David Geffen School of Medicine at University of California, Los Angeles

[6]Infectious Diseases and Microbiology Department, University of Pittsburgh

[7]Department of Medicine, Johns Hopkins School of Medicine

[8]Department of Ophthalmology, Johns Hopkins School of Medicine

Abstract

Objective—Despite effective antiretroviral therapy (HAART) and durable viral suppression, many HIV-infected individuals still do not achieve CD4+ cell count (CD4) normalization. Vitamin D has immunoregulatory functions, including inducing the development of T cells, and higher levels may improve CD4 rebound.

Design—Longitudinal study of men from the Multicenter AIDS Cohort Study who virally suppressed following HAART initiation and had pre- and post-HAART 25[OH]D and 1,25[OH]$_2$D measurements and repeated measures of CD4.

Methods—CD4 rebound was modelled using a nonlinear mixed effects model. We estimated the adjusted effect (adjusted for pre-HAART antiretroviral exposure, black race, age and CD4 at HAART initiation) of pre- and post-HAART vitamin D metabolite levels on the rate of CD4 increase and final CD4 plateau.

Address correspondence to: Alison G Abraham, Associate Professor, Johns Hopkins School of Medicine, 600 N. Wolfe Street, Baltimore MD 21287, Phone: (410) 955-6026, FAX: (410) 502-6146, alison.abraham@jhu.edu.

Conflicts of Interest

TTB has served as a consultant to Gilead Sciences, Merck, BMS, Theratechnologies and EMD-Serono. None of the other authors (AGA, LZ, KC, AT, AH, FJP, MME, LPJ, MDW, LAK) have a commercial or other association that might pose a conflict of interest.

Prior Presentations:

This work was presented at the International AIDS Conference, Durban, South Africa, July 2016

Results—Among the 263 HIV-infected HAART initiators with pre-HAART vitamin D measurements, a 1-standard deviation (SD) higher pre-HAART 25(OH) $_2$D level was associated with a 9% faster rate of rise (p= 0.02) but no gain in final CD4 plateau. In contrast, a 1 SD higher 1,25(OH)$_2$D level was associated with a 43-cell lower final CD4 (p=0.04). Among 560 men with post-HAART measurements, findings were similar to those for pre-HAART 25(OH) $_2$D with 1 SD higher level associated with faster rate of rise but no improvement in final CD4.

Conclusion—We found no evidence that higher vitamin D metabolite levels pre- or post-HAART are associated with better CD4 outcomes among HIV-infected HAART initiators. However, the value of pre-HAART 1,25(OH)$_2$D levels as an indicator of immune response dysregulation could be further explored.

Keywords

HIV infection; vitamin D; immune reconstitution

Introduction

Human Immunodeficiency Virus-1 (HIV) infection is characterized by a progressive deterioration in immune function. The advent of effective therapy (HAART) has allowed the vast majority of patients who achieve and maintain undetectable plasma HIV RNA levels to experience sustained improvements in immune function, leading to eventual recovery of their CD4+ cell count (CD4) [1–4]. However, substantial heterogeneity in the rate of increase and the apparent plateau of CD4 has been noted, and a significant proportion of individuals who start HAART fail to achieve adequate CD4 reconstitution [5–8], even after up to 10 years of treatment-mediated viral suppression [9]. While persons initiating therapy with a CD4 less than 200 cells/mm^3 are most at risk of a suboptimal CD4 recovery, an estimated 17% to 52% of patients initiating therapy with higher CD4s also fail to achieve a CD4 plateau of greater than 500 cells/mm^3 [7,8,10].

Persistently low CD4s despite virally suppressive HAART is associated with increased risk of both AIDS- and non–AIDS-related events [11–16]. Evidence suggests continuous improvements in clinical prognosis with higher CD4s maintained during therapy until 1500 cells/mm^3, at which point overall prognosis approaches that of an HIV-uninfected individual [16]. Thus, interventions targeting modifiable factors that could improve CD4 reconstitution following HAART initiation may impact AIDS- and non–AIDS-related comorbidity rates.

One potential target is vitamin D level status. Vitamin D has immunoregulatory functions, and all cells of the immune system including T lymphocytes cells have been shown to express vitamin D receptors [17]. The active form of vitamin D, 1,25(OH)$_2$D, acts as a potent anti-inflammatory agent and induces development of regulatory T cells [18]. Deficiency of 1,25(OH)$_2$D has been linked to multi-organ immune-mediated damage [19]. Given that immune activation [20–22] and reduced regulatory T cell frequencies [23] both appear to predict CD4 loss and disease progression, vitamin D status could play a role. Consistent with this hypothesis, prior studies in HIV- infected patients have shown a correlation between both pre- and post-HAART 25(OH)D levels and CD4 T-cell recovery [24,25].

In this study we measured pre- and post-HAART levels of 25(OH)D and its active form, $1,25(OH)_2D$, and evaluated their associations with the rate of CD4 reconstitution and CD4 plateau among HIV-infected participants initiating HAART in the Multicenter AIDS Cohort Study (MACS).

