Vitamin D Levels and Influenza Vaccine Immunogenicity among HIV-Infected and HIV-Uninfected Adults

Nancy F. Crum-Cianflone, MD MPH^{1,2,3}, Seunghyun Won, PhD^{1,4}, Rachel Lee, MD⁵, Tahaniyat Lalani, MBBS, MHS^{1,4,6}, Anuradha Ganesan, MBBS,^{1,4,7} Timothy Burgess, MD¹ and Brian K. Agan, MD^{1,4}

(1) Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD, (2) Scripps Mercy Hospital, San Diego, CA, (3) Naval Medical Center San Diego, San Diego, CA, (4) Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, (5) Operational Infectious Diseases, Naval Health Res. Ctr., San Diego, CA, (6) Naval Medical Center Portsmouth, Portsmouth, VA, (7) Walter Reed National Military Medical Center, Bethesda, MD

Conflict of Interest: None. The authors have no financial interest in this work or the vaccine evaluated in this clinical trial. All authors contributed to the content of the manuscript and concurred with the decision to submit it for publication.

Support for this work (IDCRP-053) was provided by the Infectious Disease Clinical Research Program (IDCRP), a Department of Defense (DoD) program executed through the Uniformed Services University of the Health Sciences. This project has been funded in whole, or in part, with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), under Inter-Agency Agreement Y1-AI-5072. [Include any other

support acknowledgements]

The views expressed are those of the authors and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences, the NIH, or the Departments of the Army, Navy or Air Force, or the Department of Defense. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. Government.

This work is original and has not been published elsewhere.

Abstract

Background: Vaccination is the most important preventive strategy against influenza, however post-vaccination antibody responses are often inadequate especially among HIV-infected persons. Vitamin D deficiency has been suggested to adversely influence immune responses and is highly prevalent among HIV-infected adults. Therefore, we evaluated the association between 25-hydroxyvitamin D [25(OH)D] levels and post-influenza vaccination responses.

Methods: We conducted a prospective cohort study evaluating the immunogenicity of monovalent influenza A (H1N1) vaccination among both HIV-infected and HIV-uninfected adults (18-50 years of age) during the 2009-2010 influenza season. Antibody titers were evaluated at baseline, day 28, and 6 months post-vaccination using hemagglutination inhibition assays. Serum 25(OH)D levels were measured at day 28. Univariate and multivariate regression analyses examined the association between 25(OH)D levels [categorized as <20 ng/ml (deficiency) vs. $\geq$ 20 ng/ml] with the primary outcome of seroconversion. Secondary outcomes included seroprotection; a $\geq$ 4-fold increase in titers; and geometric mean titers post-vaccination. Analyses were repeated using 25(OH)D levels as a continuous variable.

Results: A total of 128 adults [64 HIV-infected (median CD4 count 580 cells/mm³) and 64 HIV-uninfected] were included. Seroconversion at day 28 post-vaccination was achieved in fewer HIV-infected participants compared with HIV-uninfected participants (56% vs. 74%, $p=0.03$).

Vitamin D deficiency was more prevalent among HIV-infected persons vs. HIV-uninfected persons (25% vs. 17%), although not significantly different ($p=0.39$). There were no associations found between lower 25(OH)D levels and poorer antibody responses at day 28 or 6 months for any of the study outcomes among either HIV-infected or HIV-uninfected adults.

Conclusion: Vitamin D deficiency was common among both HIV-infected and HIV-uninfected adults, but lower levels did not predict antibody responses after H1N1 (2009) influenza vaccination. Low 25(OH)D levels do not explain poorer post-vaccination responses among HIV-infected persons.

Background

Influenza remains a leading cause of seasonal epidemic disease resulting in excess morbidity and mortality. Vaccination remains the main preventive strategy against influenza and is currently recommended for persons ≥ 6 months [1]. Protection against influenza after vaccination varies widely by the “match” with circulating strains as well as host characteristics. Immunosuppression, including HIV infection, has been associated with reduced vaccine effectiveness [2-9]. This is of particular concern since HIV-infected persons are at higher risk for influenza-related complications [10-13].

Methods to improve vaccine responsiveness among HIV-infected persons have been studied including the use of higher influenza vaccine doses (e.g., Fluzone High-Dose)[14] and use of adjuvants (e.g., AS03, MF59) [15-17]. However, neither of these potential strategies are currently recommended by vaccine guidelines [1,18].

Vitamin D may affect both the innate and adaptive immune responses, and may have an immunomodulating role in improving immune responses to vaccines mediated through its actions on antigen-presenting cells including the dendritic cells [19-25]. While a study among prostate cancer patients found evidence of an association between low baseline 25-hydroxyvitamin D [25(OH)D] levels and poorer influenza vaccine responses [26], studies in healthy persons found no associations [27,28]. Hence further data are needed especially among immunocompromised hosts [27]. Among HIV-infected patients, only three published studies have examined this potential relationship. A study (n=91) showed no relationship between baseline 25(OH)D levels and vaccine antibody responses at day 21 post-vaccination with the 2010/2011 trivalent influenza vaccine [29], while a second study [n=90] found similar

25(OH)D levels (both groups: 20 ng/ml) among responders and non-responders after 2009 H1N1 influenza vaccination at day 21 post-vaccination [30]. A third study found that use of vitamin D supplementation at the time of influenza vaccination had no effect on post-vaccination antibody levels during the 2008-2009 season, but this study did not evaluate actual 25(OH)D levels [31]. Since only one of these studies had a HIV-negative group [29], each involved differing influenza seasons, and none evaluated long-term post-vaccination responses (responses were measured at 3-8 weeks) [29-31], further research is needed.

Given that HIV-infected persons have reduced immune responses after influenza vaccination [2-9] and a high prevalence of vitamin D deficiencies [32,33], we sought to determine if low 25(OH)D levels help explain the poorer post-vaccination immune responses in this population compared with HIV-uninfected adults. Hence, the purpose of this study was to evaluate vitamin 25(OH)D levels among HIV-infected and HIV-uninfected adults and its potential relationship with influenza vaccine immunogenicity.

