Association of Suboptimal Antiretroviral Therapy Adherence With Inflammation in Virologically Suppressed Individuals Enrolled in the SMART Study

Jose R. Castillo-Mancilla,1 Andrew N. Phillips,2 James D. Neaton,3 Jacqueline Neuhaus,4 Simon Collins,4 Sharon Mannheimer,5 Sarah Pett,6,7 Veronique Touzeau-Römer,8 Mark N. Polizzotto,7 Jens D. Lundgren,9 and Edward M. Gardner10; for the INSIGHT SMART Study Group

1University of Colorado-AMC, Medicine/Infectious Diseases, Aurora, Colorado; 2Institute for Global Health, University College London, London, UK; 3University of Minnesota, School of Public Health, Minneapolis, Minnesota; 4HIV-1 Base, London, UK; 5Columbia University Medical Center, Harlem Hospital Center, New York, New York; 6University College London, London, UK; 7Kirby Institute, University of New South Wales, Sydney, Australia; 8University of Vienna Medical School, AKH, Division of Immunology, Allergy and Infectious Diseases, Vienna, Austria; 9CHIPI Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark; 10Denver Health Medical Center, Denver, Colorado

Suboptimal (ie, <100%) antiretroviral therapy (ART) adherence has been associated with heightened inflammation in cohort studies, even among people with virologic suppression. We aimed to evaluate this association among participants in the Strategies for Management of Antiretroviral Therapy (SMART) study who had virologic suppression (HIV-1 VL < 200 copies/mL) at enrollment. Based on self-reported adherence (7-day recall), plasma concentrations of interleukin 6 and D-dimer were 9% (95% confidence interval [CI], 1%–18%; P = .02) and 11% (95% CI, 1%–22%; P = .03) higher in participants who reported suboptimal vs 100% adherence, respectively. These findings confirm previous observations and support the hypothesis that suboptimal ART adherence, even in the context of virologic suppression, may have significant biological consequences.

ClinicalTrials.gov number NCT00027352

Keywords. adherence; antiretroviral therapy; inflammation; SMART study.

While suppressive antiretroviral therapy (ART) has allowed for improved survival in people living with HIV (PLWH), these individuals exhibit a state of chronic residual inflammation, immune activation, and coagulopathy that, on average, does not revert to the levels observed in their HIV-negative counterparts [1]. This residual inflammation has been associated with the development of serious non-AIDS adverse events (SNAEs) such as cardiovascular disease, cancer, and death [2, 3]. To date, the mechanisms behind this immune dysregulation remain poorly understood, which has limited the efforts to identify interventions that can successfully reverse it. Thus, it remains imperative to identify new and effective strategies to achieve this goal.

Recently, suboptimal ART adherence has emerged as a potential contributor to residual inflammation in PLWH, even if it is sufficient to achieve and sustain plasma viral suppression through conventional assays [4, 5]. Cohort-derived data suggest that suboptimal (ie, less than 100%) adherence is associated with higher levels of residual inflammation, immune activation, and activation of coagulation in PLWH who have virologic suppression while on ART [4, 5]. These observations have emphasized the potential biological differences that could exist between complete and suboptimal ART adherence in order to maximize the therapeutic benefit of ART. Whether these associations can also be identified in a large, multinational diverse population remains unknown. To evaluate this, we aimed to study the association of ART adherence with inflammation and activation of coagulation in virologically suppressed PLWH on chronic ART who were enrolled in the Strategies for Management of Antiretroviral Therapy (SMART) study.

METHODS

Participants
The SMART study (NCT00027352) was a multinational, randomized clinical trial performed in 5472 PLWH (women and men older than 13 years) between 2002 and 2006; SMART study details and main results were previously published [6]. The trial included PLWH who were or were not on ART upon enrollment without restriction according to viral load (VL) [6]. In this retrospective analysis, we evaluated participants who at enrollment: (1) were on ART, (2) had completed an adherence questionnaire, and (3) had an HIV-1 VL measurement available. Given our goal of evaluating the association between adherence and residual inflammation and coagulopathy beyond virologic suppression, we focused our study population to individuals who were virologically suppressed to at least <200 copies/mL (and to <50 copies/mL if this lower threshold was available) and limited our analysis to the enrollment visit only. All study procedures were reviewed and approved by the local institutional review boards, and all participants provided written informed consent.