Methods and Methods

The Multicenter AIDS Cohort Study (MACS)—Established in 1984, the MACS is an ongoing observational study of HIV-infected and -uninfected men who have sex with men from four recruitment sites: Baltimore, MD-Washington, DC; Chicago, IL; Los Angeles, CA; and Pittsburgh, PA [26]. At semi-annual visits participants undergo physical examinations and give blood and urine samples for laboratory analyses and storage. Standardized questionnaires are used to collect health, behavior and HIV treatment history. Each participant gives informed consent and each local institutional review board has approved the study.

For the present study, we included men with pre- or post-HAART vitamin D metabolite measurements from the MACS vitamin D substudy [27], who had at least one post-HAART CD4 measurement and evidence of viral suppression (defined as at least one HIV RNA measurement <500 copies/ml within 2 years of starting HAART) following HAART initiation. Included men were followed from HAART initiation (defined as the midpoint between last observed time not on HAART to first observed time on HAART) until viral failure (defined as HIV RNA measurement >500 copies/ml 2 years or more after starting HAART) or last CD4 measurement.

Measurement of Vitamin D—Vitamin D metabolite levels, 25(OH)D and $1,25(OH)_2D$, were measured in specimens from HAART-initiators with available blood samples at least 6 months following HAART initiation to allow sufficient time to achieve plasma HIV RNA suppression. In a subset of participants with available pre-HAART samples, pre-HAART Vitamin D measurements were also obtained as close to the time of HAART initiation as possible. Serum 25(OH)D (the sum of $25(OH)D_2$ and $25(OH)D_3$) and $1,25(OH)_2D$ (the sum of $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$) were measured using immuno-affinity purification and liquid chromatography tandem mass spectrometry [28].

Covariate Measurements—Race, smoking, alcohol use and injection drug use (IDU) were ascertained from self-reported. Body mass index (BMI) was recorded from physical examination. Plasma HIV-1 RNA levels were measured using Roche assays (Hoffman-LaRoche, Nutley, New Jersey, USA). CD4+ lymphocyte counts were measured using standardized flow cytometry. HAART was defined as three or more antiretroviral drugs consisting of one or more protease inhibitors (PI) or one non-nucleoside reverse transcriptase inhibitor (NNRTI) or nucleoside reverse transcriptase inhibitor (NRTI): abacavir or tenofovir disoproxil fumarate, or an integrase strand transfer inhibitor or an entry inhibitor [29] based on reported therapy use. We used a binary variable to account for differences in treatment in different therapy eras (pre-2000, 2000 and after). Exposure to antiretrovirals prior to HAART initiation was also captured.

Prevalent HCV was defined as a reactive HCV antibody or detectable HCV RNA level. Plasma HIV RNA levels were measured using the Roche Amplicor assay (Hoffman-LaRoche, Nutley, NJ) sensitive to 50 copies/ml. CD4 was measured by three-color flow cytometry (34). Evidence of viral suppression was defined as at least one HIV RNA measurement <500 copies/ml within 2 years of starting HAART while failure following viral suppression was defined as a HIV RNA measurement >500 copies/ml 2 years or more after starting HAART among men with evidence of post-HAART suppression.

Baseline covariate values of age and CD4 were taken from the closest visit prior to the estimated date of HAART initiation. If CD4 was missing at the closest visit, the pre-HAART nadir CD4 was used.

Statistical Analysis

Adjustment of Vitamin D levels for seasonal variation—Serum 25(OH)D levels vary by season [30]. To adjust for seasonal variation, we used a linear regression model with 25(OH)D as the dependent variable and the season of blood collection as a categorical independent variable (January–March, April-June, July–September, October–December). We estimated the seasonally-adjusted 25(OH)D value by adjusting out the seasonal variation (adding the residuals of the model to the model intercept). These estimates of the seasonally-adjusted 25(OH)D value were used in all subsequent analyses.

Modeling of CD4 rebound following HAART initiation—CD4 rebound was modelled using a nonlinear mixed effects model which described the process of CD4 increase as an exponential function of time since HAART initiation [31,32]. The model had three parameters which included the starting CD4 (starting count; S) at HAART initiation, the plateau or final level of CD4 (final count; F) following recovery, and the rate at which CD4 increased (rate of increase; R). To satisfy distributional assumptions and improve convergence, CD4 was transformed by taking the natural log and multiplying by 5. Thus the model had the following form: $5log(CD4_{ij}) = F_i + (S_i - F_i)e^{-R_i t_{ij}} + \varepsilon_{ij}$, where $CD4_{ij}$ and t_{ij} refer to the CD4 and respective time point j of each measurement for individual i. Random effects were added to each parameter to allow for individual variability in the starting count, final count and rate of increase. Each parameter (i.e. S, F, or R) could be described as a function of covariate values. To evaluate model fit and describe CD4 rebound in the MACS sample, the model was adjusted for observed CD4 at HAART initiation. Men were censored from the analysis at the time they experienced viral failure.