Methods

Study Design

A prospective cohort study was conducted to compare the immunogenicity of the monovalent 2009 influenza A (H1N1) vaccine (strain A/California/7/2009/H1N1; Novartis Vaccines and Diagnostics) among HIV-infected and HIV-uninfected adults during the 2009-2010 influenza season (Clinical Trials #NCT00996970)[9]. Vaccination was performed October 29–December 2, 2009, and participants were voluntarily enrolled at the Naval Medical Center San Diego, San Diego, California; Naval Medical Center, Portsmouth, Virginia; and Walter Reed

National Military Medical Center, Bethesda, Maryland as previously described [9]. The study was approved by a central military institutional review board and conducted in accordance with the principles of the Declaration of Helsinki and the standards of Good Clinical Practice. Each participant provided written informed consent.

Study Population

Study participants included HIV-infected adults with a positive ELISA and Western Blot tests, while HIV-uninfected participants had documented negative testing. Inclusion criteria were ages 18-50 years, military beneficiary (including active duty military members or retirees and their family members), and no serious medical conditions except HIV-infection in the former group. Exclusion criteria were pregnancy or ≤ 6 weeks post-partum; significant medical conditions as previously described [9]; allergy to eggs or influenza vaccination; or prior history of confirmed or suspected H1N1 influenza. HIV-uninfected persons were age-matched (using the categories of 18-34 years and 35-50 years) to the HIV-infected group to ensure congruent ages between the two groups.

Study Procedures

Vaccines were stored and administered in accordance with manufacturer's guidelines. Adverse events (AEs) were recorded by observing each participant for ~30 min after vaccination and via a diary card for recording local and systemic adverse reactions that occurred within 7 days of vaccination. Adverse reactions were graded on the basis of impact on

daily activities as mild (not interfering with usual function), moderate (interfering to some extent with usual function), and severe (significantly interfering with usual function) [34].

Serum samples were collected at baseline (day 0, just prior to vaccination), day 28 (+/- 4 days), and 6 months (+/- 4 weeks) post-vaccination for influenza-specific antibody responses. Testing was conducted using hemagglutination-inhibition (HAI) assays as previously described [35,36]. Testing was simultaneously conducted on the three batched samples from each time point [37,38]. Serum samples were also collected at baseline (day 0) for CD4 cell count (determined by flow cytometry) and plasma HIV RNA levels (Roche Amplicor; lower limit of detection, 50 copies/mL). For this study, subjects who had available stored serum at the day 28 post-vaccination time point for determination of serum 25(OH)D levels were included. We selected the day 28 sample as this was the time point for assessing the initial post-vaccination antibody responses and since most participants had available serum at this time point. 25(OH)D levels were performed simultaneously using batched specimens and utilized commercially available kits (Eagle Biosciences, Nashua, NH, USA) and was conducted according to the manufacturer's instructions with prior studies confirming the validity of the test [39]. Results of this testing were also validated using external quality with samples from the Vitamin D External Quality Assessment Scheme (DEQAS) [40].

Data were collected from baseline questionnaires and medical record review by study coordinators. Covariates of interest utilized in the present study included age, gender, race, body mass index (BMI), prior receipt of seasonal influenza vaccine, and number of household members which were collected from the questionnaires. Data from the medical records

included duration of HIV infection, CDC stage, nadir CD4 count, and receipt of antiretroviral therapy (ART).

The primary outcome was vaccine immunogenicity based on seroconversion, defined as a geometric mean titer (GMT) of $\geq 1:40$ after vaccination (day 28) among persons with a pre-vaccination (day 0) level of $\leq 1:10$, as measured by HAI [35,36]. We excluded persons with a baseline GMT $> 1:10$ for our primary objective in order to examine antibody levels generated by naïve persons without prior immunity to the 2009 influenza H1N1 strain. The sample size of the original study ($n=132$) was based on the primary outcome as previously described [9]. Secondary immunogenicity outcomes among all participants included a) achieving seroprotection (defined as a post-vaccination titer $\geq 1:40$) and b) ≥ 4 -fold increase in titers from before to after vaccination (regardless of post-vaccination level achieved) at day 28 post-vaccination. In addition, we evaluated geometric mean titers (GMT) at each of the 3 time points (0, day 28, and 6 months) among all study participants. Finally, we analyzed associations between 25(OH)D levels and AEs post-vaccination.

Statistical Analysis

Descriptive statistics (medians with interquartile ranges [IQRs], counts with proportions) and unadjusted group comparisons (Wilcoxon Rank-Sum tests and Fisher's exact tests for continuous and categorical variables, respectively) are shown by HIV group and vitamin D level (< 20 ng and ≥ 20 ng).

For multivariable analyses of the primary and secondary objectives, logistic regression for binary outcomes was utilized with adjusting for covariates including age, race, number of

seasonal influenza vaccinations in previous 3 years, and number of household members similar to prior analyses [9]. The exposure of interest was 25(OH)D levels which were analyzed as a dichotomous variable (vitamin D <20 ng/ml and $\geq$ 20 ng/ml) based on the literature using this value for vitamin D deficiency [41,42]. Since other values have been used to designate vitamin D insufficiency and since the optimal level to improve immune responses is unknown, we repeated analyses using cut-points for 25(OH)D levels of 30 ng/ml and 40 ng/ml. Given that immunogenicity may vary by age, subgroup analyses were performed among participants <35 and $\geq$ 35 years of age.

Exploratory analyses examining predictors of seroconversion in HIV-infected participants were performed using logistic regression. Predictors considered included the 25(OH)D level, demographic characteristics, BMI, number of household members, seasonal influenza vaccination, duration of HIV infection, baseline CD4 cell count, HIV RNA level, and receipt of ART.

Reported *P* values are 2-sided with a *P* <0.05 considered to indicate statistical significance. All analyses were conducted using SAS, version 9.4 (SAS Institute).

Results

A total of 132 participants (66 HIV-infected and 66 HIV-uninfected) were enrolled in the original study, with one HIV-infected participant excluded due to lack of follow-up at day 28 post-vaccination. In the present analysis, 3 participants (1 HIV-infected and 2 HIV-uninfected) were excluded due to lack of available serum for 25(OH)D testing. Baseline study population characteristics of the 128 participants in the current study are shown in Table 1. The HIV-

infected and HIV-uninfected groups were similar, except the former group was more likely to not have other household members. HIV-infected persons at the time of vaccination had a median duration of HIV infection of 6.2 years, median CD4 count of 580 cells/mm³, 56% had an undetectable HIV RNA level (<50 copies/ml), and 81% were receiving ART.