Adherence Assessment
Adherence was measured (within 45 days of randomization) by participant self-report using the Terry Beirn Community
Programs for Clinical Research on AIDS (CPCRA) Antiretroviral Medication Self-Report Form 065-BAS-2, which uses a 7-day global recall in which participants can respond whether they took “all,” “most,” “about one-half,” “very few,” or “none” of their pills for each specific pill in their ART regimen. This is a validated adherence measure that predicted the development of viral rebound in people with viral suppression in the SMART study [7, 8]. Adherence was labeled “suboptimal” if a participant reported any option other than taking “all of my pills” for at least 1 antiretroviral medication (calculated adherence < 100%), and “100%” if a participant reported taking “all of my pills” for all ART medications [8].

Biomarkers of Inflammation and Activation of Coagulation
Plasma levels of interleukin 6 (IL-6), high-sensitivity C-reactive protein (hsCRP), and D-dimer were measured in plasma samples collected at enrollment using EDTA tubes (and stored/shipped frozen) in a subset of the enrolled and randomized participants, as previously reported [3]. These biomarkers were measured by the Laboratory for Clinical Biochemistry Research at the University of Vermont. IL-6 was measured by ultrasensitive enzyme-linked immunosorbent assay (ELISA; Quantikine HS Human IL-6 Immunoassay; R&D Systems), with a lower limit of detection (LLOD) of 0.16 pg/mL. hsCRP was measured using the BNII nephelometer (N High Sensitivity CRP; Siemens Healthcare Diagnostics), with an LLOD of 0.16 µg/mL. D-dimer was measured using immunoturbidimetric assay (Liatest D-DI; Diagnostica Stago), with an LLOD of 0.01 µg/mL, as previously described [3].

Statistical Analysis
Participant demographic and baseline cohort characteristics were summarized using the appropriate statistical measures. Adherence was dichotomized as 100% and suboptimal (<100%). Biomarker plasma concentrations were log-transformed before analysis to address skewing of data. Initially, the relationship between ART adherence and the baseline concentrations of the biomarkers was graphically analyzed using scatterplots. We then utilized univariate and multivariable linear regression analysis to evaluate the association between adherence and the log-transformed concentrations of biomarkers at baseline, adjusting for covariates that have previously been associated with biomarkers of inflammation and coagulopathy in SMART and other cohorts [9–13], including age, race, gender, body mass index, time since start of ART, HIV exposure group, baseline viral load, baseline and nadir CD4+ T-cells, co-infection with hepatitis B or C, smoking, and ART regimen type based on anchor drug. Data are presented as fold differences (95% CI) in biomarker concentrations in participants who were suboptimally vs 100% adherent. All statistical analyses were performed using SAS version 9.4. A P value <.05 was considered statistically significant.

RESULTS

Study Participants
At the baseline visit, a total of 3963 participants were taking ART and had both a completed adherence questionnaire and an HIV-1 RNA VL measurement. Of these, 3056 participants (77%) were virologically suppressed to <200 copies/mL and were included in the analysis (Supplementary Figure). Additional demographic and baseline characteristics of the study participants are shown in Table 1.

ART Adherence and Biomarkers of Inflammation and Activation of Coagulation
Of the 3056 virologically suppressed participants analyzed, 404 (13%) reported suboptimal adherence at the baseline visit (Table 1). The distribution of time between HIV VL