The relationship between vitamin D metabolite levels and CD4 rebound—To evaluate the effect of vitamin D metabolite levels, each parameter (i.e. S, F, or R) was described as a function of vitamin D levels and/or potential confounding covariates. Pre-HAART vitamin 25(OH)D and $1,25(OH)_2D$ levels were included in the expressions for starting count, S, as well as equations for final count, F and rate of increase, R. Post-HAART vitamin 25(OH)D and $1,25(OH)_2D$ levels were included only in the expressions for final count, F and rate of increase, R, as post-HAART vitamin D levels were not expected to affect the starting CD4. Examined covariates for inclusion as possible confounders were: CD4 at HAART initiation, age at HAART initiation, BMI at HAART initiation, viral load at

HAART initiation, black race, IDU, smoking, alcohol use, hepatitis C virus infection, antiretroviral use prior to HAART initiation and pre-2000 HAART era. The final adjusted model was the most parsimonious model with the lowest Akaike Information Criterion (AIC) and with all included covariates (except the exposure of interest: 25(OH)D and $1,25(OH)_2D$ levels) having a p-value< 0.05. Thus, covariates could be included in any parameter function or none depending upon the above criteria.

The analysis was conducted using SAS 9.3.

Results

Sample Selection

645 HIV-infected men had post-HAART 25(OH)D and $1,25(OH)_2D$ measurements from the MACS Vitamin D substudy. Of these, 560 men had at least one CD4 measurement to contribute to the analysis and evidence of HIV viral suppression following HAART initiation. Among the 560, 263 men also had pre-HAART initiation vitamin D measurements. At HAART initiation the median age was 43 years and 25% were African American (Table 1). On average, men were observed for 8.1 years following HAART initiation. Comparing men included to all HAART initiators, men included were more likely to have initiated therapy before 2005, and were older at HAART initiation ($p<0.01$).

Description and correlates of pre- and post-HAART vitamin D status

The median post-HAART sample time was 2.1 years (IQR:1.7–2.3) following HAART initiation while the median pre-HAART sample time was 1.2 years (IQR: 0.9–1.7) prior to HAART initiation. The median post-HAART 25(OH)D level in the cohort was 22.9 ng/mL (IQR:16.7–30.4) (Table 1). The median post-HAART $1,25(OH)_2D$ level was 45.1 pg/mL (IQR: 35.7–55.7). Post-HAART $1,25(OH)_2D$ levels were weakly correlated with post-HAART 25(OH)D levels ($\rho=0.13$). Among those with pre-HAART initiation vitamin D measurements, the pre-HAART 25(OH)D levels were highly correlated with post-HAART 25(OH)D levels ($\rho=0.64$) while pre-HAART $1,25(OH)_2D$ levels were only modestly correlated with post-HAART $1,25(OH)_2D$ levels ($\rho=0.34$). Pre-HAART $1,25(OH)_2D$ levels were weakly correlated with pre-HAART 25(OH)D levels ($\rho=0.15$). Between tertiles of post-HAART 25(OH)D, significant differences were noted in racial distribution, age at HAART initiation, alcohol use, exposure to pre-HAART ART, current efavirenz use and CD4 at HAART initiation (Table 1). Overall, only 2% of men reported any current vitamin supplementation use at the first visit following HAART initiation and supplementation use wasn't differential by post-HAART 25(OH)D level.

Description of CD4 recovery by starting CD4

There were a median of 19 (IQR: 11–27) CD4 measurements per participant during follow-up from which to model CD4 rebound. By observed category of CD4 at HAART initiation (CD4<200, CD4 200–350, CD4>350) -- a strong determinant of CD4 rebound -- we found the median predicted final CD4 was 506, 597 and 721 cells/ml, respectively (Table 2). As a measure of the rate of CD4 gain, the median predicted time to 75% of the final CD4 was 1.9 years, 1.5 years and 0.4 years, respectively. The predicted percent of each group achieving a

CD4 of at least 500 cells/ml following HAART initiation was 53%, 79% and 92%, respectively. Having a higher baseline CD4 was correlated ($\rho = 0.2$) with a higher final CD4 and a higher baseline CD4 was correlated with a slower rate of rise ($\rho = -0.2$), as has been previously reported. The strongest correlation was between a faster rate of rise and a lower final CD4 ($\rho = -0.4$).

Relationship between vitamin D status and CD4 recovery

The final model was adjusted for ART exposure before HAART, baseline age, black race and CD4 at HAART initiation.

Among the 263 men with pre-HAART vitamin D measurements, the associations of pre-HAART 25(OH)D and $1,25(OH)_2D$ levels with final CD4 and rate of CD4 increase were examined (Table 3). We found that for the average participant, an additional 10 ng/mL 25(OH)D (equivalent to 1 standard deviation [SD] of change) was associated with a 9% faster median time to 75% of the final value (p= 0.02). In contrast, a 16 pg/mL higher pre-HAART $1,25(OH)_2D$ level (equivalent to 1 SD of change) was associated with a 29 cell lower starting CD4 (p=0.04) and a 43 cell lower final CD4 (p=0.04), holding pre-HAART 25(OH)D set at the mean pre-HAART cohort level.