Median 25(OH)D levels were similar among the two groups (26.9 vs. 28.7 ng/ml, $p=0.38$). Although more HIV-infected persons had vitamin D deficiency defined by a level <20 ng/ml than HIV-uninfected persons, this was not statistically significantly different (25% vs. 17%; $p=0.39$) (Table 1). Using alternate cut-points, values were not significantly different by HIV status: 25(OH)D levels of ≥ 30 ng/ml and ≥ 40 ng/ml in 41% and 19%, respectively, for HIV-infected persons, and 47% and 19%, respectively, among HIV-uninfected persons. We also evaluated 25(OH)D levels among the two age categories (<35 and ≥ 35 years) by HIV status and found no significant differences (data not shown).

The numbers and proportions of participants by each outcome at day 28 post-vaccination are summarized in Table 2. Seroconversion was achieved in fewer HIV-infected participants compared with HIV-uninfected participants (56% vs. 74%, adjusted p -value=0.03). Similarly HIV-infected persons were less likely to achieve the secondary outcomes (Table 2). In the multivariate analyses, significant differences by HIV status remained after adjusting for the 25(OH)D level (<20 vs. ≥ 20 ng/ml). We also examined vitamin D as a continuous variable and found similar results (data not shown).

Each group's (HIV-infected and HIV-uninfected) post-vaccination immune responses were examined by the 25(OH)D level (Table 3). Vitamin D deficiency was not associated with

either the primary or secondary outcomes for either the HIV-infected or HIV-uninfected group. Evaluating the 25(OH)D level as a continuous variable yielded similar findings (data not shown).

GMTs at each time point (baseline, day 28, and 6 months) were also evaluated by HIV status and 25(OH)D level. HIV-infected compared with HIV-uninfected persons had significantly lower GMT at day 28 (80 vs. 269, $p=0.003$) and 6 months (20 vs. 113, $p=0.0002$) post-vaccination. While both HIV-infected and HIV-uninfected participants with a 25(OH)D level <20 ng/ml had higher post-vaccination antibody levels compared with those having 25(OH)D level ≥ 20 ng/ml, none of the comparisons were statistically significant (Figure 1). We also conducted multivariate analyses examining if continuous 25(OH)D levels were associated with GMT levels at each time point, but found no significant associations (data not shown).

Since the optimal level of 25(OH)D for improving immune responses is unknown and some have favored ≥ 30 ng/ml or even higher levels (≥ 40 ng/ml), we repeated our analyses using these alternate cut-points. No significant associations were found using the 30 ng/ml cut-point for any of the post-vaccination antibody responses (data not shown). Similarly, no associations were seen for the primary or secondary immunologic outcomes using a higher cut-point of <40 ng/ml compared with ≥ 40 ng/ml (data shown in supplementary table 1). The median GMT for those with 25(OH)D levels of <40 ng/ml vs ≥ 40 ng/ml at day 28 were 160 vs. 160 ($p=0.77$) and at month 6 were 48 vs. 80 ($p=0.93$), respectively.

Among HIV-infected, factors associated with achieving the primary outcome were evaluated (Table 4). Younger age was associated with a borderline association with the primary outcome being achieved (32 vs. 45 years of age, $p=0.07$). The only HIV-specific factor found to be associated with achieving the primary outcome was a shorter duration of HIV infection (5.7

vs. 11.3 years, $p=0.09$), but again this did not meet statistical significance; CD4 counts, HIV RNA level, and receipt of ART were not associated. 25(OH)D levels, either categorical or continuous, were also not associated (Table 4).

An adverse event post-vaccination was noted among 76 (59%) participants, including 37 HIV-infected and 39 HIV-uninfected participants (57% vs. 61%, $p=0.86$) (supplementary table 2). Most commonly AEs were mild in severity and consisted of local reactions such as pain at the injection site. The occurrence of an adverse event did not vary by 25(OH)D level of <20 ng/ml compared with ≥ 20 ng/ml (59% in each group, $p=1.0$). We also evaluated the severity of AEs and the types of AEs (both local and systemic reactions), but found no associations by the 25(OH)D level.

Discussion:

Vitamin D deficiency has been increasingly recognized among persons worldwide [41]. HIV-infected persons in particular have a high prevalence of vitamin D deficiency, attributed to traditional risk factors as well as HIV-specific factors including the use of specific antiretroviral medications (e.g., efavirenz, ritonavir) [32,33]. In our study we found a relatively high percentage of both HIV-infected (25%) and HIV-uninfected persons (17%) with vitamin D deficiency (defined as <20 ng/ml).

Vitamin D may play a critical role in a variety of health conditions including infectious diseases [41]. For instance, vitamin D may affect both the innate and adaptive immune responses [19-25], and epidemiological studies have shown a link between deficiency and tuberculosis, pneumonia, and viral upper respiratory infections [19,25,43-49]. Regarding

influenza, lower 25(OH)D levels in winter months has been associated with disease seasonality, and supplementation may reduce the incidence of infection, however results have been variable [19,25, 44,50,51].

Vitamin D may also impact vaccine immune responses. Animal studies have found that 1,25-dihydroxyvitamin D₃ administered with vaccination may enhance post-vaccination responses [52,53]. Supplementation with vitamin D was subsequently found to improve tetanus responses in human studies [54], while deficiency was linked to poorer hepatitis B vaccination responses [55]. With the efficacy of influenza vaccine at an estimated 60-65%, even during the well-matched seasons (defined as vaccine strains matching those circulating in the community) [56], further data on host factors that may improve immune responses post-vaccination are a high priority, especially among immunosuppressed persons who historically generate poorer immune responses. In a small study among prostate cancer patients, low 25(OH)D levels were associated with poorer influenza vaccine responses [26]. However, other studies among healthy adults and children found no benefit of vitamin D supplementation [57,58]. Further studies evaluating baseline serum 25(OH)D levels in healthy adults >50 years [28] and children/adolescents [27] also found no associations.

Studies among HIV-infected persons are especially important give their higher risk for influenza-related complications [10-13], but historically poorer post-vaccination antibody responses [2-9]. Hence HIV-infected persons represent a critical group for novel methods to enhance vaccine efficacy. Three published studies in HIV-infected persons on the influence of 25(OH)D on influenza vaccine immunogenicity have been published to date with none showing

significant associations [29-31]. Each study had methodologic limitations including the lack of evaluating long-term antibody responses.