Table 1. Demographic Characteristics of Study Participants

| Characteristic (n = 3056) | No. (%) or Median (IQR) |
|--------------------------|------------------------|
| Demographics             |                        |
| Age, median (IQR), y     | 44 (38–51)             |
| Women                    | 827 (27%)              |
| Race                     |                        |
| White                    | 1519 (50%)             |
| Black                    | 696 (23%)              |
| Hispanic                 | 570 (19%)              |
| Asian                    | 200 (7%)               |
| Other                    | 71 (2%)                |
| Country of origin        |                        |
| Non-US                   | 1859 (61%)             |
| US                       | 1197 (39%)             |
| HIV exposure group       |                        |
| MSM                      | 1498 (49%)             |
| Heterosexual             | 1156 (38%)             |
| IDU                      | 239 (8%)               |
| Other                    | 163 (5%)               |
| BMI, median (IQR), kg/m² | 24.5 (22.1–27.4)       |
| Smoking (current)        | 1138 (37%)             |
| HBV infection            | 69 (2%)                |
| HCV infection            | 130 (4%)               |
| Time since start of ART, y|                      |
| <1                       | 72 (2%)                |
| 1–5                      | 1082 (35%)             |
| >5                       | 1895 (62%)             |
| Antiretroviral regimen   |                        |
| NNRTI-based              | 1388 (45%)             |
| PI-based                 | 1164 (38%)             |
| Other                    | 504 (16%)              |
| Baseline CD4+ T-cells, median (IQR), cells/mm³ | 649 (496–842) |
| Nadir CD4+ T-cells, median (IQR), cells/mm³ | 230 (136–329) |
| HIV VL <50 copies/mL     | 2371 (78%)             |
| Adherence (7-d)          |                        |
| 100% adherence           | 2652 (87%)             |
| Suboptimal adherence     | 404 (13%)              |

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HIV, hepatitis B virus; HCV, hepatitis C virus; IDU, injection drug users; IQR, interquartile range; MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
measured and biomarker measurement was (min, percentiles, and max): min –69 days, 1st –43 days, 5th –36 days, 10th –30 days, 25th –16 days, 50th 0 days, 75th 0 days, 90th 0 days, 95th 0 days, and 99th 14 days, max 40 days. IL-6, D-dimer, and hsCRP baseline biomarker data were available in 2763, 2776, and 2793 participants who fulfilled the other criteria, respectively. The distribution of biomarker concentrations in suboptimally vs 100% adherent participants who had available biomarker data at the baseline visit is shown in Table 2. In a univariate model, baseline IL-6, D-dimer, and hsCRP were 16% (95% CI, 1.06–1.26; P = .0005), 17% (95% CI, 1.05–1.29; P = .002), and 7% (95% CI, 0.94–1.23; P = .31) fold higher in participants who reported suboptimal adherence in comparison with those with who reported 100% adherence, respectively (Table 2). This association remained significant for both IL-6 and D-dimer in an adjusted model, where IL-6 was 9% (95% CI, 1.01–1.18; P = .02) and D-dimer was 11% (95% CI, 1.01–1.22; P = .03) higher in suboptimally adherent participants (Table 2). Similar results were observed in a sensitivity analysis restricted to participants with HIV-1 VL <50 copies/mL (n = 2371) (Supplementary Table).

DISCUSSION

In this analysis, we identified a significant inverse association between ART adherence and systemic inflammation and activation of coagulation in virologically suppressed (<200 copies/mL) PLWH at the time of their enrollment in SMART. The magnitude of this association ranged between 9% and 11% higher for levels of IL-6 and D-dimer, respectively, in participants reporting suboptimal vs 100% adherence after adjusting for multiple potential confounders such as smoking, body mass index, baseline and nadir CD4+ T-cell count, time since start of ART, and drug regimen. In addition, these findings also remained significant after restricting our analysis to participants who were suppressed to <50 copies/mL. To our knowledge, this is the first study to demonstrate an association between adherence to ART and biomarkers of inflammation and coagulopathy in a virologically suppressed diverse international population on chronic ART.

Our study reaffirms previous cohort findings in which suboptimal adherence, measured by self-report [4] and Medication Event Monitoring System [5], was associated with a similar degree of increased residual inflammation and coagulopathy, supporting the hypothesis that ART adherence has significant repercussions that extend beyond virologic suppression. However, our findings are contrary to recent studies in which short cycle ART interruptions (4 or 5 days on, 3 or 2 days off) maintained viral suppression in PLWH, and where no differences in inflammatory biomarkers were identified [14, 15]. These discrepancies could be due to differences in study populations (ie, short cycle studies generally recruited preselected participants with long-standing suppression), the small sample size, and the nonrandomized nature of most of these studies. While this therapeutic forgiveness of modern ART has usually been regarded as advantageous in clinical practice, it has de-emphasized the focus on achieving optimal adherence. It has also allowed permissiveness to missed doses, as long as virologic suppression is maintained. Further studies to better understand the role of this “suppressive adherence gap” on the pathogenesis of chronic residual inflammation in treated HIV disease are required, including additional studies evaluating treatment interruptions and studies focused on long-acting ART.