Among the 560 men with post-HAART vitamin D measurements, we found that for the average participant, an additional 10 ng/mL post-HAART 25(OH)D was associated with a 10% faster median time to 75% of the final value (p= 0.01) (Table 3). Figure 1 shows the average projected CD4 rebound by post-HAART 25(OH)D tertile and the associated variability in individual trajectories. When post-HAART $1,25(OH)_2D$ levels were added to the models, a 16 pg/mL higher post-HAART $1,25(OH)_2D$ level was associated with a 6% faster median time to 75% of the final value (p=0.003), holding post-HAART 25(OH)D set at the mean cohort level.

Predictors of CD4 recovery

We compared men with predicted final CD4 of at least 500 cells/mL to men with final CD4 less than 500 cells/ml, stratified by observed CD4 at HAART initiation to identify characteristics associated with achieving adequate immune reconstitution, independent of the CD4 category at HAART initiation. Among men with CD4 less than 200 cells/mL at HAART, non-black men were more likely to achieve 500 cells/mL (59% versus 34%). Men with lower pre-HAART $1,25(OH)_2D$ were also more likely to achieve 500 cells/mL (mean pre-HAART $1,25(OH)_2D$: 41.7 pg/mL versus 49.6 pg/mL). Among men with CD4 between 200 and 350 cells/mL, those with no prior antiretroviral exposure before HAART were more likely to achieve 500 cells/mL (86% versus 70%). Among men with CD4 greater than 350 cells/mL, the vast majority achieved 500 cells/mL. Neither levels of pre- nor post-HAART 25(OH)D were significant predictors of CD4 recovery.

Discussion

There is substantial heterogeneity in the CD4 value achieved following HAART initiation, which is only partially explained by CD4 at HAART initiation. The consequence of persistent low CD4 during treatment is the increased risk of both AIDS- and non–AIDS-

related events [11–16]. Some evidence suggests continuous improvements in a patient's overall prognosis with higher maintained CD4 during therapy until 1500 cells/mL, at which point overall prognoses approach that of HIV-uninfected individuals [16]. Finding interventions that may bolster immune rebound following HAART initiation could, therefore, have notable effects on long term morbidity. While vitamin D status is a promising target for intervention given the role of $1,25(OH)_2D$ in immune system regulation, we found little evidence to suggest that higher levels of either inactive or active vitamin D metabolites were associated with improved CD4 rebound.

Pre-HAART levels of 25(OH)D and $1,25(OH)_2D$ were examined in relation to CD4 rebound in a subset of the cohort with pre-HAART measurements. We found that higher pre-HAART 25(OH)D levels were associated with modestly faster rises in CD4 but unchanged or perhaps higher final CD4 plateaus. However, contrary to the expectation that higher levels of vitamin D metabolites would be beneficial, we found higher pre-HAART $1,25(OH)_2D$ levels were associated with a much lower final CD4 plateau – 43 cells lower per SD increase in pre-HAART active vitamin D. It has been suggested that $1,25(OH)_2D$ levels may serve as a clinical marker in autoimmune and chronic disease [33], and chronic HIV-infection may represent a similar context of long-term immune activation. Dysregulation of vitamin D metabolism either through down-regulation of vitamin D receptor activity or through increased 1α-hydroxylase activity could allow $1,25(OH)_2D$ levels to rise, with the former a common mechanism used by invading pathogens to evade the host immune response [34]. There is little prior research evaluating the active form of vitamin D and its association with CD4 rebound. A prior study of 54 HIV-infected patients and 20 controls found lower levels of $1,25(OH)_2D$ to be associated with HIV-infection but not with CD4 count, but 27 patients met criteria for AIDS, which may represent a different population and immune context [35]. Prior reports from the MACS vitamin D substudy found no difference in median $1,25[OH]_2D$ level by HIV serostatus (median 45.0 pg/mL in HIV-infected and 45.1 pg/mL in HIV-uninfected [27]). It must be emphasized that the reliability of $1,25(OH)_2D$ was modest (ρ=0.34 between repeated measures) and levels are known to be transient. Thus, the association between high pre-HAART $1,25(OH)_2D$ and poor immune reconstitution should be interpreted with caution.

Post-HAART 25(OH)D and $1,25(OH)_2D$ levels –representing the concurrent vitamin D status during CD4 recovery -- were examined in relation to CD4 rebound in the full cohort. Higher post-HAART 25(OH)D was associated with modestly faster rises in CD4 but unchanged or perhaps even lower final CD4 plateaus. Similar results were seen for Post-HAART $1,25(OH)_2D$ levels. This negative correlation between CD4 rate of rise and final resulting value was consistent across the data, suggesting that those with faster immune system gains also see a premature end to recovery, plateauing at a lower final CD4.