In our study, HIV-infected adults had significantly lower antibody responses to influenza vaccination compared with age-matched HIV-uninfected adults. HIV-infected persons also had a higher prevalence of vitamin D deficiency, although this findings was not statistically significant. Despite these observations, 25(OH)D levels did not explain the poorer responses among HIV-infected persons for the primary objective nor any of the other multiple immunogenicity outcomes including at 6 months post-vaccination. While there was a trend for 25(OH)D levels >20 ng/ml and poorer antibody responses for some of the outcomes in our study, none were statistically significant and this trend was not seen using higher 25(OH)D cut-points or continuous variable analyses.

Predictors of poorer post-vaccination immune response in HIV-infected persons in our study included increasing age and duration of HIV infection as previously noted in our original publication [9], although in this analyses these only met borderline statistical significance likely due to reduced sample size. We did not find any relationships between the CD4 count, HIV RNA level, or ART and post-vaccination immune responses. Accelerated aging and diminution of key immune factors during the course of HIV infection [59] may explain the overall poor vaccine responses achieved among HIV-infected persons, but further data are needed.

Limitations of our study included its relatively small sample size; however, given that none of the analyses suggested a relationship between low 25(OH)D levels and immune responses, it is unlikely that a larger study would have found significant differences. Further the other studies among HIV-infected persons examining baseline 25(OH)D levels and post

influenza vaccine responses included ~90 HIV-infected patients and also found no associations [29,30]. Other potential limitations include that our study did not include data on T-cell or mucosal responses which may be affected by HIV status and are important components of anti-influenza immunity. Although we collected influenza diagnoses after vaccination, the lack of events ($n=0$) precluded its use as an outcome [37]. Regarding the timing of 25(OH)D levels, these were measured at day 28, the time of the first post-vaccination antibody measurement. When evaluating 25(OH)D levels among participants with available stored specimens at day 0 (baseline, $n=81$), we found excellent correlation between the 25(OH)D measures ($r=0.762$, $p<0.001$) with median 25(OH)D levels of 31 ng/ml (baseline) and 28 ng/ml (day 28), respectively. In addition, we repeated our analyses among the 81 participants with baseline 25(OH)D levels, and found no associations between the categorical or continuous baseline 25(OH)D levels and post-vaccination antibody responses. Since some of our study outcomes were at 6 months post-vaccination, we also examined 25(OH)D levels at this time point ($n=123$) and found a high correlation with the baseline levels ($r=0.775$, $p<0.001$) with a median 25(OH)D level at month 6 of 28 ng/ml. Lastly, our HIV cohort was based in the US and had robust CD4 cell counts, hence our findings may not be generalizable to other HIV populations.

Since influenza continues to cause epidemic rates of infections amounting to an estimated 225,000 hospitalizations and ~50,000 deaths each year in the US [60] with immunosuppressed persons, including those with HIV, at high risk of infection despite vaccination [61], novel methods to prevent infections represent a public health priority. Our study evaluated whether 25(OH)D levels predicted post-influenza vaccination responses. Our study had several strengths including evaluating age-matched groups of both HIV-infected and

HIV-uninfected persons; studying multiple post-vaccination immune outcomes; and evaluating durability over a 6-month post-vaccination period. Further, we evaluated adverse events post-vaccination, a novel outcome among prior HIV studies.

In summary, vitamin D deficiency (<20 ng/ml) was common among both HIV-infected and HIV-uninfected adults in our study. However, low 25(OH)D levels did not predict antibody responses after H1N1 (2009) influenza vaccination among either group. Additionally, levels were not associated with adverse events temporally associated with vaccination. As low 25(OH)D levels do not explain poorer post-vaccination responses among HIV-infected persons, further research evaluating the causes of poorer humoral responses in this population and novel strategies for improving vaccine immunogenicity are recommended.

Acknowledgements:

The authors wish to thank the study participants who volunteered their time for this research study. We also thank the clinical site coordinators and managers as well as personnel (including Mr. Ryan Ortiguerra) at the Operational Infectious Disease laboratory, Naval Health Research Center for performing the 25(OH)D levels. We also thank the Defense Health Agency (DHA) Immunization Healthcare Branch for funding the vitamin D test kits (MILVAX grant FY13-69).

References

1. Kim DK, Bridges CB, Harriman KH, on behalf of the Advisory Committee on Immunization Practices. Advisory Committee on Immunization Practices Recommended Immunization Schedule for Adults Aged 19 Years or Older: United States, 2016 Recommended Adult Immunization Schedule: United States, 2016. *Ann Intern Med* 2016; 164:184-94.
2. Anema A, Mills E, Montaner J, Brownstein JS, Cooper C. Efficacy of influenza vaccination in HIV-positive patients: a systematic review and meta-analysis. *HIV Med* 2008; 9:57–61.
3. Atashili J, Kalilani L, Adimora A. Efficacy and clinical effectiveness of influenza vaccines in HIV-infected individuals: a meta-analysis. *BMC Infect Dis* 2006; 6:138.
4. Kunisaki K, Janoff E. Influenza in immunosuppressed populations: a review of infection frequency, morbidity, mortality, and vaccine responses. *Lancet Infect Dis* 2009; 9:493–504.
5. Tasker SA, Treanor JJ, Paxton WB, Wallace MR. Efficacy of influenza vaccination in HIV-infected persons. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1999; 131:430–3.
6. Kroon FP, van Dissel JT, de Jong JC, Zwinderman K, van Furth R. Antibody response after influenza vaccination in HIV-infected individuals: a consecutive 3-year study. *Vaccine* 2000; 18:3040–9.
7. Bickel M, Wieters I, Khaykin P, Nisius G, Haberl A, Stephan C, et al. Low rate of seroconversion after vaccination with a split virion, adjuvanted pandemic H1N1 influenza vaccine in HIV-1-infected patients. *AIDS* 2010; 24:F31–5.