In regards to potential explanatory mechanisms behind our findings, suboptimal ART adherence could contribute to residual inflammation and coagulopathy in a variety of possible ways, which include (1) residual viral replication below the limit of detection of clinically available assays (which may lead to persistently enhanced inflammation and immune activation) [16, 17], (2) ongoing viral replication at sanctuary sites (ie, lymph node), where antiretroviral drug concentrations are low as a result of limited tissue penetration and/or low adherence (leading to viral escape and subsequent inflammation) [18], or (3) intermittent episodes of viremia that are not captured at the time of viral load assessment in a clinical or research setting (ie, missed viremia resulting in intermittent bursts of inflammation and immune activation). Given the limited impact that
most interventions to date have had in reducing chronic residual inflammation in treated HIV infection, further research to understand the pathogenesis of residual inflammation that can lead to new approaches aimed at achieving this goal is needed. In this context, whether ART adherence optimization could reduce residual chronic inflammation in individuals who have achieved virologic suppression, or whether it could have a synergistic effect in conjunction with current and future ART strategies, remains unknown and should be evaluated in prospective studies.

While the association between suboptimal adherence and enhanced residual inflammation is novel and provocative, its clinical translation remains unclear, in particular as it relates to the development of SNAEs in the virologically suppressed population. As chronic residual inflammation has been associated with the development of SNAEs [2, 3], it is plausible that suboptimal adherence could also be responsible, at least in part, for the increased morbidity and mortality observed in treated PLWH who remain virally suppressed. To answer this question, future analyses evaluating the association of SNAEs with ART adherence in the virally suppressed population should be performed in large clinical cohorts. This could be coupled with a systematic evaluation of ART adherence in current and future studies aimed at reducing chronic residual inflammation in HIV, and with studies in which persistent inflammation could lead to further evaluation of adherence practices.

The main strengths of our study include a large and diverse population of PLWH obtained within the context of a multinational clinical trial and the persistently significant associations that were identified at lower viral suppression thresholds and after adjusting for multiple confounders that could contribute to residual inflammation, such as smoking, body mass index, nadir CD4+ T-cells, and others. Among its limitations are the cross-sectional nature of the analysis and the potential for self-report to overestimate adherence [19], although a more objective adherence measure could have resulted in an even more significant association than the one we observed with self-report. In addition, the thresholds for viral suppression in this study (<200 and <50 copies/mL) may not reflect what is currently used in all clinical settings, and further research to evaluate this association using lower viral load cutoffs (ie, <20 copies/mL) is required. Lastly, although this study included a wide variety of ART regimens, it did not include integrase strand-transfer inhibitors (INSTIs), which may have a more pronounced effect in reducing residual inflammation when compared with other regimens [20]. Whether the differential effect of INSTIs on chronic inflammation extends to individuals who are suboptimally adherent to ART should also be evaluated.

In conclusion, we demonstrated that suboptimal ART adherence, even if it results in virologic suppression by conventional clinical assays, is associated with enhanced residual inflammation and activation of coagulation in PLWH on chronic ART. These findings replicate previous cohort observations and highlight the importance of optimal and durable ART adherence as a potential factor to improve morbidity and mortality in HIV disease.

Supplementary Data
Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments
We would like to thank the study participants for volunteering to participate in this study.

Prior presentation. These data were partially presented at the 9th IAS Conference on HIV Science, July 23–26, 2017, Paris, France, Abstract number WEPEB0543. See N Engl J Med 2006; 355:2283–96 for the complete list of SMART investigators.

Financial support. This work was supported by National Institute of Health grants: U01AI068641, U01AI042170 and U01AI46362 (SMART) and K23AI104315 (JCM) and R21AI124859 (JCM).