However, continued gains after 4 or 5 years among even those with low baseline CD4 has been reported [3,4]. Indeed we saw that estimated median time to within 50 cells of the most recent CD4 asymptote was estimated to be around 4 years after HAART initiation and was the same regardless of the initial pre-HAART CD4. This suggests that gains for half of the population may continue beyond 4 years following HAART initiation, even in persons with the most compromised immune systems. Thus the consistency of the estimate of final time

to CD4 asymptote supports prior observations that the immune system's capacity for CD4 T lymphocyte restoration is not necessarily limited by low pre-therapy CD4 counts. The recovery of the CD4 T cell count has been reported to be hindered by residual viral replication, impaired thymic function, advanced age, enhanced T cell activation and apoptosis, and, possibly, viral coinfection [7, 10– 13]. In our sample of men who were suppressed following HAART initiation, we could not examine the impact of residual viral replication. However, neither age nor hepatitis C virus coinfection were significant predictors of attaining 500 cells/mL. Of the factors we examined, we found that a lack of prior ARV exposure, lower pre-HAART $1,25(OH)_2D$ and non-black race were the only significant predictors of attaining a plateau of at least 500 cells/mL.

There were limitations to our study worth noting. Measured 25(OH)D and $1,25(OH)_2D$ levels pre-and post- HAART initiation were used to represent long term vitamin D status that could affect CD4 rebound possibly over many years. The half-life of 25(OH)D and $1,25(OH)_2D$ levels is on the order of days for the former and hours for the latter, which could introduce substantial measurement error into the analysis if there is large variability over time. Correlations between pre- and post-HAART measurements suggests moderate stability, particularly in 25(OH)D levels. Secondly, censoring of individuals when viral load rose above 500 may have introduced informative censoring such that those with poorer response were selectively removed from the analysis. Therefore estimates of final CD4 and the percent obtaining a final count above 500 cells/mL may be overly optimistic.

In conclusion we found no evidence to suggest higher vitamin D metabolite levels are associated with better CD4 outcomes in a sample of HIV-infected men with evidence of viral suppression following HAART initiation. While vitamin D metabolite supplementation has been posited to be a potential intervention that might improve CD4 response to therapy, our data do not support this assertion. Further studies could examine whether $1,25(OH)_2D$ levels prior to HAART initiation have value as a clinical marker of immune response dysregulation.

Acknowledgments

Funding

This work was supported by grants U01-AI35039, U01-AI35040, U01-AI35041, U01-AI35042, UM1-AI35043 from the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health (NIMH). Targeted supplemental funding for specific projects in the MACS was provided by the National Heart, Lung, and Blood Institute (NHLBI), and the National Institute on Deafness and Communication Disorders (NIDCD). MACS data collection is also supported by UL1-TR001079 (JHU ICTR) from the National Center for Advancing Translational Sciences (NCATS) a component of the National Institutes of Health (NIH). The funding for this substudy was supported by the National Institute of Allergy and Infectious Diseases (R21AI109817).

We would like to thank all the collaborators, staff, and participants of the Multicenter AIDS Cohort Study (MACS). Data in this manuscript were collected by the Multicenter AIDS Cohort Study (MACS) with centers at Baltimore (U01-AI35042): The Johns Hopkins University Bloomberg School of Public Health: Joseph B. Margolick (PI), Jay Bream, Todd Brown, Barbara Crain, Adrian Dobs, Richard Elion, Richard Elion, Michelle Estrella, Lisette Johnson-Hill, Sean Leng, Anne Monroe, Cynthia Munro, Michael W. Plankey, Wendy Post, Ned Sacktor, Jennifer Schrack, Chloe Thio; Chicago (U01-AI35039): Feinberg School of Medicine, Northwestern University, and Cook County Bureau of Health Services: Steven M. Wolinsky (PI), John P. Phair, Sheila Badri, Dana Gabuzda, Frank J. Palella, Jr., Sudhir Penugonda, Susheel Reddy, Matthew Stephens, Linda Teplin; Los Angeles (U01-AI35040): University of California, UCLA Schools of Public Health and Medicine: Roger Detels (PI), Otoniel Martínez-Maza

(Co-P I), Aaron Aronow, Peter Anton, Robert Bolan, Elizabeth Breen, Anthony Butch, Shehnaz Hussain, Beth Jamieson, Eric N. Miller, John Oishi, Harry Vinters, Dorothy Wiley, Mallory Witt, Otto Yang, Stephen Young, Zuo Feng Zhang; Pittsburgh (U01-AI35041): University of Pittsburgh, Graduate School of Public Health: Charles R. Rinaldo (PI), Lawrence A. Kingsley (Co-PI), James T. Becker, Phalguni Gupta, Kenneth Ho, Susan Koletar, Jeremy J. Martinson, John W. Mellors, Anthony J. Silvestre, Ronald D. Stall; Data Coordinating Center (UM1-AI35043): The Johns Hopkins University Bloomberg School of Public Health: Lisa P. Jacobson (PI), Gypsyamber D'Souza (Co-PI), Alison, Abraham, Keri Althoff, Jennifer Deal, Priya Duggal, Sabina Haberlen, Alvaro Muoz, Derek Ng, Janet Schollenberger, Eric C. Seaberg, Sol Su, Pamela Surkan. Institute of Allergy and Infectious Diseases: Robin E. Huebner; National Cancer Institute: Geraldina Dominguez. The MACS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health (NIMH). Targeted supplemental funding for specific projects was also provided by the National Heart, Lung, and Blood Institute (NHLBI), and the National Institute on Deafness and Communication Disorders (NIDCD). MACS data collection is also supported by UL1-TR001079 (JHU ICTR) from the National Center for Advancing Translational Sciences (NCATS) a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH), Johns Hopkins ICTR, or NCATS. The MACS website is located at http://www.statepi.jhsph.edu/macs/macs.html. None of the authors have a commercial or other association that might pose a conflict of interest