8. Tebas P, Frank I, Lewis M, Quinn J, Zifchak L, Thomas A, et al. Center for AIDS Research and Clinical Trials Unit of the University of Pennsylvania. Poor immunogenicity of the H1N1 2009 vaccine in well controlled HIV-infected individuals. *AIDS* 2010; 24:2187–92.
9. Crum-Cianflone NF, Eberly LE, Duplessis C, Maguire J, Ganesan A, Faix D, Defang G, Bai Y, Iverson E, Lalani T, Whitman T, Blair PJ, Brandt C, Macalino G, Burgess T. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine in an immunocompromised population: a prospective study comparing HIV-infected adults with HIV-uninfected adults. *Clin Infect Dis* 2011; 52:138-46.
10. Neuzil KM, Reed GW, Mitchel EF Jr, Griffin MR. Influenza-associated morbidity and mortality in young and middle-aged women. *JAMA* 1999; 281:901–7.
11. Lin JC, Nichol KL. Excess mortality due to pneumonia or influenza during influenza seasons among person with acquired immunodeficiency syndrome. *Arch Intern Med* 2001; 161:441–6.
12. Safrin S, Rush JD, Mills J. Influenza in patients with human immunodeficiency virus infection. *Chest* 1990; 98:33–7.
13. Centers for Disease Control and Prevention (CDC). Increase in pneumonia mortality among adults and the HIV epidemic in New York City, United States. *MMWR Morb Mortal Wkly Rep* 1988; 37:593–6.
14. McKittrick N, Frank I, Jacobson JM, White CJ, Kim D, Kappes R, et al. Improved immunogenicity with high-dose seasonal influenza vaccine in HIV-infected persons: a single-center, parallel, randomized trial. *Ann Intern Med* 2013; 158:19-26.

15. Nielsen AB, Nielsen HS, Nielsen L, Thybo S, Kronborg G. Immune response after one or two doses of pandemic influenza A (H1N1) monovalent, AS03-adjuvanted vaccine in HIV infected adults. *Vaccine* 2012; 30:7067-71.
16. Durier C, Desaint C, Lucht F, Girard PM, Lévy Y, May T, et al; ANRS 151 study group and the REIVAC network. Long-term immunogenicity of two doses of 2009 A/H1N1v vaccine with and without AS03(A) adjuvant in HIV-1-infected adults. *AIDS* 2013;27:87-93.
17. Fabbiani M, Di Giambenedetto S, Sali M, Farina S, Sansonetti P, Tamburrini E, et al. Immune response to influenza A (H1N1)v monovalent MF59-adjuvanted vaccine in HIV-infected patients. *Vaccine* 2011; 29:2836-9.
18. Aberg JA, Gallant JE, Ghanem KG, Emmanuel P, Zingman BS, Horberg MA; Infectious Diseases Society of America. Primary care guidelines for the management of persons infected with HIV: 2013 update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2014; 58:1-10.
19. Khoo AL, Chai L, Koenen H, Joosten I, Netea M, van der Ven A. Translating the role of vitamin D3 in infectious diseases. *Crit Rev Microbiol* 2012; 38:122-35.
20. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006; 311:1770–3.
21. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O’Garra A. 1 α ,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol* 2001; 167: 4974–80.
22. Chen S, Sims GP, Chen XX, Gu YY, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol* 2007; 179:1634–47.

23. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol* 2005; 97:93–101.
24. Lang PO, Aspinall R. Can we translate vitamin D immunomodulating effect on innate and adaptive immunity to vaccine response? *Nutrients* 2015;7:2044-60.
25. Lang PO, Samaras N, Samaras D, Aspinall R. How important is vitamin D in preventing infections? *Osteoporos Int* 2013;24:1537-53.
26. Chadha MK, Fakih M, Muindi J, Tian L, Mashtare T, Johson CS, Trump D. Effect of 25-Hydroxyvitamin D Status on Serological Response to Influenza Vaccine in Prostate Cancer Patients. *Prostate* 2011; 71:368-372.
27. Science M, Maguire JL, Russell ML, Smieja M, Walter SD, Loeb M. Serum 25-hydroxyvitamin d level and influenza vaccine immunogenicity in children and adolescents. *PLoS One* 2014; 9:e83553.
28. Sundaram ME, Talbot HK, Zhu Y, Griffin MR, Spencer S, Shay DK, Coleman LA. Vitamin D is not associated with serologic response to influenza vaccine in adults over 50 years old. *Vaccine* 2013; 31:2057-61.
29. Bickel M, Lassmann C, Wieters I, Doerr HW, Herrmann E, Wicker S, et al. Immune response after a single dose of the 2010/11 trivalent, seasonal influenza vaccine in HIV-1-infected patients and healthy controls. *HIV Clin Trials* 2013; 14:175-81.
30. Momplaisir F, Frank I, Meyer W 3rd, Kim D, Kappes R, Tebas P. Vitamin D levels, natural H1N1 infection and response to H1N1 vaccine among HIV-infected individuals. *J AIDS Clin Res.* 2012;3:152.

31. Cooper C, Thorne A; Canadian Hiv Trials Network Ctn Influenza Vaccine Research Group.

Vitamin D supplementation does not increase immunogenicity of seasonal influenza vaccine in HIV-infected adults. *HIV Clin Trials* 2011; 12:275-6.

32. Dao CN, Patel P, Overton ET, Rhame F, Pals SL, Johnson C, Bush T, Brooks JT; Study to Understand the Natural History of HIV and AIDS in the Era of Effective Therapy (SUN)

Investigators. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D Levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clin Infect Dis* 2011; 52:396-405.

33. Oyedele T, Adeyemi OM. High prevalence of vitamin D deficiency in HIV-infected adults: what are the future research questions? *Curr HIV/AIDS Rep* 2012; 9:1-4.

34. Division of AIDS table for grading the severity of adult and pediatric adverse events (version 1.0-December 2004) Available at: <http://rcc.tech-res.com/safetyandpharmacovigilance/>. Accessed 1 March 2016.

35. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J Hyg (Lond)* 1972; 70:767-77.

36. Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. *Virus Res* 2004; 103:133–8.

37. Crum-Cianflone NF, Iverson E, Defang G, Blair PJ, Eberly LE, Maguire J, et al. Durability of antibody responses after receipt of the monovalent 2009 pandemic influenza A (H1N1) vaccine among HIV-infected and HIV-uninfected adults. *Vaccine* 2011; 29:3183-91.

