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BRIEF REPORT

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

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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.

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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

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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/m^2	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), $cells/mm^3$	649 (496–842)
Nadir $CD4^+$ T-cells, median (IQR), $cells/mm^3$	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; HBV, 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.

measurement 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

Table 2. Distribution of Biomarker Concentrations in Participants With Available Baseline Data According to Adherence Category and Fold Difference in Available Baseline Inflammatory and Coagulopathy Biomarker Plasma Concentrations in Suboptimally Adherent, Virologically Suppressed PLWH on ART Enrolled in SMART

					Unadjusted Analysis			Adjusted Analysis[a]		
	100% Adherence		Suboptimal Adherence							
Biomarker	No.	Mean (SD)	No.	Mean (SD)	Fold Higher Level Compared With 100% Adherence[b]	95% CI	*P* Value	Fold Higher Level Compared With 100% Adherence[b]	95% CI	*P* Value
IL-6, pg/mL	2372	2.60 (6.93)	391	2.93 (4.53)	1.16	1.06–1.26	.0005	1.09	1.01–1.18	.02
D-dimer, µg/mL	2382	0.30 (0.59)	394	0.38 (1.06)	1.17	1.05–1.29	.002	1.11	1.01–1.22	.03
hsCRP, µg/mL	2397	3.72 (7.08)	396	4.27 (7.51)	1.07	0.94–1.23	.31	1.04	0.91–1.17	.58

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; PLWH, people living with HIV.

[a]Models were adjusted for covariates including age, race, gender, body mass index, time on ART, HIV exposure group, baseline viral load, baseline and nadir CD4$^+$ T-cells, hepatitis B or C co-infection, smoking, and regimen type.

[b]100% adherence defined as no report of any missed doses for any drug in the preceding 7-day period.

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 copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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