References

1. Tarwater PM, Margolick JB, Jin J, et al. Increase and plateau of CD4 T-cell counts in the 3(1/2) years after initiation of potent antiretroviral therapy. J Acquir Immune Defic Syndr 1999. 2001; 27:168–175.
2. Gras L, Kesselring AM, Griffin JT, et al. CD4 cell counts of 800 cells/mm3 or greater after 7 years of highly active antiretroviral therapy are feasible in most patients starting with 350 cells/mm3 or greater. J Acquir Immune Defic Syndr 1999. 2007; 45:183–192.
3. Hunt PW, Deeks SG, Rodriguez B, et al. Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. AIDS Lond Engl. 2003; 17:1907–1915.
4. Mocroft A, Phillips AN, Gatell J, et al. Normalisation of CD4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. Lancet Lond Engl. 2007; 370:407–413.
5. García F, de Lazzari E, Plana M, et al. Long-term CD4+ T-cell response to highly active antiretroviral therapy according to baseline CD4+ T-cell count. J Acquir Immune Defic Syndr 1999. 2004; 36:702–713.
6. Moore RD, Keruly JC. CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. Clin Infect Dis Off Publ Infect Dis Soc Am. 2007; 44:441–446.
7. Kaufmann GR, Perrin L, Pantaleo G, et al. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. Arch Intern Med. 2003; 163:2187–2195. [PubMed: 14557216]
8. Le Moing V, Thiébaut R, Chêne G, et al. Long-term evolution of CD4 count in patients with a plasma HIV RNA persistently <500 copies/mL during treatment with antiretroviral drugs. HIV Med. 2007; 8:156–163. [PubMed: 17461859]
9. Kelley CF, Kitchen CMR, Hunt PW, et al. Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment. Clin Infect Dis Off Publ Infect Dis Soc Am. 2009; 48:787–794.
10. Palella FJ, Armon C, Chmiel JS, et al. CD4 cell count at initiation of ART, long-term likelihood of achieving CD4 >750 cells/mm3 and mortality risk. J Antimicrob Chemother. 2016; 71:2654–2662. [PubMed: 27330061]
11. Kaufmann GR, Furrer H, Ledergerber B, et al. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/microL in HIV type 1-infected individuals receiving potent antiretroviral therapy. Clin Infect Dis Off Publ Infect Dis Soc Am. 2005; 41:361–372.
12. Baker JV, Peng G, Rapkin J, et al. CD4+ count and risk of non-AIDS diseases following initial treatment for HIV infection. AIDS Lond Engl. 2008; 22:841–848.