38. Kendal AP, Pereira MS, Skehel JJ. Concepts and procedures for laboratory-based influenza surveillance. Geneva: World Health Organization; 1982.
39. Eagle Biosciences Vitamin 25 OH ELISA Package Insert. Available at:
http://www.eaglebio.com/content/VID31-K01_Vitamin_D_25_OH_ELISA_v2.pdf. Accessed on March 23, 2016.
40. DEQAS (Vitamin D External Quality Assessment Scheme). Endocrine Laboratory - Charing Cross Hospital - Fulham Palace Road - London W6 8RF – UK. Available at:
<http://www.deqas.org/>, Accessed on March 23, 2016.
41. Holick MF. Vitamin D Deficiency. *N Engl J Med* 2007; 357:266-81.
42. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008;87:1080S-6S.
43. Sundaram ME, Coleman LA. Vitamin D and influenza. *Adv Nutr* 2012; 3:517-25.
44. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, Garland CF, Giovannucci E. Epidemic influenza and vitamin D. *Epidemiol Infect* 2006; 134:1129-40.
45. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML. Serum 25-Hydroxyvitamin D and the Incidence of Acute Viral Respiratory Tract Infections in Healthy Adults. *PLoS One* 2010; 5:e11088.
46. Science M, Maguire JL, Russell ML, Smieja M, Walter SD, Loeb M. Low serum 25-hydroxyvitamin D level and risk of upper respiratory tract infection in children and adolescents. *Clin Infect Dis* 2013; 57:392-7.
47. Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients* 2015; 7:4240-70.

48. Pletz MW, Terkamp C, Schumacher U, Rohde G, Schütte H, Welte T, Bals R; CAPNETZ-Study Group. Vitamin D deficiency in community-acquired pneumonia: low levels of 1,25(OH)₂ D are associated with disease severity. *Respir Res* 2014; 15:53.
49. Watkins RR, Lemonovich TL, Salata RA. An update on the association of vitamin D deficiency with common infectious diseases. *Can J Physiol Pharmacol* 2015; 93:363-8.
50. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clin Nutr* 2010; 91:1255-60.
51. Bergman P, Lindh AU, Björkhem-Bergman L, Lindh JD. Vitamin D and respiratory tract infections: A systematic review and meta-analysis of randomized controlled trials. *PLoS One* 2013; 8:e65835.
52. Ivanov AP, Dragunsky EM, Chumakov KM. 1,25-Dihydroxyvitamin D₃ enhances systemic and mucosal immune responses to inactivated poliovirus vaccine in mice. *J Infect Dis* 2006; 193:598-600.
53. Enioutina EY, Visic D, McGee ZA, Daynes RA. The induction of systemic and mucosal immune responses the subcutaneous immunization of mature adult mice: characterization of the antibodies in mucosal secretions of animals immunized with antigen formulations containing a vitamin D₃ adjuvant. *Vaccine* 1999; 17:3050-64.
54. Heine G, Drozdenko G, Lahl A, Unterwalder N, Mei H, Volk HD, Dorner T, Radbruch A, Worm M. Efficient tetanus toxoid immunization on vitamin D supplementation. *Eur J Clin Nutr* 2011; 65:329-34.

55. Zitt E, Sprenger-Mahr H, Knoll F, Neyer U, Lhotta K. Vitamin D deficiency is associated with poor response to active hepatitis B immunization in patients with chronic kidney disease. *Vaccine* 2012; 30:931-5.
56. Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; 12:36-44.
57. Kriesel JD, Spruance J. Calcitriol (1,25-dihydroxy-vitamin D3) coadministered with influenza vaccine does not enhance humoral immunity in human volunteers. *Vaccine* 1999; 17:1883-8.
58. Principi N, Marchisio P, Terranova L, Zampiero A, Baggi E, Daleno C, Tirelli S, Pelucchi C, Esposito S. Impact of vitamin D administration on immunogenicity of trivalent inactivated influenza vaccine in previously unvaccinated children. *Hum Vaccin Immunother* 2013; 9:969-74.
59. Deeks SG. HIV Infection, Inflammation, Immunosenescence, and Aging. *Annu Rev Med* 2011; 62:141–55.
60. Dushoff J, Plotkin JB, Viboud C, Earn DJ, Simonsen L. Mortality due to influenza in the United States--an annualized regression approach using multiple-cause mortality data. *Am J Epidemiol* 2006; 163:181-7.
61. Klein MB, Lu Y, DelBalso L, Cote´ S, Boivin G. Influenza virus infection is a primary cause of febrile respiratory illness in HIV-infected adults, despite vaccination. *Clin Infect Dis* 2007; 45:234–40.

Table 1: Baseline Characteristics* and Influenza Antibody Levels by HIV Status

		<i>Total</i> <i>(n=128)</i>	<i>HIV- Uninfected</i> <i>(n=64)</i>	<i>HIV-Infected</i> <i>(n=64)</i>	<i>p- value**</i>
Age (years)		34.5 (14.5)	34.5 (11.0)	35.0 (19.0)	0.4935
Gender	Male	117 (91.4%)	57 (89.1%)	60 (93.8%)	0.5302
Race	Caucasian	76 (59.4%)	42 (65.6%)	34 (53.1%)	0.1305
	African-American	32 (25.0%)	11 (17.2%)	21 (32.8%)	
	Other	20 (15.6%)	11 (17.2%)	9 (14.1%)	
BMI, kg/m²		26.5 (5.0)	27.0 (4.0)	26.0 (5.0)	0.7430
Enrollment Location***	Site 1	111 (86.7%)	64 (100%)	47 (73.4%)	<0.001
	Site 2	13 (10.2%)	0 (0%)	13 (20.3%)	
	Site 3	4 (3.1%)	0 (0%)	4 (6.3%)	
2009 seasonal influenza vaccine receipt		122 (95.3%)	63 (98.4%)	59 (92.2%)	0.2076
No. of other household members	0	36 (28.1%)	11 (17.2%)	25 (39.1%)	0.0045
	1	35 (27.3%)	15 (23.4%)	20 (31.3%)	
	2	23 (18.0%)	14 (21.9%)	9 (14.1%)	
	≥3	34 (26.6%)	24 (37.5%)	10 (15.6%)	
HIV history					
HIV duration (years)				6.2 (11.4)	.
CDC stage	A			49 (76.6%)	
	B			10 (15.6%)	
	C			5 (7.8%)	
Nadir CD4 count (cells/mm³)				305.5 (204.5)	.
	< 200			11 (17.2%)	
	200 - 349			27 (42.2%)	
	>= 350			26 (40.6%)	
CD4 cell count at vaccination (cells/mm³)				580.0 (363.5)	.
	< 350			7 (10.9%)	
	350 - 499			15 (23.4%)	
	500 - 749			22 (34.4%)	