Potential conflicts of interest. A.P. received speaker fees for 2 presentations in 2015. The other authors reported no conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References
1. Neuhaus J, Jacobs DR Jr, Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis 2010; 201:1788–95.
2. Borges AH, Silverberg MJ, Wentworth D, et al. INSIGHT SMART; ESPRIT; SILCAAT Study Groups. Predicting risk of cancer during HIV infection: the role of inflammatory and coagulation biomarkers. AIDS 2013; 27:1433–41.
3. Borges AH, O’Connor JL, Phillips AN, et al. INSIGHT SMART Study and ESPRIT Groups. Interleukin 6 is a stronger predictor of clinical events than high-sensitivity C-reactive protein or D-dimer during HIV infection. J Infect Dis 2016; 214:408–16.
4. Castillo-Mancilla JR, Brown TT, Erlandson KM, et al. Suboptimal adherence to combination antiretroviral therapy is associated with higher levels of inflammation despite HIV suppression. Clin Infect Dis 2016; 63:1661–7.
5. Castillo-Mancilla J, Morrow M, Boum Y, et al. Greater ART adherence is associated with less inflammation in HIV-suppressed Ugandans. Poster Presented at: CROI February 13–16, 2017, Seattle, WA. Abstract 675.
6. El-Sadr WM, Lundgren J, Neaton JD, et al; Strategies for Management of Antiretroviral Therapy Study Group. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med 2006; 355:2283–96.
7. O’Connor JL, Gardner EM, Esser S, et al; International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) Strategies for Management of Antiretroviral Therapy (SMART) Study Group. A simple self-reported adherence tool as a predictor of viral rebound in people with viral suppression on antiretroviral therapy. HIV Med 2016; 17:124–32.
8. Mannheimer S, Friedland G, Matts J, et al. The consistency of adherence to antiretroviral therapy predicts biologic outcomes for human immunodeficiency virus-infected persons in clinical trials. Clin Infect Dis 2002; 34:1115–21.
9. Borges AH, O’Connor JL, Phillips AN, et al; INSIGHT SMART and ESPRIT Study Groups and the SILCAAT Scientific Committee. Factors associated with plasma IL-6 levels during HIV infection. J Infect Dis 2015; 212:585–95.
10. Kilper LH, Tracy R, Belloso W, et al; INSIGHT SMART Study Group. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 2008; 5:e203.
11. Wada N, Jacobson LP, Margolick JB, et al. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS 2015; 29:463–7.
12. McKibbon RA, Margolick JB, Grinspoon S, et al. Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection. J Infect Dis 2015; 211:1219–28.
13. Hunt PW, Lee SA, Siedner MJ. Immunologic biomarkers, morbidity, and mortality in treated HIV infection. J Infect Dis 2016; 214(Suppl 2):S44–50.
14. Group BT. Weekends-off efavirenz-based antiretroviral therapy in HIV-infected children, adolescents, and young adults (BREATHER): a randomised, open-label, non-inferiority, phase 2/3 trial. Lancet HIV 2016; 3:e421–30.
15. de Truchis P, Assoumou L, Landman R, et al. No increase in HIV-1 reservoir and inflammation markers in four days a week short-cycles maintenance therapy: the ANRS 162-4D trial. Poster Presented at: IAS 2017: 9th IAS Conference on HIV Pathogenesis Treatment and Prevention; July 23–26, 2017; Paris, France.
16. Mavigner M, Delobel P, Cazabat M, et al. HIV-1 residual viremia correlates with persistent T-cell activation in poor immunological responders to combination antiretroviral therapy. PLoS One 2009; 4:e7658.
17. Ostrowski SR, Katzenstein TL, Pedersen BK, et al. Residual viraemia in HIV-1-infected patients with plasma viral load <20 or =20 copies/ml is associated with increased blood levels of soluble immune activation markers. Scand J Immunol 2008; 68:652–60.
18. Lorenzo-Redondo R, Fryer HR, Bedford T, et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 2016; 530:51–6.
19. Pearson CR, Simoni JM, Hoff P, et al. Assessing antiretroviral adherence via electronic drug monitoring and self-report: an examination of key methodological issues. AIDS Behav 2007; 11:161–73.
20. Hileman CO, Kinley B, Scharen-Guivel V, et al. Differential reduction in monocyte activation and vascular inflammation with integrase inhibitor-based initial antiretroviral therapy among HIV-infected individuals. J Infect Dis 2015; 212:345–54.