13. El-Sadr WM, Lundgren JD, et al. Strategies for Management of Antiretroviral Therapy (SMART) Study Group. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med. 2006; 355:2283–2296. [PubMed: 17135583]
14. Weber R, Sabin CA, Friis-Møller N, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med. 2006; 166:1632–1641. [PubMed: 16908797]
15. Gutierrez F, Padilla S, Masiá M, et al. Clinical outcome of HIV-infected patients with sustained virologic response to antiretroviral therapy: long-term follow-up of a multicenter cohort. PloS One. 2006; 1:e89. [PubMed: 17183720]
16. Lewden C, Chene G, Morlat P, et al. HIV-infected adults with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population. J Acquir Immune Defic Syndr 1999. 2007; 46:72–77.
17. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys. 2000; 374:334–338. [PubMed: 10666315]
18. Jeffery LE, Burke F, Mura M, et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol Baltim Md 1950. 2009; 183:5458–5467.
19. Gratz IK, Campbell DJ. Organ-specific and memory treg cells: specificity, development, function, and maintenance. Front Immunol. 2014; 5:333. [PubMed: 25076948]
20. Hunt PW, Martin JN, Sinclair E, et al. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis. 2003; 187:1534–1543. [PubMed: 12721933]
21. Deeks SG, Kitchen CMR, Liu L, et al. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood. 2004; 104:942–947. [PubMed: 15117761]
22. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, Giorgi JV. CD8+ T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. J Acquir Immune Defic Syndr Hum Retrovirology Off Publ Int Retrovirology Assoc. 1998; 18:332–340.
23. Marziali M, De Santis W, Carello R, et al. T-cell homeostasis alteration in HIV-1 infected subjects with low CD4 T-cell count despite undetectable virus load during HAART. AIDS Lond Engl. 2006; 20:2033–2041.
24. Ross AC, Judd S, Kumari M, et al. Vitamin D is linked to carotid intima-media thickness and immune reconstitution in HIV-positive individuals. Antivir Ther. 2011; 16:555–563. [PubMed: 21685543]
25. Aziz M, Livak B, Burke-Miller J, et al. Vitamin D insufficiency may impair CD4 recovery among Women's Interagency HIV Study participants with advanced disease on HAART. AIDS Lond Engl. 2013; 27:573–578.
26. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. Am J Epidemiol. 1987; 126:310–318. [PubMed: 3300281]
27. Zhang L, Tin A, Brown TT, et al. Vitamin D Deficiency and Metabolism in HIV-Infected and HIV-Uninfected Men in the Multicenter AIDS Cohort Study. AIDS Res Hum Retroviruses. 2017; 33:261–270. [PubMed: 27700140]
28. Strathmann FG, Laha TJ, Hoofnagle AN. Quantification of 1α,25-dihydroxy vitamin D by immunoextraction and liquid chromatography-tandem mass spectrometry. Clin Chem. 2011; 57:1279–1285. [PubMed: 21768219]
29. [Accessed 28 December 2015] What's New in the Guidelines?. Adult and Adolescent ARV Guidelines. Available at: https://aidsinfo.nih.gov/
30. Karohl C, Su S, Kumari M, et al. Heritability and seasonal variability of vitamin D concentrations in male twins. Am J Clin Nutr. 2010; 92:1393–1398. [PubMed: 20943799]
31. Chen D-T, Chan W, Francis DJ, Shaywitz SE, Shaywitz BA. Application of Two-Level Negative Exponential Model to Children's Learning Curve in Reading. Commun Stat - Simul Comput. 2002; 31:279–299.

32. Burke CT, Shrout PE, Bolger N. Individual differences in adjustment to spousal loss: A nonlinear mixed model analysis. Int J Behav Dev. 2007; 31:405–415.

33. Blaney GP, Albert PJ, Proal AD. Vitamin D metabolites as clinical markers in autoimmune and chronic disease. Ann N Y Acad Sci. 2009; 1173:384–390. [PubMed: 19758177]

34. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. Inflamm Res. 2014; 63:803–819. [PubMed: 25048990]

35. Haug CJ, Aukrust P, Haug E, Mørkrid L, Müller F, Frøland SS. Severe deficiency of 1,25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunological hyperactivity and only minor changes in calcium homeostasis. J Clin Endocrinol Metab. 1998; 83:3832–3838. [PubMed: 9814454]

Key Points

- Higher post-HAART vitamin D metabolite levels were not associated with better CD4 outcomes following HAART initiation, indicating vitamin D supplementation would not improve CD4 rebound.
- 1,25(OH)2D levels pre-HAART initiation may serve as a clinical marker of immune response dysregulation

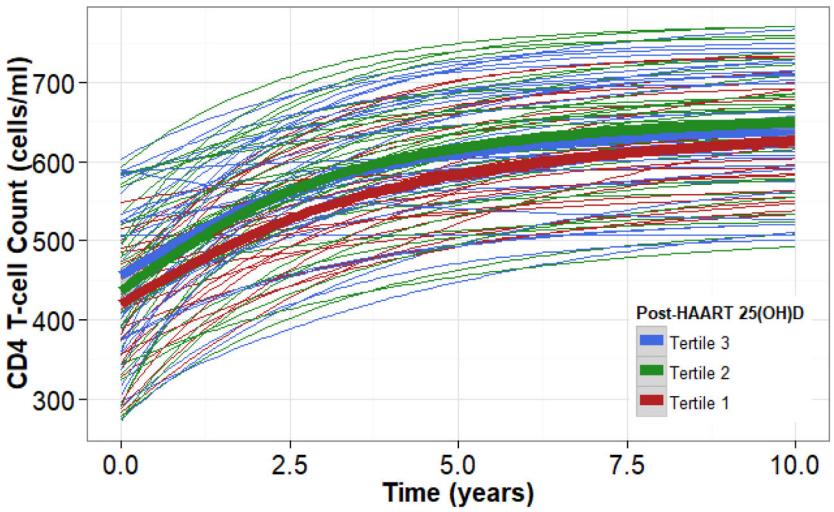

Figure 1.

Predicted CD4 T cell count rise following HAART initiation by post-HAART 25(OH)D tertile from the adjusted model. Thick lines are the smoothed average trajectory for each post-HAART 25(OH)D tertile while thin lines are individual predicted trajectories representing the interquartile range of predicted trajectories for each post-HAART 25(OH)D tertile.

AIDS. Author manuscript; available in PMC 2019 May 15.

Table 1

Demographic and clinical characteristics of the 560 HIV-infected men at the time of HAART initiation.