Table 1: Baseline Characteristics* and Influenza Antibody Levels by HIV Status

	Total (n=128)	HIV- Uninfected (n=64)	HIV-Infected (n=64)	p- value**
>= 750			20 (31.3%)	
HIV RNA at vaccination (log₁₀ copies/ml)			1.7 (0.7)	.
HIV RNA level <50 copies/ml			36 (56.3%)	
Currently receiving ART			52 (81.3%)	
Vitamin D level****				
25(OH)D level	27.9 (17.0)	28.7 (17.3)	26.9 (15.2)	0.3848
<20 ng/ml	27 (21.1%)	11 (17.2%)	16 (25.0%)	0.3865
≥20 ng/ml	101 (78.9%)	53 (82.8%)	48 (75.0%)	

*Count(proportion) or median (interquartile range)

** Wilcoxon Rank-Sum test and Fisher's exact test were used for continuous and categorical variables, respectively

***Enrollment location was San Diego for site 1 and Portsmouth and Bethesda for sites 2 and 3.

****At day 28 post-vaccination (time of first antibody response determination)

Table 2: Antibody Responses to the H1N1 Vaccine in HIV-uninfected and HIV-infected Participants

	<i>Total</i>	<i>HIV- Uninfected</i>	<i>HIV- Infected</i>	<i>Unadjusted P-value</i>	<i>Adjusted OR**</i>	<i>P-value</i>
Primary Endpoint*						
Titer ≥ 1:40 at day 28	51/80 (63.8%)	26/35 (74.3%)	25/45 (55.6%)	0.104	0.26 (0.07 – 0.88)	0.031
Secondary Endpoints						
≥ 4-fold increase in all participants	93/124 (75.0%)	52/62 (83.9%)	41/62 (66.1%)	0.037	0.29 (0.10 – 0.78)	0.015
Titer ≥ 1:40 in all participants	93/124 (75.0%)	51/62 (82.3%)	42/62 (67.7%)	0.096	0.25 (0.09 – 0.70)	0.003
Subgroup analyses for primary outcome						
Age <35 years (n=48)	28/39 (71.8%)	12/16 (75.0%)	16/23 (69.6%)	>.999	1.06 (0.15 – 7.44)	0.953
Age ≥ 35 years (n=46)	23/41 (56.1%)	14/19 (73.7%)	9/22 (40.9%)	0.058	0.11 (0.01 – 1.24)	0.074

* (n=80 with prevaccine level ≤1:10)

**Adjusted for HIV status, age, race, seasonal influenza vaccinations in previous 3 years, household members, and 25(OH)D level (<20 ng/ml vs. ≥20 ng/ml).

Unadjusted p-values from two-sided Fisher's exact test; Adjusted p-values from logistic regression. All data represent

Table 3: Immunogenicity at Day 28 post H1N1 Influenza Vaccination by HIV and Vitamin D Status

		<i>HIV-uninfected</i>	<i>25(OH)D <20 ng/ml</i>	<i>25(OH)D ≥ 20 ng/ml</i>	<i>P-value</i>	<i>HIV-infected</i>	<i>25(OH)D <20 ng/ml</i>	<i>25(OH)D ≥ 20 ng/ml</i>	<i>P-value</i>
Primary Endpoint	Titer ≥ 1:40 at day 28	26 (74.3%)	4 (80.0%)	22 (73.3%)	>.9999	25 (55.6%)	7 (70.0%)	18 (51.4%)	0.4728
Secondary Endpoints	a. ≥4-fold increase	52 (83.9%)	9 (90.0%)	43 (82.7%)	>.9999	41 (66.1%)	13 (86.7%)	28 (59.6%)	0.0659
	b. Titer ≥ 1:40	51 (82.3%)	9 (90.0%)	42 (80.8%)	0.6743	42 (67.7%)	12 (80.0%)	30 (63.8%)	0.3462
Subgroup analyses for primary outcome	a. Age <35 years	12 (75.0%)	2 (66.7%)	10 (76.9%)	>.9999	16 (69.6%)	4 (66.7%)	12 (70.6%)	>.9999
	b. Age ≥ 35 years	14 (73.7%)	2 (100.0%)	12 (70.6%)	>.9999	9 (40.9%)	3 (75.0%)	6 (33.3%)	0.2643

Table 4: Factors Associated with Achieving Primary Endpoint among HIV-infected Participants

		Total (N=45)	Endpoint Not Achieved (n=20)	Endpoint Achieved (n=25)	OR*	P- value*
Age (years)		34.0 (19.0)	45.0 (21.0)	32.0 (15.0)	0.94 (0.89 – 1.01)	0.0719
Gender	Male	43 (95.6%)	19 (95.0%)	24 (96.0%)	1.26 (0.07 – 21.5)	0.8718
Race	White	23 (51.1%)	9 (45.0%)	14 (56.0%)	Ref.	0.5006
	Black	16 (35.6%)	7 (35.0%)	9 (36.0%)	0.83 (0.23 – 3.02)	
	Other	6 (13.3%)	4 (20.0%)	2 (8.0%)	0.32 (0.05 – 2.13)	
BMI		27.0 (5.0)	27.0 (4.5)	27.0 (5.0)	1.04 (0.91 – 1.19)	0.5516
HIV infection duration (years)		5.8 (12.2)	11.3 (20.3)	5.7 (11.0)	0.93 (0.86 – 1.01)	0.0902
Nadir CD4 cell count (cells/mm³)	<200	5 (11.1%)	4 (20.0%)	1 (4.0%)	Ref.	0.3056
	200-349	20 (44.4%)	8 (40.0%)	12 (48.0%)	6.00 (0.56 – 63.98)	
	>=350	20 (44.4%)	8 (40.0%)	12 (48.0%)	6.00 (0.56 – 63.98)	
Baseline CD4 cell count (cells/mm³)	<350	4 (8.9%)	3 (15.0%)	1 (4.0%)	Ref.	0.1721
	350-499	11 (24.4%)	6 (30.0%)	5 (20.0%)	2.50 (0.19 – 32.19)	
	500-749	17 (37.8%)	4 (20.0%)	13 (52.0%)	9.75 (0.78 – 121.8)	
	>=750	13 (28.9%)	7 (35.0%)	6 (24.0%)	2.57 (0.21 – 31.71)	
HIV RNA level (copies/ml)	<50	25 (55.6%)	11 (55.0%)	14 (56.0%)	1.04 (0.32 – 3.40)	0.9465
	≥50	20 (44.4%)	9 (45.0%)	11 (44.0%)	Ref.	
Currently taking ART	No	9 (20.0%)	5 (25.0%)	4 (16.0%)	Ref.	0.4563
	Yes	36 (80.0%)	15 (75.0%)	21 (84.0%)	1.75 (0.40 – 7.63)	
No. of other household members	0	18 (40.0%)	8 (40.0%)	10 (40.0%)	Ref.	0.8868
	1	13 (28.9%)	5 (25.0%)	8 (32.0%)	1.28 (0.30 – 5.48)	
	2	7 (15.6%)	4 (20.0%)	3 (12.0%)	0.60 (0.10 – 3.50)	

Table 4: Factors Associated with Achieving Primary Endpoint among HIV-infected Participants

		<i>Total (N=45)</i>	<i>Endpoint Not Achieved (n=20)</i>	<i>Endpoint Achieved (n=25)</i>	<i>OR*</i>	<i>P- value*</i>
	>=3	7 (15.6%)	3 (15.0%)	4 (16.0%)	1.07 (0.18 – 6.21)	
No. of seasonal vaccine in previous 3 years	<3	16 (35.6%)	6 (30.0%)	10 (40.0%)	Ref.	0.4874
	>=3	29 (64.4%)	14 (70.0%)	15 (60.0%)	0.64 (0.19 – 2.24)	
25(OH)D level (ng/ml)		28.7 (15.7)	30.3 (15.2)	25.4 (14.7)	0.98 (0.94 – 1.02)	0.3803
	<20 ng/ml	10 (22.2%)	3 (15.0%)	7 (28.0%)	2.20 (0.49 – 9.93)	0.3040
	≥20 ng/ml	35 (77.8%)	17 (85.0%)	18 (72.0%)	Ref.	

*Unadjusted OR with its 95% CI and p-values from logistic regression

Supplementary Table 1: Immunogenicity at Day 28 post H1N1 Influenza Vaccination by HIV Status and 25(OH)D Level
Using a Cut-point of 40 ng/ml

		<div> <div>25(OH)D</div> <div>HIV-uninfected</div> </div> <div> <div>25(OH)D</div> <div><40 ng</div> </div> <div> <div>25(OH)D</div> <div>≥40 ng</div> </div> <div> <div>P-value</div> </div>				<div> <div>25(OH)D</div> <div>HIV-infected</div> </div> <div> <div>25(OH)D</div> <div><40 ng</div> </div> <div> <div>25(OH)D</div> <div>≥40 ng</div> </div> <div> <div>P-value</div> </div>			
Primary Endpoint	Titer ≥ 1:40 at day 28	26 (74.3%)	18 (69.2%)	8 (88.9%)	0.3907	25 (55.6%)	19 (54.3%)	6 (60.0%)	>.999
Secondary Endpoints	a. ≥4-fold increase	52 (83.9%)	41 (82.0%)	11 (91.7%)	0.6704	41 (66.1%)	35 (68.6%)	6 (54.6%)	0.4852
	b. Titer ≥ 1:40	51 (82.3%)	40 (80.0%)	11 (91.7%)	0.6747	42 (67.7%)	35 (68.6%)	7 (63.6%)	0.7353
Subgroup analyses	a. Age <35 years	12 (75.0%)	11 (78.6%)	1 (50.0%)	0.4500	16 (69.6%)	13 (65.0%)	3 (100.0%)	0.5257
for primary outcome	b. Age ≥ 35 years	14 (73.7%)	7 (58.3%)	7 (100.0%)	0.1060	9 (40.9%)	6 (40.0%)	3 (42.9%)	>.999

Supplementary Table 2: Adverse Reactions Temporally Associated with H1N1 Influenza Vaccination by 25(OH)D Level

		Total (n=128)	< 20ng/ml (n=27)	≥20ng/ml (n=101)	P-value*
Any adverse reactions		76 (59.4%)	16 (59.3%)	60 (59.4%)	>.9999
Number of AE per participant	None	52 (40.6%)	11 (40.7%)	41 (40.6%)	0.5237
	One	29 (22.7%)	8 (29.6%)	21 (20.8%)	
	Two or More	47 (36.7%)	8 (29.6%)	39 (38.6%)	
Severity	Mild	69 (53.9%)	14 (51.9%)	55 (54.5%)	0.8311
	Moderate	28 (21.9%)	5 (18.5%)	23 (22.8%)	0.7953
	Severe	8 (6.3%)	2 (7.4%)	6 (5.9%)	0.6752
Local Reactions	Any reaction	47 (36.7%)	11 (40.7%)	36 (35.6%)	0.6573
	Pain	43 (33.6%)	11 (40.7%)	32 (31.7%)	0.4917
	Erythema	13 (10.2%)	2 (7.4%)	11 (10.9%)	0.7346
	Bruising	8 (6.3%)	1 (3.7%)	7 (6.9%)	>.9999
	Induration	8 (6.3%)	3 (11.1%)	5 (5.0%)	0.3640
Systematic Reactions	Any reaction	53 (41.4%)	10 (37.0%)	43 (42.6%)	0.6646
	Headache	38 (29.7%)	8 (29.6%)	30 (29.7%)	>.9999
	Malaise	30 (23.4%)	5 (18.5%)	25 (24.8%)	0.6137
	Myalgias	21 (16.4%)	5 (18.5%)	16 (15.8%)	0.7720
	Nausea	14 (10.9%)	1 (3.7%)	13 (12.9%)	0.2982
	Chills	13 (10.2%)	1 (3.7%)	12 (11.9%)	0.2979
	Fever	3 (2.3%)	0 (0%)	3 (3.0%)	>.9999
	Axillary node tenderness	2 (1.6%)	0 (0%)	2 (2.0%)	>.9999
	Vomiting	1 (0.8%)	0 (0%)	1 (1.0%)	>.9999

*P-values from two-sided Fisher's exact test

Figure 1: Geometric Mean Titer (GMT) at Each Study Visit by HIV Status and 25(OH)D Level