	Post-HAART 25(OH)D Group			
	Overall (N=560)	**Tertile 1 (<18.3 ng/mL)**	**Tertile 2 (18.3 – 27.3 ng/mL)**	**Tertile 3 (>27.3 ng/mL)**
	Median or %	**Median or %**	**Median or %**	**Median or %**
Demographic and Clinical Characteristics at HAART initiation				
Black	24.6	**50.9**	**14.2**	**11.3**
Age at HAART initiation	42.8	**41.9**	**42.8**	**44.2**
BMI (kg/m^2)	24.6	25.4	24.1	24.4
Year of HAART initiation				
HAART pre-2000 era	39.1	31.4	40.5	44.6
HAART 2000–2004 era	39.3	47.4	38.4	32.8
HAART post 2005 era	21.6	21.1	21.1	22.6
HCV Positive	7.3	10.9	6.3	5.1
Injection Drug Use	2.1	3.4	2.1	1.0
Current smoker	16.1	13.7	17.4	16.9
Current moderate, heavy or binge drinker[§]	30.3	**26.4**	**35.8**	**28.8**
Pre-HAART antiretroviral exposure	50.9	**44.6**	**48.9**	**58.5**
Current tenofovir use	10.9	13.7	8.4	10.8
Current efavirenz use	24.8	**34.3**	**23.2**	**17.9**
Current ritonavir use	17.3	16.0	14.2	21.5
Viral load at HAART initiation (copies/mL)	30183	27931	35226	27254
Description of CD4 Count (cells/mL) data				
# of post-HAART measurements	19	19	19	21
CD4 count at HAART initiation	337	**321**	**337**	**363**
Vitamin D description (25D: ng/mL; 1,25D: pg/mL)				
Pre-HAART[*]				
25(OH)D (ng/mL)	24	**17.2**	**24.1**	**29.3**
1,25(OH)$_2$D (pg/mL)	42.8	44.8	42.3	42.4

	Post-HAART 25(OH)D Group			
	Overall (N=560)	**Tertile 1 (<18.3 ng/mL)**	**Tertile 2 (18.3 – 27.3 ng/mL)**	**Tertile 3 (>27.3 ng/mL)**
	Median or %	**Median or %**	**Median or %**	**Median or %**
Post-HAART				
25(OH)D (ng/mL)	22.9	**13.8**	**22.5**	**32.7**
1,25(OH)$_2$D (pg/mL)	45.1	**43.3**	**45.1**	**45.9**

§Moderate, heavy or binge drinker defined as 3 or more drinks/day more than once a month

*N=263

Bold indicates differences across Post-HAART Serum 25(OH)D tertiles with significance p<0.05

Table 2

Description of estimated CD4+ T cell count rebound following HAART initiation by category of observed CD4+ T cell count at HAART initiation

Estimated parameters	Observed CD4 at HAART initiation			
	Overall	CD4<200 (N=188)	CD4 200–350 (N=192)	CD4>350 (N=175)
Median Final CD4 [IQR]	628 [519, 773]	506 [437 584]	597 [509, 685]	721 [611, 866]
Median years to 75% of asymptote [IQR]	1.3 [0.2, 2.6]	1.9 [1.2, 3.6]	1.5 [0.7, 2.7]	0.4 [0.0, 1.5]
Median years to asymptote [IQR]	4.1 [2.5, 7.8]	4.5 [2.6, 7.8]	4.4 [2.5, 8.5]	3.8 [2.4, 7.0]
Percent with final CD4 >500 (%)	76%	53%	79%	92%

Abbreviations: CD4 - CD4+ T cell count (cells/mL); IQR- Interquartile range

AIDS. Author manuscript; available in PMC 2019 May 15.

Table 3

Estimated effects of pre- and post-HAART vitamin D metabolites on rate of rise and final plateau of CD4 following HAART initiation.

Estimated Parameter[1]	Estimated effect on CD4 rebound	P-value
Effect of Post-HAART 25(OH)D (per 1 SD or 10 ng/mL)		
Final CD4	17 cells lower on average	0.133
Time to 75%	10% faster median time	0.006
Effect of Post-HAART 1,25(OH)2D (per 1 SD or 16 pg/mL) holding Post-HAART 25(OH)D constant		
Final CD4	11 cells lower on average	0.303
Time to 75%	6% faster median time	0.003
Effect of Pre-HAART 25(OH)D (per 1 SD or 10 ng/mL) in 263 men with pre-HAART measurements		
Starting CD4	14 cells higher on average	0.290
Final CD4	9 cells higher on average	0.679
Time to 75%	9% faster median time	0.016
Effect of Pre-HAART 1,25(OH)2D (per 1 SD or 16 pg/mL) holding Pre-HAART 25(OH)D constant in 263 men with pre-HAART measurements		
Starting CD4	29 cells lower on average	0.042
Final CD4	43 cells lower on average	0.038
Time to 75%	7% faster median time	0.163

Abbreviations: CD4 - CD4+ T cell count (cells/mL)

[1]Adjusted for ART exposure before HAART, baseline age, black race and CD4 at HAART initiation.